ABSTRACT
BOOK

SETAC SciCon

SETAC Europe 30th Annual Meeting

Open Science for Enhanced Global Environmental Protection

3–7 May 2020

Online Meeting

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Society of Environmental Toxicology and Chemistry Europe (SETAC Europe)

ABSTRACT BOOK
SETAC Europe 30th Annual Meeting

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This book compiles the abstracts from the platform and poster session presentations at the 30th Annual Meeting of the Society of Environmental Toxicology and Chemistry - Europe (SETAC Europe), conducted as a virtual conference, from 3–7 May 2020.
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 underlined.

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SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY
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®, to timely and effective communication of research, and to 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). Environmental Toxicology and Chemistry 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.

Keynote Lecture Abstracts

K1
A Sunday evening opinion on SETAC's role in the EU Green Deal
Annemarie van Wezel, University of Amsterdam, Netherlands
Mid December BC (Before Corona) the EU GreenDeal was presented by Frans Timmermans, with amongst others ambitious goals with regard to a toxic-free environment. In this opening lecture, during your Sunday (#veggie?) roast at the #virtual and thus #very21stCentury #SETAC2020 meeting, I'll discuss the various topics of relevance to SETAC of this EU Green Deal. What are the knowlegde gaps and what is the scientific consensus with regard to the various relevant topics related, such as the one-substance/one-assessment paradigm? What could be essential elements to come to a chemicals strategy for sustainability? And what role can our tripartite society (composed of members from academia, government and industry) play so that new scientific evidence is rapidly reflected in the regulatory frameworks.

K2
Safety Assessment without Animal Testing: Progress in Industry
Matthew Dent**, Unilever, United Kingdom**
Safety is a prerequisite for any consumer product. Worldwide, there is an ever-increasing desire to bring safe products to market without animal testing. In recent years it has become clear that trying to develop safety assessments that simply replace animal test data with some form of equivalent 'alternative' data will not work. Instead, a completely new approach to consumer safety is needed. 'Next Generation Risk Assessment' (NGRA), defined as an exposure-led, hypothesis driven risk assessment approach that integrates in silico, in chemico and in vitro approaches, provides such an approach. This talk will outline scientific progress in the development and adoption of NGRA and discuss how we can build acceptance of new assessment approaches for both human and environmental safety within the scientific community.

K3
Biodiversity and the role of big data: Using data to drive pollinator conservation in Ireland
Úna FitzPatrick, National Biodiversity Data Centre, Ireland
Pollination is a free service provided by nature, that delivers a healthy environment and landscape. Data shows that one third of Ireland's 98 wild bee species are threatened with extinction and that common bumblebees have experienced a 14.2% decline in abundance since 2012. Rare species are disappearing through habitat loss and common species are struggling because of the way we currently manage the rest of the landscape. Pollinators are in enormous difficulties, but we don't have to accept that fate. The All-Ireland Pollinator Plan was published in 2015www.pollinators.iemaking Irelandone of the first countries in Europe with an approach to address this problem. It is a voluntary plan that is supported by more than 100 governmental and non-governmental organisations and it has identified 81 actions to make Ireland more pollinator-friendly. Clear, accessible evidence-based guidelines have been written for all sectors explaining what actions can be taken to help. These have been widely and voluntarily adopted by farmers, local authorities, gardeners, community groups, businesses and schools. The All-Ireland Pollinator Plan is still in the early stages, but it has demonstrated that you can engage across sectors, and you can bring people together to address a biodiversity crisis. Importantly, it has shown that data is the impetus for change; allows identification of the correct evidence-based actions, and through tracking and monitoring creates the momentum to facilitate real progress.

K4
Science Communication
Estelle Robichaux, COMPASS, United States

Platform Abstracts

Advances in Invertebrate Endocrine Toxicology

1.01.1
Levels and trends of tributyltin (TBT) and intersex in common periwinkle (Littorina littorea) in Vikkilen, Norway from 2005 to 2018
M. Schøyen, Norwegian Institute for Water Research (NIVA) / Centre for Coastal and Marine Research; L.A. Tveiten, Norwegian Institute for Water Research NIVA / Marine Biology; D. Hjermann, Norwegian Institute for Water Research NIVA / Environmental Data Science; S. Øxnevad, Norwegian Institute for Water Research NIVA / Environmental Contaminants
TBT causes disruption of sexual development in gastropods, and the masculinization occur as intersex or imposex. While imposex is closely linked to contamination by organotin compounds, intersex can also be caused by natural estrogens (e.g. estradiol), synthetic oestrogens (e.g. ethinyl estradiol) and non-hormones (e.g. octylphenol and nonylphenol). Intersex can occur in both sexes and is development of spermatocytes in the ovary or oocytes in the testis. Intersex can be caused by TBT concentrations in seawater as low as 10-15 ng/L. 50 years after the first signs of imposex in marine snails, there were no imposex in dogwhelk (*Nucella lapillus*) along the Norwegian coast (SETAC Europe, Helsinki in 2019). These results were confirmed for 2018 data. *N. lapillus* exists in wave exposed areas, while common periwinkle (*Littorina littorea*) also lives in the fjords. Here we present new data on intersex in *L. littorea* from Vikkilen in Grimstad, a fjord in Norway. Sediment in the inner part of Vikkilen has for years been severely contaminated by TBT due to pollution from to the local shipyard industry. Concentrations as high as 60 000 µg TBT/kg have been detected in the sediment, which is the highest in Norway. The biological effect in *L. littorea* is quantified by the Intersex Sequence Index (ISI) and average Female Prostate Length (FPrL). Levels and trends of TBT and intersex (ISI) in *L. littorea* have been monitored since 2005 at six stations. Subsequently, 50 specimens from each station were analysed individually for intersex/ISI and pooled (only females) for TBT. There were high levels of intersex from 2005 to 2010, and then the levels decreased. There was no intersex (ISI=0) in *L. littorea* in 2018, or the last year intersex was investigated, and the TBT concentrations were low. There were significant downward trends for intersex (ISI) at the stations in the Inner fjord (Skjeviga, Shipyard, and Vikkilen), or low levels (Båtstø). There were good correlations between TBT and ISI, but no correlations between TBT and FPrL, or ISI and FPrL. There was a significant reduction in TBT concentrations in blue mussel in Vikkilen. These results show that the remediation in 2016 and legislation banning use of TBT in 2008 have been effective in reducing intersex in *L. littorea*. This monitoring data confirm the remediation and rationale of implementing strict international regulations on industrial chemicals when these can be linked to ecological perturbations in coastal ecosystems.

1.01.2
DEHP acts as an endocrine disrupting chemical in the pest moth Spodoptera littoralis and affects post-embryonic development, adult male sexual behaviour and offspring development
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Bis(2-ethylhexyl) phthalate (DEHP) is a widely produced plasticiser found in many everyday life products. As DEHP is not covalently bound to plastic, it is found everywhere in the environment, in particular in sediments, soils and plants. DEHP is a recognised endocrine disrupting chemical (EDC) in vertebrates, where it affects steroid levels and induces, amongst others, developmental and behavioural impairments. While invertebrates represent most of animal biodiversity and play critical roles in the aquatic and terrestrial ecosystems, fewer studies have focussed on the effects of DEHP on those species and particularly in terrestrial insects. However, studies on *Chironomus riparius* aquatic larvae found that DEHP could reduce the expression of the ecdysteroid receptor (EcR). In holometabolous insects, the ecdysteroids are one of the major lipidic hormone family and play a crucial part in post-embryonic development. In this context, we used the Egyptian Cotton leafworm *Spodoptera littoralis* as a model to study the effects of larval exposure to DEHP on post-embryonic development, male sexual behaviour and offspring's development in a terrestrial insect. Larvae were exposed to three environmentally relevant concentrations (1.1mg, 3.2mg and 19.7mg per gram of food) and three higher concentrations (447.2mg, 4.3mg and 39.5mg per gram of food) of DEHP via food. An integrative approach was used, combining post-embryonic and male sexual behaviour monitoring, hormonal and DEHP titrations, gene expression measurements and metabolomics. We showed that, DEHP acts as an EDC in this species by affecting ecdysteroid titration and ecdysteroid response genes' expressions in both larvae and adult males. Besides, DEHP was also observed to affect post-embryonic development, inducing, for example, an increased food consumption with no effect on larval growth curves, and affecting the metabolic profile two days before metamorphosis. DEHP also affected male sexual behaviour, and delayed mating and shortened mating time in DEHP-treated females mated with control males. Finally, intergenerational experiments showed that DEHP altered the development of the offspring of DEHP-treated females. Altogether, our results show that DEHP acts as an EDC in *Spodoptera littoralis* resulting in observed effects in different life stages and on offspring's development.

1.01.3
Multigenerational effects of the lipid lowering drug Simvastatin in the keystone amphipod Gammarus locusta
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Hypocholesterolaemic drugs such as statins are among the most prescribed human pharmaceuticals in western European countries and United States. In vertebrates, this therapeutic class disrupts the cholesterol synthesis by inhibiting the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR), responsible for the limiting step in the mevalonate pathway. Furthermore, the catalytic domains of metazoan HMGRs and the mevalonate metabolic pathways is highly conserved among metazoans. In crustaceans, the down stream pathway (Sesquiterpenoid biosyntetic pathway) synthesize the isoprenoid methyl farnesoate (MF) which has a central role in crustacean's reproduction. Importantly, in a previous work of ours we demonstrated that chronic partial life-cycle simvastatin (SIM) exposure disrupts crustacean reproduction and development at environmentally relevant concentrations. Here, we expand our early studies, adressing the multigenerational effects of SIM in ecological relevant endpoints, and the link of the findings with tissue MF levels and change in gene transcription. *Gammarus locusta*, used in this study, were derived from a permanent stock culture settled at the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) since 2009. To investigate the multigenerational (F0 and F1) effects of environmentally relevant concentrations of SIM, *G. locusta* was continuously maintained under SIM exposition during two successive generations to 64 and 320 ng/L. We found significant effects in F0 at 320 ng/L, i.e., decrease of reproductive output and growth, and depression of female growth at 64 ng/L. In F1, both growth and reproduction were impacted at 64 and 320 ng/L. Levels of MF in males were significantly increased at 320 ng/L in F0 and 64 ng/L in F1. In contrast, levels of MF were depressed in F0 females from the 64 ng/L treatment and in F1 females from both tretament groups. The transcriptomic analysis identified multiple affected pathawys, mostly related with cuticle synthesis and fatty acid metabolism.The present results demonstrate that chronic low-levels exposures of one of the most prescribed pharmaceutical impacts key ecologoical endpoints at least for two consecutive exposed generations. The ecological relevances of the findings will be discussed and integrated with current risk assesment frameworks of chemicals of emerging concern.

1.01.4
Effects of a potent estrogen mimic on wild populations of leeches
K.A. Kidd, McMaster University / Department of Biology and School of Geography and Earth Sciences; S. Graves, University of Saskatchewan / Toxicology Centre; G. McKee, McMaster University / Department of Biology; C. Podemski, Fisheries and Oceans Canada
Leeches are widespread, found in a variety of freshwater habitats, and have diverse dietary habits, ranging from those that will prey on invertebrates to those that are ectoparasites. Despite their prevalence and close phylogenetic relationships to Mollusca, a phylum with species known to be affected by exogenous estrogens, it is unclear whether Hirudinea may also be impacted. A whole lake experiment was done at the Experimental Lakes Area (ELA) in Ontario, Canada, to assess whether the estrogen in the birth control pill (17a-ethynylestradiol; EE2) affected reproduction and numbers of fish and other organisms in the food web. Herein, we examined whether EE2 in the experimental lake impacted leech community composition, species abundance, cocoon production, growth rates and gonad size when compared to reference lakes using a BACI design. Each month baited leech traps were set overnight in the littoral zone at 10 sites around experimental Lake 260 and two reference lakes, and all individuals were anaesthetized, identified, weighed and measured. In addition, artificial substrates were left in the littoral zone over the summer months and regularly assessed for cocoon production. The common species *Haemopis marmorata* was dissected (n=25/lake/month) and male and female organs were

measured, dried and weighed to examine changes in gonadosomatic indices (GSI). Across all lakes and dates, 9 species representing 3 families were collected. There were no apparent effects of EE2 on total numbers, species richness or community composition. Condition was highest in June and July and lowest in May and August, and 1 of 5 species showed a significant increase after EE2 exposures (ANOVA BACI). Total GSI (all male and female organs), and the GSI for all male or all female organs for *H. marmorata* were not affected by EE2 additions. However, some individual reproductive organs were affected, including relative sperm sac length, relative epididymis weight, relative vaginal bulb length, relative ovisac length and relative albumen length. Finally, cocoon laying was dominated by *Nephelopsis obscura* (88-96%) and we observed some effects of EE2 on the timing (earlier), but not overall, output. In summary, few individual through community measures of leeches showed differences after exposures to low ng/L concentrations of EE2, suggesting that species of Hirudinea are not as sensitive to this endocrine disruptor as other invertebrates and vertebrates.

Advantages of Using Laboratory Model and Field Collected Invertebrates in Ecotoxicology: New Insights for Environmental Risk Assessment

1.02.1
iNVERTOX: Linking metabolomics to behavioural changes in Gammarus pulex upon exposure to psychoactive drugs
T.H. Miller, K. Ng, Kings College London / Environmental Research Group; M. Santos, J. MacRae, The Francis Crick Institute / Metabolomics; N. Bury, University of Suffolk / School of Engineering, Arts, Science and Technology; S. Owen, AstraZeneca / Global Sustainability; L. Barron, Kings College London / Analytical, Environmental and Forensic Science
The (pseudo)persistence of emerging contaminants and other organic micropollutants in the environment represents a risk for the organisms that are exposed to them. Omics technologies are providing a powerful tool within environmental toxicology to better understand the effects of these exposure scenarios. However, application of metabolomics in environmental toxicology is currently very limited and the subsequent interpretation of metabolite data for the understanding of toxicological responses is challenging. Furthermore, behavioural endpoints are generating discussion for their use in risk assessment as a sub-lethal indicator of effect. Thus, we characterised metabolomic changes in a freshwater invertebrate, *G. pulex,* upon exposure to several psychoactive drugs. Behaviour was monitored across a 7-day exposure period to 5 concentrations of selected drugs ranging from environmentally relevant to non-relevant levels. Animals were sampled across the exposure phase to determine both internalised drug concentrations and altered endogenous metabolites to establish links between cause and effect relationships. The results indicated that both endogenous metabolites and activity of the animals were altered depending on psychoactive drug treatments. Additionally, endogenous metabolites were shown to be affected by laboratory acclimatisation, moulting cycle and biological sex of the animals. Behaviour was affected during the first 24-hours of the experiment suggesting that handling of animals can cause significant effects for subsequent behavioural measurements. Internal concentrations of drugs reached steady-state very quickly and experimentally estimated bioconcentration data suggested that these organisms are capable of rapid turnover of these drugs. 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.

1.02.2
Effect of tidal movement on the toxicity of chlorpyrifos:: is current risk assessment applicable to tidal areas with high environmental values?
S. Prévalet, Wageningen University & Research / Environmental Toxicology; H.H. van den Berg, Wageningen University / Division of Toxicology; N. van den Brink, Wageningen University / Dept of Toxicology
Tidal areas are of extremely high value for marine ecosystems. Fish breed in high numbers, and large numbers of birds use such areas as feeding ground, especially during migration. Current testing protocols to assess risks of chemicals to marine ecosystems however, do not include the potential interactive effects of tidal movements. Since organisms in tidal areas are exposed to extremely variable environmental conditions, including temperature, salinity, solar radiation and desiccation, risk assessments based on test systems with overlaying water may not be relevant for tidal areas. To test the potential effects of tidal movement on the toxicity of chemicals a novel test system was developed in which marine benthic fauna can be tested under environmentally relevant conditions with alternating high and low tides appearing two times a day. In the presentation the details of the system will be presented. Using the new systems, exposure experiments were performed to assess the differential effects of Chlorpyrifos on the marine bivalve *Cerastoderma edule*, a cockle. Treatments with tidal movement were compared to treatments under stagnant water. Chlorpyrifos was selected because this is a well-studied neurotoxic compound with known mode of action via inhibition of AChE activity. *C. edule* were exposed 18 days to nominal concentrations of 0 to 3 mg*l^{-1}. The LC50 for the Stagnant Water systems (SW) and Tidal Flow systems (TF) at 18 days of exposure were > 3.0 and 1.0 mg*l^{-1}, respectively. Survival was significantly higher in SW for CPF concentrations of 1 to 3 mg.l^{-1}. The SW tended to have higher mean filtration rate than the TF but this was only significant at 0.1 mg.l^{-1} CPF. From 1 to 3 mg.l^{-1} CPF the mean burrowing of *C. edule* per treatment decreased more importantly in TF and, in contrast to SW, no recovery was observed over time. Overall it can be concluded that, although not all response variables showed significant differences between the systems, the toxicity of CPF to *C. edule* is higher under tidal conditions. This is a first indication of the potential effects of tidal movement on the toxicity of chemicals to benthic marine organisms. The results warrant for further research on different chemicals and different ecological receptors to verify the applicability of current methods to address risks of chemicals for extremely valuable marine habitats, with high conservation status.

1.02.3
In situ evaluation of pesticide mixture effects on embryo-larval stages of the Pacific oyster (Magallana gigas)
E. Rozmankova, EPOC, University of Bordeaux / RECETOX, Faculty of Science; M. Barré, University of Bordeaux / EPOC; L. Blaha, Masaryk University, Faculty of Science / Faculty of Science, RECETOX; J. Cachot, Université Bordeaux / EPOC; B. Morin, University of Bordeaux / EPOC; P. Gonzalez, University of Bordeaux / UMR EPOC CNRS 5805
Pesticides are widely used throughout the world in many agricultural applications. Once applied, they may end up in aquatic ecosystems and harm non-target species. Moreover, complex mixtures of various substances and metabolites are formed, whose toxicity may vary depending on the interactions between molecules and organisms (synergism, addition, antagonism). This study is based on the case of the Arcachon Bay, situated in south-west France, and influenced by growing anthropogenic pressures. Numerous substances have been detected in the waters of the Bay. The mixture's impact on local fauna is unknown, especially on the Pacific oyster, which represents a great ecological and economic value for the Aquitaine region, and which has been encountering farming-related problems in the last years. In situ experimentations allow us to evaluate directly the complex effects of the actual situation in the ecosystem. Thus, we designed a caging device consisting of 4l HDPE bottles closed by 20 µm mesh NITEX filters on both sides, which was kept on three different sites of the Bay for two days. Four devices on every site encaged each approx. 660000 oyster embryos (issued from four different mature oyster couples). The in situ exposure was followed in the laboratory by a battery of tests: larvae malformation evaluation, locomotion analysis (2 min videos analyzed for the maximal and average speed and the type of trajectory followed by the larvae) and evaluation of expression levels of 14 selected genes via qRT-PCR (β-actin, ef1a, rpl7 were used as housekeeping genes). Concentrations of selected pesticides in the water on the caging sites were measured by LC-MS/MS. This study successfully used the designed device for the caging of oyster embryos and larvae in the Arcachon Bay in France. The larvae presented no significant mortality or developmental malformations regardless of the sampling site which revealed a good water quality in the Arcachon Bay. Although not significant, locomotion analysis showed an increase in abnormal swimming behaviour and in swimming speed on two sites of the Bay, which encounter strong anthropogenic pressure, in comparison with the reference site situated near the open ocean. Statistically significant effects were found on the molecular level, especially regarding oxidative stress defense (genes cat, sodMn, gpx), detoxification function (genes mt1, mt2), mitochondrial metabolism (genes 12S, cox1) and DNA damage and repair (genes gadd45, rad51).

1.02.4
Effect of the reproduction cycle period on energy metabolism responses in a model organism (Dreissena polymorpha) exposed to cadmium: what consequences for biomonitoring?
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Aquatic environments quality can be assessed measuring biomarkers in species used as model for biomonitoring studies. In freshwater ecotoxicology, the zebra mussel *Dreissena polymorpha* is a freshwater bivalve commonly chosen. To develop new biomarkers integrative of the general health status of organisms, energy metabolism provide relevant candidates. Among metabolic pathways, processes of synthesis and energy management may give possibilities. The energetic status of an organism is modulated by biotic (such as reproductive stage) and abiotic (temperature, oxygen, etc) parameters fluctuations. When the organism is subject to environmental stress, a part of its available energy will be allocated to defence mechanisms, at the expense of other essential functions such as growth or reproduction. In order to understand how the reproductive cycle may

influence responses of organisms to a chemical stress, the effect of a metal exposure on cellular energy metabolism was investigated in the zebra mussel at different breeding periods. Dreissenids were exposed to cadmium (10 $\mu g.L^{-1}$) and at two different temperatures for 7 days, during rest (November) and reproduction (June) periods. Several cellular energy (ATP) management processes have been targeted (oxidative phosphorylation, adenylate balance and regulation, mitochondrial density). The results showed responses differing according to the reproductive stage. After 24 hours of Cd exposure, glycogen content decreased and cellular damages were observed in reproduction period. In contrast, no effect of Cd was noticed during the rest period. These observations could be explained by the energy status of the organisms during the rest period, since organisms restore their energy reserves and are then more able to counteract the metal stress without associated noticeable damages. This study highlights the importance of considering the cycle time of organisms for their use in biomonitoring.

Alternative Approaches to Animal Testing for Aquatic Ecotoxicity Assessments and Environmental Risk Assessments

1.03.1

Update of TG203: what is further needed to address fish welfare

I. Katsiadaki, Cefas / Environment and Animal Health; T. Ellis, Cefas; C. Green, DEFRA / Environmental Quality Directive

The Fish Acute Toxicity Test (TG203) is one of the most frequently conducted regulatory ecotoxicology tests using animals (1) and raises welfare concerns for animal suffering as it determines the concentration of chemical required to kill 50% of fish (LC50). Recognising these concerns, the UK and Switzerland worked in partnership at the OECD to modernise the TG leading to the publication of an updated version in 2019 . This intended to allow those conducting the test to euthanize fish that are considered “moribund” – exhibiting clinical signs indicating that death is inevitable – to end suffering early. However, it was not possible to introduce this more humane endpoint due to opposition from some countries who felt that there was currently insufficient evidence to link sub-lethal clinical signs of toxicity to death and that moribundity is ill defined. The revised TG has therefore enhanced the recording of the sublethal clinical signs that fish may exhibit in these tests to help us fill the data gap and improve our ability to predict death. It is hoped that this will form the scientific basis by which we can achieve our aim of determining a standardised definition of morbundity based on sublethal clinical signs and incorporating it into TG203 in the future to minimise animal suffering. However, a mechanism for collecting, holding and analysing the data on clinical signs generated by the new TG need to first be determined to make progress towards achieving this aim.

1.03.2

Reducing, refining and replacing fish acute toxicity tests

B. Labram, N. Gellatly, F. Sewell, N. Burden, NC3Rs

Acute toxicity tests are conducted as part of regulatory risk assessment and hazard classification packages for industrial chemicals and agrochemicals. Historically, the aim of acute toxicity tests has been to determine an LD_{50} dose, i.e. the dose which would be lethal to 50% of the animals treated. They are therefore associated with suffering in the test animals. Large numbers of animals can be used for this purpose; for example, OECD Test Guideline (TG) 203 (fish acute toxicity) studies are the most widely conducted regulatory vertebrate ecotoxicology tests. There is great scope to apply the 3Rs principles – the reduction, refinement and replacement of animals – in this area of testing. An expert Ecotoxicology Working Group, led by the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), including members from government, academia and industry, reviewed global fish acute test data requirements for the major chemical sectors. We identified the key challenge and opportunity areas related to reducing, refining and replacing traditional fish acute toxicity tests without compromising environmental protection. Data analysis exercises have been carried out to explore whether the number of animals used could be reduced, without compromising the scientific objectives. For example, it may be possible to align varying global regulatory requirements and decrease the number of different species that require acute testing. The need for testing formulations in addition to active substances has also been considered. There are opportunities to refine these tests, by moving away from using lethality towards more early and humane endpoints. There is now a requirement within OECD TG 203 to record sublethal clinical signs in a systematic way, which may in future have utility in predicting moribundity and death. Novel approaches which replace the use of late life stage animals, such as those using fish embryos (e.g. OECD TG 236) and fish gill cell lines, are now being further developed for use in integrated approaches (e.g., OECD project 2.54), and may aid in reducing the number of *in vivo* fish acute toxicity studies needed. This presentation will highlight the ongoing initiatives and provide an overview of the key challenges and opportunities associated with replacing, reducing and/or refining fish acute toxicity studies.

1.03.3

Predictive acute fish toxicity models and applicability within the industrial context

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There is an ever increasing need for in silico alternatives to animal testing for the assessment of chemical hazardous properties in the environment. The advantage of these alternative methods is primarily the reduction of animal use, but they aim also at improving the predictability. However, in most cases, existing models have a limited applicability domain because the training sets of the existing tools are quite limited, and they have not been updated since several years. Moreover, many existing in silico models also have limited potential for regulatory acceptance, like for REACH submissions, due to the lack of understanding of their underlying concepts by the users and authorities, which require that a model complies with the five OECD principles. We generated a new fish acute toxicity (LC50) model (ISIDA Consensus) based on a large collection of thoroughly curated and publicly available data, counting 1813 compounds (around double that of other available models). A specific emphasis has been put on the simplicity of use and understanding of the model’s output by including features like: evaluation of the reliability, verification of applicability domain and visualization of which molecular fragments of the substance influenced the prediction (ColorAtom). This last utility provides a valuable enabler for the understanding of the model’s prediction, thus allowing further mechanistic interpretation. Internal and external validation have been carried out following the OECD principles. The performance of the model was benchmarked against existing tools on a set of 103 compounds which were considered more representative and relevant for the chemical space of the current Chemical Industry. Our model scored first on data coverage and second in prediction accuracy. This model and the associated utilities, as well as other models predicting properties such as bioaccumulation and ready biodegradability, are freely accessible through the online ISIDA/Predictor platform, at the Laboratory of Chemoinformatics webpage: http://infochim.u-strasbg.fr/cgi-bin/predictor_reach.cgi.

1.03.4

Building bridges between the world of traditional lab animal science and environmental research: better science and improved animal welfare

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Despite advances in the development and validation of in vitro techniques, animals are still used in large numbers for research, development and testing to meet today’s environmental challenges. In recent years, the use of animals in research and testing has come under scrutiny from scientists themselves, who are concerned about poor study design and reproducibility, in addition to low translatability from animals to human medicine. These concerns come in addition to animal-related issues, which have created a need for better welfare indicators. Transposition of EU Directive 2010/63 has resulted in a number of new legal requirements, including severity classification of experimental procedures. Many of these issues have, until now, largely been discussed in relation to the mammalian species that are commonly used in the laboratory. There are large knowledge gaps related to the use of fish in research, made worse by the fact that “fish” are often treated as one group. This situation has created a need for readily available information and guidance on the use of fish and other aquatic species for scientific purposes. Until recently, efforts to lessen the reproducibility crisis have tended to focus on improving the reporting of animal studies. This is too late in the process to have any immediate effect on research quality. Guidance for planning preclinical research is now available. The PREPARE guidelines consist of two parts: the first is a checklist which is intended to be an aide memoire from day one of planning, rather than an additional bureaucratic hurdle. Importantly, the checklist is supplemented by a comprehensive website containing sections which explain each of the 15 items on the checklist. These sections contain links to detailed expert guidance on each topic, collected from expert working group reports, the general scientific literature and multi-author consensus statements. We are concerned about the two-way knowledge gap and lack of communication which seems to exist, to varying degrees, between laboratory animal specialists and scientists in other disciplines. Since both groups are now acknowledging that there is a “reproducibility crisis”, concerted efforts must be made to bridge this gap, in the interests of improved animal welfare and better science, since the one leads to the other. Hopefully the PREPARE guidelines will help this process.

1.03.5

Are two negative controls needed when a solvent is used in an aquatic study?

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In order to receive regulatory approval to market certain substances such as biocides, pesticides and industrial chemicals, a battery of studies must be conducted to establish the environmental safety of the product and the safety of the animals who are exposed to the chemical. Since drift and runoff bring the chemicals into adjacent waterways, numerous aquatic studies are performed. With difficult to test substances, as discussed in OECD Guidance Document 23, it is sometimes found necessary to use a low concentration of a solvent in order to maintain an adequate and stable supply of the test chemical throughout the study. Most regulatory test guidelines (TGs) for such aquatic studies require both a water and a solvent control when a solvent is used. The same concentration of the solvent is used in all concentrations of the test chemical and in the solvent control. An international team of scientists led by the European Commission Joint Research Centre and the International Council on Animal Protection in OECD Programmes (ICAPO) is exploring the question of whether both controls are needed in all aquatic studies that use solvents If the water control is not needed, then omitting it would reduce animal use and would be an ethically sound decision. The 2019 revised OECD Test Guideline (TG) 203 describing fish, acute toxicity, contains the statement "The dilution water control can be omitted, and the test conducted and evaluated with a solvent control only, provided it is appropriate when considering the needs for these data and the requirements of the relevant regulatory authorities." The purpose of this presentation is to explore the justification for omitting the water control in this and certain other TG studies. The biological, statistical, and regulatory concerns regarding the need for, and use of, both water and solvent controls in these studies are explored and evidence is presented to show that the omission of the water control is justified at least for some aquatic guideline studies. The focus is on OECD TG 203, but some discussion of TGs 212 and 236 is included. The power to detect or estimate statistically effects that are biologically relevant are explored, as is a critical understanding of the usefulness of having two controls to determine study acceptability. This work draws on and, in some instances, extends results from earlier papers.

1.03.6
COMBASE, a computational platform for the assessment of biocidal active substances of ecotoxicological concern
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Biocides are substances employed to prevent or attenuate the action of harmful organisms, or even destroy them. However, their effects may not be limited to the pathogens to which they are intended, but may also affect human health and the environment. In order to favour the sustainable use of biocides, their commercialization and use have to strictly follow the EU Regulation No. 528/2012 ("Biocidal Products Regulation", BPR), thus guaranteeing the protection of people and environment. This regulation presents the inconvenience that it involves a large number of animal tests to meet the information requirements. The use of alternative methods to animal experimentation has numerous advantages over traditional laboratory tests, allowing to reduce the number of animals slaughtered or even replace them, and are completely valid at the regulatory level. The BPR itself promotes the use of such methods. Among them, Computational Toxicology stands out, which consists of the development and application of algorithms and models that allow predicting various biological and environmental parameters based on the chemical structure of substances. In this context, the objective of the European project COMBASE ("Computational tool for the assessment and substitution of biocidal active substances of ecotoxicological concern", www.life-combase.com) was to develop a computational tool integrating predictive aquatic toxicity models for biocidal substances at four trophic levels: bacteria, algae, crustaceans and fish. This tool allows to predict the eco-toxic effects of biocides during their useful life, as well as metabolites and degradation products generated from active substances. COMBASE is highly innovative and it is the first time in Europe that a database with specific ecotoxicological information focusing on biocides is integrated, coupled with a set of experimentally validated computational models to confirm its effectiveness and with regulatory validity.

1.03.7
Potentials and pitfalls of transient in vitro reporter bioassays
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Regulatory directives drive the demand for animal testing. Agencies and stakeholders on the other hand are promoting alternatives and implementing the concept of 21st century tox. Future guidelines propose the integration of effect-based tools and bioassays (WFD re-evaluation). Within aquatic toxicology, coverage of certain MOAs and AOPs is scarce and most applications are based on mammalian or yeast models, not reflecting realistic exposure scenarios. The use of transient reporter gene assays in organisms of interest (fish cells) could be a quick and inexpensive solution. However, interference with cellular homeostasis may impact the system beyond the function of the manipulated gene and lead to non-specific results. We describe how varying vector geometry and different regulatory gene elements on plasmids used for transfection may lead to a large difference in sensitivity. Zebrafish embryonic fibroblasts (ZF4) and hepatocytes (ZFL) were seeded on 96-well plates, co-transfected with a Nrf2-responsive Firefly luciferase reporter plasmid and 8 different combinations of Renilla luciferase normalization vectors. Transfected cells were exposed to increasing concentrations of oxidative stress inducer metazachlor. In response, Nrf2-dependent luciferase induction was recorded via a plate reader. Alternatively, various endpoints of cellular stress (mitochondrial metabolism, membrane stability, protein amount, proliferation) were assessed using non-transfected or transiently transfected cells with constructs of increasing sizes. The results indicate that plasmid geometry and gene-regulatory units have an effect on the outcome and potency of the reporter gene assay after co-transfection. Differences in relative induction are a result of the applied normalization vectors, specifically their constitutive promotors and backbones. Using the ZF4 cell-line and co-transfecting with the pRLx series resulted in stronger signals. Of all combinations tested, the reporter vector pGL4.37 together with the normalization vector pRL CMV gave the strongest signal. Viability tests showed that transfection itself increases cellular stress in a construct-size-dependent matter. Given that the final signal measured will always be the result of a synergistically acting black-box, precautionary decisions must be taken in plasmid vector design to display weak points and overcome intrinsic faults. In the regulatory context, awareness is important, in order to choose suitable bioassays.

1.03.8
First generation annotation of the rainbow trout RTgutGC cell line transcriptome
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While the RTgutGC cell line has been used in several toxicological studies, there is limited information on its comparability to its intestinal tissue of origin. The distal intestine of fish has been used to investigate dietary uptake of numerous compounds ex vivo via preparatory methods such as the "gut sac", however data on intestinal composition in terms of proteins or RNA is minimum. Using a transcriptomic approach (RNAseq), confluent monolayers of RTgutGC were compared to freshly isolated distal intestine to establish basal levels of RNA expression in both experimental systems and establish similarities and differences between. Therefore, the aim of this study is to characterise how comparable this in vitro model is to its in vivo tissue. Following sequencing, genome alignment and quality control steps, 18,853 genes were selected for differential expression analysis with significant overlap observed between the cell line and the tissue (~95 %). Of the 18,853 genes identified, 8,542 genes were differentially expressed (3,682 up-regulated and 4860 down-regulated). Validation of a subset of identified genes (n= 11) via RT-qPCR confirmed RNA-seq results. Gene ontology over-representation suggests an over-representation of genes involved in catalytic activity (representing 39 % of genes) in the molecular function group which is synonymous with enzymatic activity. Of DEG's, only 304 were identified as proteins suitable for KEGG analysis with 89 enriched KEGG pathways identified. The characterisation provides a meaningful contribution to publicly available fish cell line information and provides a first glimpse into the metabolic nature of this animal replacement system.

1.03.9
The roles of the fish gill in uptake and clearance of organic environmental contaminants as revealed by an in vitro gill cell culture epithelium
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As part of chemical hazard and risk assessment, it is necessary to determine the likelihood of bioaccumulation. As part of this assessment, the bioconcentration factor (BCF) is a common metric used in environmental assessment of chemicals and is generally determined from laboratory experiments using fish according to the OECD TG 305. However, typically these studies use high numbers of fish, therefore it is impracticable to the test thousands of chemicals produced annually using this method. Alternative, in vitro methods are currently being developed to provide information to predict BCFs with a focus on determining in vitro biotransformation rates. Yet, most studies investigating biotransformation focus on the liver as the main site of action, despite the gill constituting a major contact site for chemicals that are largely soluble in water. Therefore, in this study we used the fish gill cell culture system (FIGCS) to explore the biotransformation of chemicals at the gill. Five chemicals were exposed to the FIGCS system over a 24 hour period. At each specific time point, chemical extraction occurred from the apical and basolateral media compartments and the cells, summed up to provide the total amount of parent compound in the system; as well, the isolated amounts in the apical or basolateral compartment were evaluated separately, providing the additional information of permeability. All five chemicals studied crossed the gill epithelium; and for three of the five tested biotransformation was observed. Overall, these data demonstrated the use of the FIGCS to assess the biotransformation and uptake of chemicals across the gill, highlighting the importance in informing and strengthening conceptual and mathematical models, which will ultimately result in far fewer animal tests carried out, and allowing a much more efficient testing of the thousands of chemicals produced annually.

1.03.10
The use of a fish gill cell culture model to investigate the uptake of acidic and basic drugs: accurately reporting pH is essential to understand and develop predictive models of bioaccumulation.
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Water pH is predicted to effect ionisable pharmaceutical uptake and toxicity in fish. The current study used an in vitro primary fish gill cell culture system (FIGCS) to assess the effect of pH in the range of 4.5 – 9 on propranolol (pKa 9.42) and ibuprofen (pKa 4.91) uptake rate. At water pHs where either drug is ionised uptake rate is significantly reduced, but not completely abolished. If drug uptake rate were solely dependent on passive diffusion of the unionised species across the epithelium, then the rate would be expected to correlate to the concentration of the unionised fraction. Ibuprofen did not follow this pattern with only a minimal change in uptake when the ionised fraction dropped from 92.5 to 28% and a precipitous 94% drop off in uptake rate as the % the ionised fraction went from 92.5 to 100%. Similarly, propranolol uptake rate rapidly dropped off by 99% between 72.5% to 99.9% ionisation. Indeed, uptake rate plotted as a function of the unionised fraction showed saturation uptake kinetics with a K_m of 47.9 nM and V_{max} of 120.3 pmole/(cm^{-2} x h) for ibuprofen and K_m 0.27nM and V_{max} 0.32 pmole/(cm^{-2} x h) for propranolol, suggesting a facilitated uptake route of drugs across the gill epithelium. The rapid decline in uptake rate of both the basic drug propranolol and acidic drug ibuprofen occurred between pH 6 to 8, the range of pH of most of our freshwaters, as well as the range, pH 6.0 – 8.5, in the guidelines for a valid OECD 203 Fish, acute toxicity test and OECD 305 Bioaccumulation in fish: aqueous and dietary exposure test. Our data suggests there is large variation in uptake rate of drugs associated with only small fluctuations in pH. This demonstrates the importance of accurately measuring and reporting test media pH to help regulators to interpret drug accumulation and toxicity studies and implies that a better understanding of how pH influences drug uptake is required if robust models to predict drug absorption in fish are to be developed.

1.03.11
Developing in-silico predictions of toxicity
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Human activities have increased the abundance of variable toxins in the environment, but not all compounds can be tested, thus in-silico approaches such as quantitative structure-activity relationship (QSAR) models, have been widely used to assess this issue. These types of models generally predict with high accuracy but lack biological understanding. While the structure of the molecules is responsible for their physical, chemical and biological properties, generalized QSAR models are difficult to establish due to the large variability of available chemical structures. To address this issue, we exposed Zebrafish embryos to 158 compounds, including endocrine disruptors, pharmaceuticals and industrial pollutants, at multiple concentrations. Dose-response curves were generated, including phenotypic characterizations at multiple concentrations for up to 120hpf. Subsequent exposure at specific concentrations (LC5 and below) were then used to establish quantitative heartrate measurements as well as transcriptomics data for up to 5 concentrations. A QSAR model predicting LC50 across all compounds showed a reduced accuracy suggesting the large variation of structures reduces accuracy. Dragon 7 software is used to generate a list of molecular descriptors, that provide numerical information about the structure of the compound. Chemicals were clustered on the basis of their chemical structural descriptors and molecular response and compared. Robust clusters were identified and QSAR models developed for each cluster improving overall accuracy. Further integration of the molecular data in each cluster highlights specific functional differences between the identified compound sets.

Epigenetic and Evolutionary Effects of Environmental Stressors on Environmental and Human Health

1.04.1
Transgenerational response to prochloraz in Daphnia magna. A 3-generation study
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For most chemicals, toxic effects are only investigated for one generation and possibly as number of offspring. Scientific evidence for transgenerational effects is however increasing, challenging the dogmas of time perspectives of ecotoxicity. In this study, we investigated the transgenerational effect of the azole fungicide prochloraz over three generations of parthenogenically reproducing *Daphnia magna.* The study included two scenarios; one where all three generations were continuously exposed to prochloraz (100 ug/L) and one where only the first generation (F0) was exposed. We studied effects at different levels of biological organization combining transcriptomics, metabolomics, general CYP enzyme activity and key phenotypic effects, such as growth and reproduction. For the continuously exposed animals, compensatory mechanisms and acclimation was seen over the three generation with changes in size and number of offspring in F0 and F1, but with no differences from control animals in F2. The metabolome was also significantly affected in F1, but not in F2. Cytochrome P450 ECOD activity was strongly inhibited in F1 and F2 but RNA sequencing in F2 showed only few significant differences when compared to controls. However, aromatase, of which inhibition is the currently accepted molecular initiating event for adverse outcomes of prochloraz exposure, was significantly upregulated in F2, indicating a compensatory response. Contrary to expectations, effects of F0-exposure seemed to skip a generation, as decreased enzyme activity and significantly increased number of off-spring was observed in F2, with no such effects present in F1. Furthermore, RNA sequencing in F2 showed 127 up-regulated genes compared to the non-exposed control animals. Analysis of gene ontology (GO) terms revealed that all enriched GO-terms were directly related to the chemical characteristics of prochloraz i.e. interaction between the azole moiety and heme-iron. Hence, the study corroborates that this type of interaction with essential proteins, is the molecular initiating event in an adverse toxic outcome of prochloraz and that the same mode of action is relevant across generations and beyond exposure. Upregulation of aromatase both in continuously exposed animals and in the grand-maternally exposed, furthermore, links the effect in *Daphnia* to the currently accepted AOP for prochloraz. The study underlines the importance of timing of exposure and effect assessment in studies of ecotoxicity.

1.04.2
Deciphering DNA methyltransferase inhibitor mediated transgenerational effects on Daphnia: high-throughput analyses and Adverse Outcome Pathway assembly
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Epigenetic marks can in many cases reflect the life-time exposure history of an organism to environmental stressors. While the rapid development of the OMICS techniques allows measurements of genome-wide epigenetic changes, the high costs for such analyses still limit our ability to fully understand the epigenetic effects across doses/concentrations, exposure durations and multiple generations in a population. Targeted high-throughput (HT) epigenetic bioassays are needed to allow the inclusion of more life stages and exposure conditions to yield comparative concentration-response data on a temporal scale, and to support universal mechanistic models such as Adverse Outcome Pathways (AOPs). The present study has therefore integrated and refined several HT bioassays to understand the relationships between chemical-mediated DNA methyltransferase (DNMT) inhibition, promoter and gene body methylation, gene expression and reproduction, and to develop a novel epigenetic AOP for DNMT inhibitor-mediated transgenerational effects in aquatic organisms. Adult female *Daphnia magna* were exposed to 0-80 µM of 5-azacytidine for 7 days and recovered in clean media for another 7 days. F1-F3 offspring were sub-cultured in clean media to investigate potential transgenerational effects. A suite of HT bioassays were employed, such as the total DNMT activity assay, methylation-sensitive high-resolution melt analysis (MS-HRM), and qPCR. The test genes are well-known biomarker genes or key regulators involved in major biological pathways. Cumulative fecundity was recorderd for each generation. After 7-day exposure, the total DNMT activity in F0 decreased in a concentration-dependent manner, with 80 µM 5-azacytidine caused significant reduction in DNMT acitvity. The majority of the test genes showed reduced promoter methylation and increased transcription in F0 after 7-day exposure, whereas increased promoter methylation and down-regulation in F0 after recovery. In F3, most of the genes displayed increased promoter methylation. The cumulative fecundity decreased in a concentration-dependent manner in F0 after exposure and recovery, and in the succesive generations. On the basis of the results, a conceptual AOP on DNMT inhibition leading to oocyte apoptosis associated population decline was proposed. The present study has developed a novel analytical pipeline for targeted HT analyses of epigenetic effects, and has unravelled the relationships between DNMT inhibition, DNA promoter methylation, gene body methylation, gene expression and transgenerational reproduction in *D. magna* exposed to 5-azacytidine. The world's first epigenetic AOP was proposed for chemical-mediated transgenerational population effects in aquatic organisms.
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1.04.3
Inhibition of Methyltransferase Activity of Enhancer of Zeste 2 Leads to Enhanced Lipid Accumulation and Altered Chromatin Status in Zebrafish

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Early life exposure of environmental chemicals are associated with late onset of metabolic diseases, possibly via changes to epigenetic marks. These changes alter transcription of genes leading to an imbalance in lipid homeostasis that might eventually cause obesity, type II diabetes or fatty livers. Epigenetic changes might be mediated by enzymes involved in epigenetic mechanisms such as Enhancer of Zeste 2 (Ezh2), a histone H3K27 methyltransferase that has been implicated to play a role in lipid metabolism and adipogenesis. Here, we investigated the role of Ezh2 on lipid metabolism via altered chromatin status following developmental exposure to a specific Ezh1/2 inhibitor, PF-06726304 acetate, in zebrafish. Tributyltin (TBT) was used as a positive control, as this chemical is known to act on lipid metabolism via EZH-mediated pathways in mammals. Exposure to non-toxic concentrations of PF-06726304 acetate (5 µM) and TBT (1nM) showed increases in lipid accumulation. We used the assay for transposase accessible chromatin sequencing (ATAC-seq) on PF-06726304 acetate exposed embryos (from 0.5 to 5.5 hpf (50 % epiboly)) and observed 349 altered chromatin regions. These regions were predominantly located at H3K27me3 loci, and showed more accessible chromatin in PF-06726304 acetate exposed samples. GO term analysis revealed that genes associated to these loci were linked to metabolic pathways. Additionally, gene expression analysis of ATAC positive loci and a selection of genes involved in lipid homeostasis and adipogenesis were differentially expressed after both TBT and PF-06726304 acetate exposure at 5 dpf, but not at 50 % epiboly stage. An in depth analysis of the differentially expressed gene cebpa showed a strong decrease in underlying H3K27me3 marks at 50 % epiboly following chromatin immunoprecipitation analysis, although it did not show a change in chromatin accessibility. Our results indicate that Ezh2 inhibition leads to increased lipid metabolism via an altered chromatin state and that TBT shows a gene expression profile which resembles that of the Ezh2 inhibitor. This first time assessment of chromatin status in a toxicological setting provides valuable information about epigenetic mechanisms involved in metabolic disorders. However, our in depth assessment of the cebpa locus does indicate that other epigenetic marks might be important to study as well.

1.04.4

Transcriptomic effects of chemical and temperature stress in amphipods from Lake Baikal in Siberia

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Lake Baikal in Eastern Siberia, a UNESCO World Heritage Site, is the largest and oldest freshwater lake in the world. It is a global biodiversity hotspot with a high degree of endemicity. The amphipods of Baikal are a particularly species-rich taxon. The permanently cold (6°C mean) and superoligotrophic water is generally highly pristine. Anthropogenic pollution is currently increasing from growing tourism and the related increase in wastewater influx and from increasing contamination of Baikal´s main tributary, the Selenga River, with heavy metals. A rise in water temperature due to global change may challenge Baikal biota further. We addressed the question how chemical and thermal stress may affect Baikal amphipods in comparison to a non-Baikal species. Upon exposure to chemical (cadmium, phenanthrene) and temperature (LT10) stress the transcriptomic responses were examined using Illumina NGS sequencing. The reads could be assembled to 30 – 40 k contigs, from which about 10% could be identified as amphipod sequences. In stressor exposed amphipods transcripts of cell function related gene ontology (GO) terms were less and those of stress response related GO terms were more represented. Our results reveal a pronounced response of Lake Baikal endemic amphipods to stress indicating that those species have the cellular capacity to mitigate stress impact.

1.04.5

Poster spotlight: Resurrected Daphnia as a model organism to assess evolution of natural populations to environmental stressors

S.E. Crawford, Goethe University Frankurt / Department of Evolutionary Ecology and Environmental Toxicology

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1.04.6

Poster spotlight: Multigenerational exposures of Daphnia magna to pristine and aged silver nanoparticles: epigenetic changes and phenotypical ageing related effects

L.A. Ellis, The University of Birmingham / GEES

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1.04.7

Poster spotlight: Applying DNA Methylation as a Biomarker to Assess Latent Effects of Acute Exposure of Benzene in Simulated Chemical Accident Using Zebrafish Model

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1.04.8

Deciphering the molecular mechanisms of pesticide tolerance of the soil engineer biodiversity

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Environmental diffuse pollution by agrochemicals has become a major soil threat. In cultivated fields, non-target organisms, such as earthworms are frequently exposed to different pesticide applications. Although soil engineer biodiversity declines in agro-ecosystems, certain endogeic species are still abundant and persist in intensively cultivated fields, suggesting they become tolerant to long-term anthropogenic pressure. We thus raised a first work hypothesis that populations of *A. caliginosa* earthworms derived from conventional managed field developed a tolerance to pesticides compared to those from organic managed field. If *A. caliginosa* species is tolerant, another correlative question is to decipher whether worm tolerance is based on physiological acclimation or whether it involves genetic adaptation, which can be inherited across generations. A multigenerational evolutionary toxicology experiment (F0, F1 and F2) to obtain 3 generations of earthworms and a non-targeted strategy combining transcriptomics and proteomics are both proposed to resolve the issue. *A. caliginosa* populations used in this study originated from two agricultural fields (conventionally field compared to organic field). Adult worms have been cultured in chemical-free soil in the laboratory and experimentaly exposed to a model fungicide (epoxiconazole for 28 days). The soil epoxiconazole concentration at the beginning of the exposure experiment was shown to be 193.3 and 194.6 ng/g for the naïve and pre-exposed populations, respectively. After 7 days of exposure, a decrease in soil epoxiconazole concentration is observed for the naïve (3%) and pre-exposed (18%) populations. Epoxiconazole concentration in earthworm tissues of naïve and pre-exposed is respectively 3.8 and 2.9 times higher, compared to the soil epoxiconazole concentration. Transcriptomics and proteomics analyses have been done and data analysis are in progress. Our expectations are to create *de novo* a protein database involved in pesticide response of soil engineer and identify the genes and proteins network responsible for tolerance in *A. caliginosa* species. We also intend to provide evidence of ongoing acclimation/adaptation of soil fauna to chronic organic pollution by pesticides through multigenerational studies. This study will improve our understanding of the long-term impact of chronic exposure of soil engineers to low-dose multi-pollutants and assess the costs associated with this tolerance for populations.

1.04.9

Contaminants contributing to oxidatively induced DNA damage of exposed Dreissenid mussels in Detroit River

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The National Oceanic and Atmospheric Administration (NOAA), National Ocean Service (NOS), Mussel Watch Program (MWP) uses dreissenid mussels to monitor chemical contamination in the Laurentian Great Lakes basin. For assessment of biological consequences of an exposition to the legacy and contaminants of emerging concern (CECs) present in water and mussels' tissues we applied gas chromatography-tandem mass spectrometry (GC-MS/MS) with isotope dilution methodology developed at National Institute of Standards and Technology (NIST) to quantitate biomarkers of oxidatively induced damage in

mussels' DNA isolateed from animals collected from several sites of Detroit River. We found significant differences in the concentrations of these potentially mutagenic and/or lethal lesions in the DNA of mussels from the sites exposed to industry/agriculture/communal waste water derived pollutants as compared to the animals collected at relatively clean sites.

1.04.10
Genetics and genomics of resistance of salmon lice to deltamethrin and emamectin benzoate
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Infestations of farmed salmon by parasitic caligid sea lice (Copepoda: Crustacea) constitute a major health challenges for commercial salmon production. The salmon louse Lepeophtheirus salmonis is the main caligid species infecting farmed and wild salmonids in the northern hemisphere. Continued use of a restricted range of drugs has led to the evolution of resistance against most available licensed anti-sea louse treatments in populations of the caligid Lepeophtheirus salmonis (salmon louse) in the North Atlantic. In order to provide insights into the genetic basis of resistance to the anti-sea louse agents deltamethrin and emamectin benzoate, F2 mapping crosses were performed between drug-susceptible and multidrug resistant L. salmonis . In the obtained families, deltamethrin resistance was mainly inherited maternally, with all parasites derived from crosses of resistant dams and susceptible sires being highly resistant, whereas few lice from crosses from the inverse orientation were resistant. Analysis of the mitochondrial genome (mtDNA) in parasites from the cross, as well as field isolates from different regions of Scotland, revealed that all deltamethrin resistant salmon lice had virtually identical sequences, contrasting diverse mtDNA sequences in susceptible parasites. In contrast, the distribution of emamectin resistance was more complex, with F2 generations comprising lice of different degrees of susceptibility ranging from highly susceptible to highly resistant. Selected families from the cross were subjected to ddRAD-seq. Single nucleotide polymorphism (SNP) markers were identified and a genetic map of 15 linkage groups prepared. Quantitative trait loci (QTL) analysis revealed that emamectin benzoate resistance was affected by two regions of the same linkage group, suggesting a multigenic mechanism. Pool-seq analyses were performed for eight farm populations of L. salmonis of sites differing in the type of sea lice management measures applied. Preliminary results indicate the presence of selective sweeps related to the use of salmon delousing agents. Ongoing analyses are assessing the relationship of these selective sweeps to the above QTL regions associated to emamectin resistance, and to target site mutations in an acetylcholine esterase and a sodium channel subunit described in earlier studies. The results suggest that the use of veterinary drugs for L. salmonis control at salmon farming sites has led to positive selection that has caused the expansion of specific mitochondrial haplotypes and resulted in selective sweeps surrounding putative resistance loci in the nuclear genome.

1.04.11
Poster spotlight: Zebrafish differential sensitivity to environmental stressors during early development and implications for subsequent exposures
P. Robinson, University of Exeter
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1.04.12
Poster spotlight: Identification of Differentially Methylated Regions in zebrafish eleutheroembryos exposed to TBT
L. Navarro-Martin, Institute of Environmental Assessment and Water Research / Environmental Chemistry
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Fish Model Species in Human and Environmental Toxicology

1.05.1
Long-term and transgenerational neurobehavioral effects of the insecticide permethrin in zebrafish.
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Permethrin is a pyrethroid insecticide and used for medication to treat scabies and lice. Human exposure to pyrethroids has been associated with Parkinson´s disease onset and generally with cognitive defects. In addition, permethrin was shown to alter epigenetic patterns over several generations in rats. In the present work, we wanted to investigate if early-life exposure of zebrafish to permethrin (PERM) induces epigenetic disruption that contributes to the transfer of neurobehavioral impairments through generations. Zebrafish were exposed during 28 days to 10 µg/l pf PERM (F0 generation). At 28 days post-fertilization, F0 fish were transferred to clean water. F1/F2 generation was obtained by pair-cross spawning of adult F0/F1 and grown in clean standard conditions. General activity and anxiety behavior of larvae (photomotor response) and adults (novel tank) were monitored in F0, F1 and F2 generations. In addition, we performed transcriptomic analyses of the adult fish brains using RNA-sequencing.We observed hypoactive behavior in F1 and F2 larvae but not in F0. However, F0 adult fish displayed strong hypoactive behavior as well. In F1 and F2 generation, adult fish showed a strongly reduced anxiety response in the novel tank diving test. On the other hand, brain transcriptome analyses revealed increasing changes in PERM F0 to F2 generations when compared to controls. Especially, epigenetic mechanisms and neural differentiation pathways were enriched in all generations. Besides, key-genes related to circadian rhythm were differentially regulated from F0 to F2 generations, with strong regulation of the circadian clock pathway observed in F1 and F2.Reduction in larval activity along with circadian rhythm modulation was previously observed after exposure to flutolanil. In addition, circadian rhythm modulates dopamine and activity levels. Studies previously reported the impact of PERM on dopaminergic system modulation and our results show a significant regulation of the dopamine pathway in F2. Therefore, we propose a connection between epigenetic mechanisms, circadian rhythm and locomotor activity, possibly via the modulation of the dopaminergic system and synaptic activity. However, further studies are required to investigate underlying mechanisms. Particularly, the role of epigenetic changes in the observed effects is under investigation with identification of differentially methylated regions in brain using bisulfite sequencing.

1.05.2
Neurotoxicity profiling of organophosphate pesticides at different biological levels using zebrafish embryos
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Neurological disorders are challenging for the affected people and their families and cost governments billions of dollars, as there is no cure available. It has been assumed that exposure to neurotoxic substances especially during neurogenesis may cause brain damages, resulting in such disorders. Omnipresent pesticides, such as organophosphate (OP) pesticides, have long been suspected to increase the risk to suffer from neurological disorders like e.g. the Attention-Deficit Hyperactivity Disorder (ADHD). But a causal relationship between OP exposure and neuronal disorders has not been established yet. To do so it is necessary to identify all molecular mechanisms by which OPs act and to understand the impact on different levels of organisation within an organism. So far most research focuses on the predicted mode of action, the Acetylcholinesterase (AChE) inhibition. But recently it has been suggested that AChE inhibition alone might not be an appropriate biomarker to detect (developmental-) neurotoxicity of OPs, since different OPs can cause differing effects. Within this work we aim to establish a neurotoxicity profile for a set of commonly used OP´s by investigating different biological levels using zebrafish embryos (*Danio rerio*). Therefore, zebrafish embryos were exposed to different concentrations of OP´s. To identify the OP´s impact on different biological levels, behavioural, enzymatic, transcriptomic and metabolomic changes were analysed. Most OP's did reduce AChE levels and alter behaviour. But we could also identify changes not directly linked to those effects suggesting that other mechanisms also play a role in OP neurotoxicity.

1.05.3
Behavioral responses and cell type-specific transcriptomic and proteomic profiling of zebrafish embryos (D. rerio) exposed to copper nanoparticles
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Metal oxide nanoparticles such as copper-based engineered nanomaterials (ENMs) are used in a wide range of commercial products and applications. Herein, we investigated the impact of newly synthesized CuO-ENMs by assessing different behavioral responses of zebrafish (*Danio rerio*) larvae (ZFL) and the underlying mechanisms, in particular the impact of CuO-ENMs on hair cells, which are the mechanical receptors and sensory cells of the lateral line. ZFL were short-term exposed (4 hours) starting at 96 hours post fertilization (hpf) to non-toxic concentrations of CuO-ENMs. Upon exposure, neither locomotion (swimming distance) nor the startle response (escape reaction upon vibration stimulus) were affected. However, CuO-ENMs impacted parameters indicating rheotactic behavior (counter flow swimming) of ZFL. These finding led us to further investigate the impact on hair cells and the underlying mechanisms we hypothesize to be the main target of the CuO-ENMs. Thus, the hair cells of ZFL were stained with a cell-type specific fluorescent dye (DASPEI), and the labeled cells were collected using fluorescence activated cell sorting (FACS). Transcriptomics (SMART-Seq v4 and TruSeq mRNA) and proteomics (HRM ID and mass spectroscopy) is performed with both tissue from whole embryos and FACS-sorted hair cells. OMICs data analysis is supported by using a computable

network model of neuromast toxicity. The combination of hair cell-specific OMICs and development of a neuromast network model will reveal new insights of the molecular pathways and underlying mechanisms.

1.05.4
Exposure to Thirdhand Smoke induces developmental and metabolic changes in zebrafish embryos
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Tobacco smoke is a ubiquitous outdoor and indoor environmental pollutant that has been overlooked to date by the environmental and health policies. Most of the smoke gases and particles deposit and remain for long periods of time in fabrics, surfaces and dust forming the so-called **thirdhand smoke** (THS), a less studied source of tobacco exposure that especially affects children living with smokers. THS components can react with oxidants and other atmospheric compounds to yield secondary contaminants, some of them with increased toxicity. This is the case of nicotine, which reacts to form tobacco-specific nitrosamines (TSNAs), a leading class of highly carcinogenic compounds in tobacco products. House dust is an important route of toxicant exposure, especially for children under 5-years-old, who spend most of their time indoors and are estimated to ingest about 100 mg of indoor dust per day. Despite the evidence of THS toxicity and health effects observed in exposed cells and murine models to date, the effects of THS toxicants in early development are not fully characterised. To accomplish this aim, we exposed wildtype zebrafish embryos from 6 hours post fertilization (hpf) and 24 hpf, till 120 hpf, to THS toxicants at concentrations mimicking children exposure in smokers' and non-smokers' homes. Further, *fli1*:EGFP zebrafish embryos were also exposed for the study of blood vessel development. The alterations caused by these exposures were evaluated by developmental observation combined with untargeted metabolomics and lipidomics analysis. The exposure to the highest concentration of THS toxicants resulted in total lethality. Embryos exposed to the next highest concentration presented malformations and more than 50% lethality. No differences were observed at the lowest concentrations in comparison with the control group. The exposure of *fli1*:EGFP zebrafish embryos did not show significant differences in the development of blood vessels. Comparable results were found between 6 hpf and 24 hpf starting points. Metabolomic analysis showed upregulation of exposure metabolites and alterations in different endogenous metabolites. Dysregulations in the lipid pattern were also observed in THS exposed embryos. These results demonstrate that the exposure to THS affects early development, generating phenotypic and metabolic alterations. In order to validate these results, future work will focus on the effects of the exposure to THS extract samples.

1.05.5
Seasonal variation of health biomarkers in perch from the Baltic Sea-Connection to naturally produced brominated compounds?
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The Baltic Sea wildlife shows signs of declining health, including population declines and negative trends for some health biomarkers. One of the species affected is the perch and the causes are currently poorly understood. The Baltic Sea suffers from eutrophication which has favoured the growth of cyanobacteria and filamentous algae such as the red algae Ceramium tenuicorne. During the summer these filamentous algae produce halogenated compounds, for example hydroxylated polybrominated diphenyl ethers (OH-PBDEs). In toxicity testing using zebrafish embryos with developmental delay as the measured endpoint, the OH-PBDEs, and especially 6-OH-BDE47 and 6-OH-BDE85, were shown to be potent toxins. The OH-PBDEs disrupt the energy production in the cell by interfering with the oxidative phosphorylation (OXPHOS). When comparing the levels found in Baltic Sea biota to the lowest observed effect concentrations (LOECs) from the zebrafish embryo assay, they are of the same magnitude. Thus, it is important to further investigate if the OH-PBDEs are causing negative health effects of wildlife in the Baltic Sea. In the present study, seasonal variation of health biomarkers and potential correlations to the OH-PBDE exposure were investigated. Perch was sampled at Nämdö (59°11´N, 18°42´E), a remote site far from point sources in the Stockholm archipelago, Sweden. Sampling was conducted once a week from May until October, with a total of 18 occasions. Several health biomarkers such as ethoxyresorufin-O-deethylase (EROD) activity, glutathione reductase activity, glutathione S-transferase activity, catalase activity, glucose and lactate were measured in the fish as well as the concentrations of OH-PBDEs. There is an exposure peak of OH-PBDEs for the perch in the middle of the summer and at the highest exposure time point the concentrations are approximately ten times higher than in the spring and autumn. The highest measured individual concentration was 14 ng/g fresh weight. The glucose levels are at a constant high level throughout the whole sampling period at twice the concentrations measured in the yearly environmental monitoring program. There are large variations in the EROD activity but the activity seems to be higher in the spring compared to the middle of the summer. Neither the glucose level nor the EROD activity show any obvious connection with the concentration of OH-PBDEs.

1.05.6
Assessment of water quality through an active biomonitoring approach using three-spined stickleback (Gasterosteus aculeatus)
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The Interreg DIADeM program (development of an integrated approach for the DIAgnosis of the water quality of the river Meuse) was created in order to develop new tools to evaluate the quality of water bodies in the Meuse waterway across the French-Belgian border. The project aimed to bring together the various cross-border stakeholders concerned with the protection of aquatic environments (academics, operators, users) while informing citizens of the risks caused by the degradation of water bodies. The use and the relevance of the multibiomarker approach in different model species (fish, crustacean, mussel) to assess the water quality was an important part of the program. Among the different model species used, the three-spined stickleback has gained interest for biomonitoring for several years, due to its ubiquity and its tolerance to pollution. For many years, a new active strategy based on the importation of caged sticklebacks in a study site have been developed. Within the scope of the DIADeM program, the caging strategy was used with stickleback as model species to discriminate some sites in a large scale on the Meuse basin. In that way, sticklebacks were caged for 21 days upstream and downstream the wastewater treatment plants (WWTPs) of Namur (Belgiun), Charleville-Mézière (France), Bouillon (Belgium) and Avesnes-sur-Helpe (France). A battery of biomarkers was assessed to represent different physiological functions, such as innate immunity (leucocyte mortality and distribution, phagocytosis activity, respiratory burst, antioxidant system (GPx, CAT, SOD and GSH content) and oxidative damages (TBARS), biotransformation enzymes (EROD, GST), synaptic transmission (AChE) and reproduction system (spiggin and vitellogenin concentration). Physiological responses were compared between each site but also with biomarkers levels measured in sticklebacks under control conditions. Study highlighted a decrease of innate immune system downstream the WWTPs, especially through an increase of leucocyte mortality and a decrease in phagocytosis activity. A slight induction of EROD activity was also noticed downstream the WWTP of Bouillon and Namur. Moreover, the use of active biomonitoring clearly discriminated the different study sites from the reference but also amongst themselves, demonstrating the efficiency of used tools to assess the quality of water and characterize types of physiological impacts on fish.

1.05.7
Exploring the use of zebrafish embryo models as alternatives to rodent testing for investigating inflammatory responses to nanomaterials (NMs)
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Nanomaterials (NMs), defined as particles or materials with at least one dimension measuring 100nm or less, exhibit unique physicochemical properties making them highly attractive for use in a variety of consumer products, with the nanotechnology market set to be worth $125 billion (USD) by 2024. There are uncertainties regarding the potential adverse impacts of engineered NMs on human health. The safety of engineered NMs must therefore be assessed to ensure the responsible exploitation of nanotechnology, thus NMs must undergo thorough toxicity testing to ensure their safe use. Assessment of NM safety currently relies on the use of rodents, which is associated with disadvantages such as expense and ethical implications. There is an urgent need to better align nanotoxicology with the principles of the 3Rs: the replacement, reduction, and refinement of the use of animals in research. Few studies have used zebrafish (Danio rerio) larvae as a model for studying the impacts of NMs on human health. Therefore, we propose that the use of this model is increased, as an alternative to rodents. In rodent studies, neutrophil accumulation (e.g. in the lung) is commonly used as a marker of NM toxicity. Therefore, in the zebrafish, we have exploited transgenic larvae with fluorescently-labelled neutrophils (Tg mpx:EGFP) to assess inflammatory responses to NMs. This model has been used to investigate the dynamics of

inflammatory responses to injury and pathogen exposure previously. We have assessed neutrophil accumulation following aqueous exposure to NMs post-injury (transection of tail), and following microinjection of NMs directly into specific target sites, such as the otic vesicle. Preliminary data from the tail transection study shows that post-injury exposure to NMs enhances and prolongs the neutrophil response in the larvae, with greater numbers of neutrophils being present in the wound area for longer than in injured larvae with no exposure to NMs. The methods employed in our study will feed into the development of a tiered testing strategy for NM safety assessment that promotes the more widespread adoption of non-rodent, alternative models to make nanotoxicology testing more ethical, physiologically relevant, and cost and time efficient.

1.05.8
Distribution of chemicals between the yolk sac and the embryonic body in the zebrafish embryos
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The zebrafish embryo is an important model in toxicological studies. The yolk sac of the embryos is a major body part during embryonic development. Its proportions decreases from 80 % to 50% of the whole dry weight (body+yolk) between 24 and 72 hours post fertilization (hpf) as the body grows and consumes the yolk. As the targets for toxic effects of chemicals are in the embryonic body rather than in the yolk, it is important to know whether or not chemicals are evenly distributed between the body and the yolk. If determined at all, internal concentrations are usually analyzed from the whole embryo rather than from the body alone; this approach would overestimate the internal concentration, provided that test chemicals have a higher affinity to the yolk. In this study seven chemical compounds with different physicochemical properties (neutral: 4-iodophenol, diuron, carbamazepine, 1,2,4-tribromobenzene, 2-ethylpyridine; ionic: bromoxynil, paroxetine) are explored for their uptake into zebrafish embryos and their distribution between yolk and embryonic body over 96 hours of exposure. The distribution was analyzed by LC-MS after mechanical removal of the yolk. The amount in the yolk was then calculated by subtraction. 95% of the total internal amount was found in the yolk for the exposure with 4-iodophenol at 26 hpf and this decreased to 80% at 74 hpf (67% at 26 hpf to 33% at 74 hpf for bromoxynil). This decrease was attributed to the decrease in dry weight of the yolk. Thus, in early life stages (up to 74 hpf) of zebrafish embryo, the amount of a chemical in the embryonic body is much lower than what is found in the whole body. Also concentrations in the yolk were higher compared to the embryonic body for all compounds, suggesting compositional differences. Furthermore, it was investigated whether the absorbed amount in the yolk can be predicted. Equilibrium concentrations for the yolk were measured by a dialysis experiment. However, the observed steady-state concentration and equilibrium concentration differed within one log unit for three out of the five neutral compounds possibly due to aging of the yolk in the dialysis experiment. Our findings show that there is a substantial amount absorbed by the yolk and that the distribution of absolute amounts is mainly influenced by the developmental stage.

1.05.9
Multilevel effects of intergenerational exposure to glyphosate and glyphosate based herbicides on rainbow trout (Oncorhynchus mykiss).
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Introduction: Glyphosate, one of the most common active substance (AS) found in herbicide products used to increase agriculture productivity, is highly prevalent in continental waters and oceans worldwide. If opinions published by the European Food Safety Authority suggest that this AS has a limited impact on aquatic organisms, several studies reported deleterious effects after a glyphosate or Glyphosate Based Herbicides (GBHs) exposure and raise the issue of the toxicity of adjuvants formulated in these GBHs. Methodology and results: Our objective was to investigate the potential multilevel effect of an intergenerational chronic exposure to glyphosate on rainbow trout (RT), Oncorhynchus mykiss, comparing the toxicity of the AS alone and of two commercial GBHs. Forty-eight genitors were daily exposed to an environmental concentration of glyphosate (1 µg.L-1) over one year and a first generation of offspring was produced. Several parameters of this offspring were analyzed to reveal potential impacts at different levels of biological organization (behavior, response to viral infection, immune parameter, metabolic pathways,…). At the individual scale, swimming behavior and capacity to survive to an experimental infection with infectious haematopoietic necrosis virus (IHNV) were altered in offspring issued from genitors chronically exposed to glyphosate. Induction of disturbance of the energetic metabolism by glyphosate and adjuvants was strongly suggested through the observation of modifications of both aerobic and anaerobic pathways. Finally, modulations in the activities of enzymes like acetylcholine esterase, anhydrase carbonic and those involved in the anti-oxidative defense in offspring of genitors exposed to glyphosate could explain cellular effects responsible for the physiological perturbations observed in vivo. Conclusions: Our complex experimental design was fully appropriated to compare the impact of intergenerational chronic exposure to AS and GBH. The higher sensitivity to viral infections and modification of swimming behavior observed in offspring issued from genitors contaminated by glyphosate could be associated to a modification of energetic metabolism and several cellular mechanisms (e.g. inhibition of acetylcholine esterase). Taken together, all these data will allow to have an overall vision about the global impacts of glyphosate and GBHs and to reassess risks associated with these molecules.

1.05.10
Identification of additional key events of organophosphorus poisoning by using zebrafish larva as a model
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Organophosphorus (OPs) are organic compounds widely used, for example as pesticides or plasticizers, which poses a serious public health problem in many countries because of their lethality or because they can cause neurological alterations. The acute toxicity of OPs results from the irreversible inhibition of acetylcholinesterase (AChE), whose inactivation leads to a major cholinergic syndrome that associates peripheral manifestations and epileptic seizures. In addition, exposure to neuropathic OPs is well-known to induce OP-induced delayed neuropathy, a neurodegenerative disorder characterized by a delayed onset of prolonged ataxia and upper motor neuron spasticity. Neuropathic OPs may target the neuropathic target esterase (NTE/PNPLA6) but their mechanism of action (MoA) is not entirely resolved and still controversial. Zebrafish larvae are validated, easy-to-use, inexpensive and fast in vivo vertebrate models with highly conserved biological processes related to human neuron diseases and organophosphorus poisoning [1,2]. OPs that inhibit the enzymatic activity of AChE and/or NTE were used for discovering new keys events (KEs) involved in the neurotoxicity of these molecules. Methods involving behavioral endpoints were performed. Visual motor response (VMR) was used to determine the effects of selected OPs on central nervous system functions, including the ability of the larvae to respond to light stimuli. The peripheral nervous system impairment was evaluated by using the electric field pulse motor response (EFPMR) as zebrafish larvae had a very stereotypical locomotor response to an electric stimulation. As an example, TCP isomers including ToCP, were studied with the purpose of elucidating their mechanism of action. A very specific phenotype was retrieved after ToCP exposure showing hyperactivity during several hours and a so-called multiple C-bend spaghetti behaviour. Exposure to picrotoxin recapitulated some aspects of these phenotypic features, suggesting that GABAA receptor modulation is one of the key events in the mechanism of action of this type of OP on the nervous system. [1]. Babin PJ et al. 2014. Zebrafish models of human motor neuron diseases: Advantages and limitations. Prog. Neurobiol. 118C:36-58. [2]. Faria et al. 2015. Zebrafish Models for Human Acute Organophosphorus Poisoning. Scientific Reports. 5:15591.

1.05.11
Identification of biomarker candidates for discriminating endocrine disruption from hepatotoxicity in zebrafish (Danio rerio)
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Nowadays there is much concern about substance-induced hepatic toxicity interference with the ascribed apical endpoints for assessing reproductive impairment by endocrine disrupting chemicals (EDC) in fish. To this end, our study aimed at the identification of protein biomarker candidates indicating substance-induced liver toxicity in zebrafish. Adult zebrafish (*Danio rerio*) were exposed to the known hepatotoxic substance acetaminophen for 21 days, in compliance with the fish short-term reproduction assay (FSTRA). Molecular perturbations induced by acetaminophen were studied in the liver and in the gonads by applying a previously developed combined FSTRA and proteomics approach. We observed a significant decrease in egg numbers, an increase in plasma hyaluronic acid, and the presence of single cell necrosis in the liver tissue, thus suggesting hepatotoxic effects on reproduction. Consistent with these changes in apical endpoints, proteomics analyses revealed an intense impairment in liver function ranging from stress induction in endoplasmic reticulum,

dysfunctional mitochondria, and glutathione depletion to excessive accumulation of reactive oxygen species. Comparing the results with those observed upon exposure to the endocrine disrupting substance fadrozole, we identified specific targets for both MoAs and three potential biomarkers for accessing liver injury i.e. *cahz*, *c3a.1* and *atp5f1b*. In conformity with apical endpoints, the proteome response to fadrozole exposure indicated a significant regulation in estrogen synthesis, reduced vitellogenin synthesis, perturbed binding of sperm to zona pellucida in the ovary and including the identification of cystathionine gamma-lyase (*cth*) protein, as a potential sex unspecific biomarker for evaluating deficiency in estrogen synthesis and impaired reproductive endocrine disorder. Our study demonstrates that biomarkers identified and quantified by proteomics can serve as additional weight-of-evidence for the discrimination of hepatotoxicity and endocrine disruption, which is necessary for hazard identification in EU legislation and to decide upon the option for risk assessment.

1.05.12
Poster spotlight: Oestrogenic effects in wild fish in English Rivers: comparing contemporary and historical impacts
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Human and Environmental Lipid Disruptors

1.06.1
The Echinodermata PPAR: functional characterization and exploitation by the model lipid homeostasis regulator tributyltin
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The wide ecological relevance of lipid homeostasis modulators in the environment has been increasingly acknowledged. Tributyltin (TBT), for instance, was shown to cause lipid modulation, not only in mammals, but also in fish, molluscs, arthropods and rotifers. In vertebrates, TBT is known to interact with a nuclear receptor heterodimer module, formed by the retinoid X receptor (RXR) and the peroxisome proliferator-activated receptor (PPAR). These modulate the expression of genes involved in lipid homeostasis. In the present work, we isolated for the first time the Echinodermata (*Paracentrotus lividus*) gene orthologues of PPAR and RXR and evaluated the ability of a model lipid homeostasis modulator, TBT, to interfere with the lipid metabolism in this species. Our results demonstrate that TBT alters the gonadal fatty acid composition and gene expression patterns: yielding sex-specific upregulation of long-chain acyl-CoA synthetase (*acsl*), *ppar* and *rxr*. Furthermore, organotins (TBT and TPT (Triphenyltin)) repressed the heterodimer PPAR/RXR, *in vitro*, in a concentration-dependent manner. Together, these results suggest that TBT acts as a lipid homeostasis modulator at environmentally relevant concentrations in Echinodermata and highlight a possible conserved mode of action via the PPAR/RXR heterodimer.

1.06.2
Effects of the exposure to various EDCs on the lipid metabolism of zebrafish embryos
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The European project ENDpoiNTs is developing new testing and screening methods to identify endocrine disrupting chemicals (EDCs) that induce human developmental neurotoxicity (DNT). Clear evidence supports associations between endocrine disrupting chemical (EDC) exposure and impaired neurodevelopment. Yet, current hazard assessment of EDCs does not address developmental neurotoxicity. This is due to a lack of scientific knowledge on how endocrine disruption is linked to developmental neurotoxicity (DNT). Thus there is an urgent need for novel testing and screening tools to address ED-induced DNT, based on new scientific knowledge. The current study is generating new scientific knowledge on the link between endocrine disruption and DNT at the molecular, organism and behavioural level using zebrafish (*Danio rerio*) embryos. The focus of the current paper will be on the lipid metabolism of 5 day old zebrafish embryos that were exposed to various EDCs. Zebrafish embryos were exposed to eight different EDCs. The exposure levels were selected based on earlier studies and were generally below the NOEC for visiual malformations,. The EDCs include pesticides and metabolites (aldicarb, permethrin, 3-PBA), PFAS (PFOS, PFOA, PFHxS), and phthalates and metabolites (DIDP, DEP) . Exposure to EDCs resulted in altered lipid metabolism in zebrafish embryos, but different lipid pathways were affected by different EDCs. The most commonly affected pathway were the glycerophospholipids, but also the glycerolipid and sphingolipid metabolisation were affected. Various EDCs (e.g. aldicarb, PFOS and PFOA) caused effects on fatty acid metabolism, such as elaidic acid, nervonic acid, and lauric acid, but the strongest effects were seen on other lipids classes. Two distinguished groups could be observed. One group of EDCs (PFhxS, permethrin, 3-PBA) mainly affected the PC and PE lipid metabolisation, another group (PFOS, aldicarb) mainly triacylglycerol lipids, and DEP affected both PC, PE and TAG lipids.

1.06.3
Lipidomic profiling of plasma and endoplasmic reticulum fraction of Atlantic cod liver for the detection of metabolic dysfunctions associated to pollutants exposure
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The Atlantic cod (Gadus morhua) is a major fisheries species in the North Atlantic and an attractive model for analysing the impact of environmental contaminants. As the site of xenobiotic biotransformation and main storage site of fat, the responses in cod liver is of particular interest. The dCod 1.0 project aims to better understand how the Atlantic cod respond and adapt to pollutant stressors. In order to partially characterize the lipidome of plasma and the endoplasmic reticulum (ER) fraction of cod liver following pollutants exposure, we have developed a methodology based on the use of Flow injection analysis coupled to high-resolution mass spectrometry (FIA-HRMS). In this study, fish were intraperitoneally injected with a mixture of ten contaminants (chlorpyriphos, perfluorinated compounds (PFOA, PFOS), bis(2-ethylhexyl) phthalate, trans-nonachlor, 4-tert-octylphenol, bisphenol A, p,p'-dichlorodiphenyltrichloroethylen, and polychlorinated biphenyls (PCB153, PCB138)) at environmentally relevant concentrations, and sampled after 12 days. The targeted analysis allowed the quantification of about 150 lipid species in plasma, mainly triacylglycerols (TG), cholesterol esters (CE) and phosphatidylcholines (PC). In the ER fraction, TGs, PCs and phosphatidylethanolamines (PE) dominated. Interestingly, phosphatidylinositols (PI), phosphatidylserines (PS) and PEs were downregulated in the ER fraction of exposed cod, indicating a major effect of the xenobiotic mixture in de novo synthesis of phospholipids, together with a concomitant increase in some TGs. The lipidomic dataset was further processed with the proFIA package followed by tentative annotation with LIPIDMAPS® to seek for dysregulated features that had not been considered in the targeted analysis. This untargeted strategy provided complementary evidence of the segregation of control and exposed samples and highlighted glycosphingolipids, fatty acid conjugates and derivatives, plasmenyl/plasmanyl forms of PCs, PSs, PIs; and phosphatidic acids as additionally altered lipids. Overall, these results demonstrate the disturbance of the ER lipidome in exposed cod. ER is a critical site of lipid metabolism, and a perturbation of ER homeostasis can contribute to metabolic dysregulation that is shown in alterations in plasmatic lipids. The applied methodology represents a promising tool for the discovery of new lipid biomarkers in ecotoxicological studies.

1.06.4
Untargeted lipidomics of zebrafish (Danio rerio) embryos exposed to bisphenol A and estradiol using comprehensive 2D-LC-HRMS
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There is an increasing interest in investigating possible adverse effects of endocrine-disrupting chemicals (ECDs) on aquatic organisms and infer this knowledge to human populations[1]. This study focuses the exposure of zebrafish embryos to an exogenous endocrine disruptor, bisphenol A (BPA), and a natural hormone, estradiol (E2). The main aim is to determine which effects are provoked by the estrogenic effect of BPA, and which are specific to this compound. The high complexity of biological samples has led to the development of multidimensional separation platforms, with a greater separation power than mono-dimensional approaches[2]. Among them, LC × LC–MS seems especially suitable for untargeted metabolomic analysis. In this study, an RP×HILIC-HRMS method was optimized for the analysis of lipid extracts, employing Active Solvent Modulation (ASM) to deal with the solvent incompatibilities between the two separations[3]. Zebrafish embryos were exposed to BPA and E2 from 48 to 144 hours post-fertilization (hpf). Exposure concentrations were chosen after toxicity tests to ensure working at sub-lethal doses. Three concentration levels were used in each case: 4.4, 17.5 and 26.3 µM for BPA, and 1, 4 and 8 µM for E2. Finally, embryos were collected at 96 and 144 hpf. Two types of studies were performed depending on the target lipid family. The first study focuses on the effects caused on phospholipids whereas the second on the changes on the sphingolipidome. As LC × LC produces very complex datasets, advanced chemometric approaches are

highly recommended to process the data. ROIMCR[4] strategy was used for that purpose, which is composed by two main steps: 1) Regions of interest (ROI), a compression and arrangement of the raw LCxLC-MS data; 2) Multivariate Curve Resolution–Alternating Least Squares (MCR-ALS), a resolution of the LC×LC elution profiles and mass spectra to detect the main features related to the changes in the lipidome caused by the exposition. After lipid identification, Kyoto Encyclopedia of Genes and Genomes (KEGG) platform was used for the identification of metabolomic pathways affected and the biological impact the exposure produced on the organisms. The combination of a high resolving power separation technique (LC×LC-HRMS) with the chemometrics-based ROIMCR data analysis allowed the detection and identification of a wide number of lipids, markers of the exposure to the studied endocrine disruptors. [1] Diamanti-Kandarakis, E. et al. 2009. Endocr. Rev. 30: 293–342. [2] Pirok, B. W. J., et al. 2019. Anal. Chem. 91: 1:240-263. [3] Stoll, D. R., et al. 2017. Anal. Chem. 89: 9260–9267. [4] Gorrochategui, E., et al. 2019. BMC Bioinformatics 20:1–17.

1.06.5
Oil exposure disrupts lipid metabolism during diapause in calanoid copepods
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Calanoid copepods are keystone zooplankton species in marine ecosystems, primarily due to their ability to transform ingested prey into nutritious lipids. Copepod presence in surface waters is seasonal, as several months of the year is spent at depth in a dormant state called diapause. The termination of diapause, which involves the ascent to surface waters to feed and reproduce, may be dependent on content and/or composition of lipids in the lipid sac. Very little is known about lipid catabolism during diapause, and how environmental stressors, like oil pollution, may influence this. We performed a study where we exposed copepods terminating diapause to the water-soluble fraction (WSF) of a crude oil, and investigated effects on lipid metabolism. Our results suggest that WSF-exposure during diapause termination can results in a temporary reduction in lipid catabolism. WSF-exposed copepods i) utilized stored lipids more slowly and ii) had more down-regulated genes in the ß-oxidation pathway than control copepods. As this reduction in lipid catabolism resulted in excess lipid stores in the copepodites, termination of diapause could be delayed. This delay could have detrimental consequences for marine ecosystems.

1.06.6
Spatially resolved lipidome profiling of freshwater sentinel species Gammarus fossarum by shotgun lipidomics and mass spectrometry imaging
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Lipids are important component in living organisms and play vital roles in various biological functions. In aquatic biomonitorig, disruptions in lipid homeostasis of bioindicators induced by various pollutants have been recently illurstrated by NMR and LC-MS analyses. However, both analytical methods require a lipid extraction process which sacrifices the spatial information of the lipid species. Mass spectrometry imaging (MSI) is a well recognised technique for in situ mapping of a variety of molecular species within a tissue section. To obtain a comprehensive lipid profile of a freshwater sentinel species Gammarus fossarum, we developed a spatially resolved lipidomics workflow combining shotgun lipidomics and MSI. Gammarids which were collected from a referenced site were used for all the experiments. Shotgun lipidomics was performed using nano ESI coupled to a hig-resolution mass spectrometer (Q Exactive). MALDI (matrix assisted laser desorption ionisation) was utilised to map the whole-body gammarid tissue sections and secondary ion mass spectrometry (SIMS) was applied to scrutinise specific organs and structures with high spatial resolution. Various lipid species including glycerolipids, glycerolphospholipids, sphingolipids were detected and identified from the lipid extracts by shotgun lipidomics. The imaging of whole-body gammarids and targeted regions of interest shows accumulation of different lipid classes in different organs. Overall, our results indicate a both diverse and dynamic lipid composition during the reproduction cycle of G. fossarum. This methodology will be applied to examine the lipidome in gammarids exposed to aquatic pollutants that are are especially knonw to disrupt lipid metabolism. We envisage that cellular localisation of the pollutants and lipids by this spatially resolved lipidomics will contribute significantly to deciphering the molecular mechanism of the toxicity effect induced by environmental pollution.

Neuroendocrine and Neurochemical Disruption in Vertebrate and Invertebrate Models

1.07.1
Single and combined low concentrations of neuro-active drugs disrupt key neurological signalling pathways in Daphnia magna affecting reproduction and lipid metabolism
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Assessing the risk of neuro-active pharmaceuticals in the environment requires an understanding of their joint effects at low concentrations across species. Here, we assessed reproductive, transcriptional and lipidomic effects of single and mixture equi-effective mixture exposure to fluoxetine, propranolol, diazepam and carbamazepine on the crustacean Daphnia magna at environmentally relevant concentrations. We also included previous obtained knockout clones lacking serotonin since they had the opposite phenotype of animals treated with fluoxetine. The four compounds enhanced reproduction in adults, and induced specific transcriptome and lipidomic changes in pre-adolescent individuals. Knock out clones showed the opposite responses. Comparison of the results from single exposures to a ternary equi-effective mixture of the three compounds, showed additive action. Functional transcriptomics analysis indicated that the four drugs shared major de-regulated signalling pathways implicated on energy, growth, reproduction, and neurologically- related processes, which may be responsible for the observed reproductive effects. Lipodomic analysis also evidenced downregulation of specific lysophospolipids in animals exposed to the four drugs and the opposed trend in knockout animals . Thus, our study showed additive effects at the transcriptional and physiological level and a probably similar key or initiating event linked with the serotonergic pathway.

Not all Oil Spills Behave the Same: Unconventional Oils, Advanced Characterization and Ecotoxicity

1.08.1
Dispersant application increases adverse long-term effects of oil on Northern shrimp larvae after six hours exposure
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The application of chemical dispersants is one option of oil spill response (OSR) and substantial information exists on the acute toxicity of mechanically dispersed oil (MDO) and chemically dispersed oil (CDO) on different aquatic species generated from standard laboratory studies. However, these studies use exposure durations of 24-96 h, although short exposure times (1-8 h) are considered most representative for oil spill scenarios. Therefore, we exposed Northern shrimp (Pandalus borealis) larvae to short exposure durations of 6 h and 1 h to field-realistic concentrations of CDO (~ 10 mg L-1 THC), MDO (~ 7 mg L-1 THC), and dispersant only (D; 4%). A control with clean seawater served as reference. P. borealis is an ecologically and economically important key species of northern ecosystems, along the Norwegian coast and in the Arctic up to Svalbard. Higher northern latitudes and Arctic areas are becoming more opened to ship traffic and exploration activities by the oil and gas industry. Therefore, data on the sensitivity of northern key species and particularly larval stages to petroleum discharges is needed as the risk of accidental oil spills increases. Increased knowledge helps to tailor OSR capabilities to this region. Spill impact mitigation assessment for the Arctic marine environment relies on a trade-off approach such as the Net Environmental Benefit Analysis (NEBA) to use the less damaging OSR for the environment. Here, endpoints relevant for NEBA, considering their ecological meaning for the recruitment and population dynamics of Northern shrimp, were investigated. Generally, the short exposure to oil had long lasting effects on Northern shrimp larval fitness. However, there were differences with exposure time and between endpoints. Feeding was impaired directly after oil exposure, while all other endpoints were affected during recovery. After 1 h exposure to oil, only minor effects on survival and feeding were found, whereas 6 h exposure affected all endpoints. Overall, the data indicated a greater sensitivity of Northern shrimp larvae to CDO than MDO. Differences in oil droplets size and fractions of dissolved oil components in the water potentially explain the greater sensitivity to CDO compared to MDO. The data are relevant for the NEBA approach and for spill responders in taking the best OSR decision i.e. with the best net environmental benefit.

1.08.2
Acute toxicity of individual polycyclic aromatic compounds and compound mixtures to American lobster (Homarus americanus) larvae using a passive dosing system
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The American lobster (*Homarus americanus*) is a species of both cultural and economic importance on the east coast of Canada and the United States. Stage I larval lobsters are a particularly vulnerable life stage when they are seasonally present as they are both sensitive to environmental contaminants and reside near

the surface of the water column. This study examined the acute toxicity of 13 individual PACs and three PAC mixtures on stage I lobster larvae using a passive dosing system to predict the impact of an oil spill on lobster habitat. The chosen PACs cover a range of compound classes and K_{ow} values (i.e. PAH, alkyl-PAH, heterocycles and oxidized hydrocarbons). Each compound and mixture was loaded into PDMS O-rings to partition PACs into seawater and maintain concentration over the course of the exposure period. Exposed lobster larvae were assessed for survival and affected mobility at 3, 6, 12, 24, 36 and 48 hours of exposure. Passive dosing was effective for acute exposures to PAH, alkyl-PAHs and heterocycle compounds. However, passive dosing was ineffective for the oxidized hydrocarbons tested due to low solubility in methanol. Results provided a linear relationship between the log (LC_{50}) and log (K_{ow}) values with a slope similar to the universal slope of type 1 narcotic compounds (slope = -0.97) described in the Target Lipid Model (TLM). Stage 1 lobster larvae proved to be very sensitive to type 1 narcosis based on the critical body burden derived from the TLM. The collected toxicity data will improve oil spill models used by response organizations, fishery managers and industry stakeholders to make science-based decisions in the case of an oil spill within critical lobster habitat and seasonally when stage I larvae are present.

1.08.3

The use of standard toxicity test methods in a modified process for the approval of oil spill treatment products in the UK

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Current experimental protocols for the toxicity assessment of oil spill treatment products in the UK have been established since the 1970s. To address health and safety, cost and scientific robustness issues the UK approach for dispersant testing and approval has been reviewed and updated for implementation during 2020. The aim is to provide more robust scientific advice for risk assessments that enable effective decision making on the use of oil remediation products in the event of a spill and promote more effective cross-institute comparisons of toxicity. It is preferable that environmentally relevant test species are used but, as the scientific literature provides little conclusive evidence of a taxa-specific trend in sensitivity, species selection based on sensitivity alone was not justified. Eight dispersants, commonly stockpiled in the UK, were tested independently and in combination with a representative crude oil. Testing of dispersants in combination with oil has historically provided more variable results so this study has considered the benefits of this versus product only testing. Core test species included the harpacticoid copepod, *Tisbe battagliai*, and the algae, *Skeletonema* sp., as both have cost-effective internationally standardised methods, whilst also being environmentally representative and easily cultured under laboratory conditions with no seasonality. Other candidate test species, such as oyster embryos, had limitations in applicability due to seasonal issues. Fish testing was not considered as there was no ethical reasoning for vertebrate testing due to the absence of taxa-specific toxicity. Results showed that, if oil is excluded from the assessment, *Skeletonema* sp. and *Tisbe battagliai,* can produce reliable, reproduceable and interpretable results. This suggests that product only testing is suitable for ranking products based on toxicological hazards. The redevelopment of the UK guideline to use standardised testing and environmentally relevant test organisms will increase the quality and reliability of data used to underpin the UK oil spill treatment testing and approval scheme. The adoption of this approach will enable an approved list of products for use in UK waters to be maintained. However, the decision for dispersant use in any given scenario will still need to use a risk assessment approach taking account of incident-specific physical and environmental sensitivity.

Novel Developments in Endocrine Disruptor Testing with Vertebrates

1.09.1

Interlaboratory OECD Validation of the Rapid Androgen Disruption Adverse outcome Reporter (RADAR) assay

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In the past ten years, increasing interest has been paid to the identfication of chemicals interefering with the normal functioning of the androgen axis. Two key *in vitro* studies identified 66/200 and 37/134 pesticides tested as anti-androgenic. However, the effects of many of these pesticides have yet to be confirmed *in vivo* due to the absence of medium throughput *in vivo* assays. With this in mind, we developed a transgenic medaka line capable of revealing the level of activity of the androgen axis by emmision of green fluorescence. This line harbours a portion of the spiggin1 gene promoter upstream of GFP coding sequence. The spiggin1-GFP line has been demonstrated to respond specifically to androgens and to be capable of identifying pro-androgenic and anti-androgenic chemicals acting via a variety of modes of action (MoAs). The sensitivity of the line is similar to that of the 21-day androgenised female stickleback screen. Using eleuthero-embryonic life stages of this transgenic line, which are non-compliant with the EU definition of a laboratory animal, we developed the 72 h Rapid Androgen Disruption Adverse outcome Reporter (RADAR) assay which is carried out in six-well plates. This assay is currently under OECD validation as a potential test guideline. In an OECD interlaboratory validation study, the RADAR assay was performed in five different laboratories in France, the UK, Switzerland, Germany and Japan. **1. 2. 3.** The RADAR assay was successfully transferred to the four partner laboratories. The laboratories in Switzerland and Japan reared spiggin1-gfp fish to adulthood and bred them to produce eleuthero-embryos which they performed the tests with. The laboratories in the UK and Germany received embryos from WatchFrog which they used for testing. The results were coherent between each of the partner laboratories and showed a similar sensitivity. The transferability of the RADAR assay has been confirmed as well as its ability to be read using a variety of different fluorescence imaging systems. This transferability includes the ability to perform it either with embryos shipped from a supplier or with embryos bred on site. The reproducibility of the assay has also been demonstrated with a high level of coherence between the results from the different partners. Evaluation of the results by the OECD will indicate the next steps towards the possible publication as an OECD test guideline.

1.09.2

A new standard for endocrine disruptor testing in fish - The integrated Fish Endocrine Disruptor Test (iFEDT)

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Various EU research projects deal with the optimization of aquatic vertebrate 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 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 to 78.125 mg/L; n = 4) was tested as a model substance for the inhibition of thyroid hormone synthesis.In addition to endpoints established in 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. Although ongoing, the analysis of PTU effects already revealed that PTU not only impairs the reproductive performance of adult fish and reduces the growth of juvenile fish, but also induces thyroid follicle proliferation. Further endpoints are under investigation in both juvenile and adult fish: hormone and vitellogenin (VTG) levels, histopathology of the eyes, gonads and thyroid follicles, as well as swimbladder inflation. Based on preliminary results, the merged test design iFEDT already appears to be a promising tool to integrate various important ED modalities in fish into one assay.This study receives funding from the European Commission under contract no. 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").

1.09.3

Refinement of an OECD test guideline for evaluating the effects of EDCs on aromatase gene expression and reproduction using novel transgenic cyp19a1a-eGFP zebrafish

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Transgenic fish are powerful models that can provide mechanistic information regarding the endocrine activity of test chemicals. In this study, our objective was to use a newly developed transgenic zebrafish line expressing eGFP under the control of the *cyp19a1a* promoter in the OECD Fish Short Term Reproduction Assay (Test Guideline 229) to provide additional mechanistic information on tested substances. For this purpose, we exposed adult transgenic zebrafish to a reference substance of the TG 229, i.e. prochloraz (PCZ; 1.7, 17.2 and 172.6 µg/L). In addition to “classical” endpoints used in the TG 229 (reproductive outputs, vitellogenin), the fluorescence intensity of the ovaries was monitored at 4 different times of exposure using *in vivo* imaging. Our data revealed that the highest PCZ concentration significantly decreased the number of eggs laid per female per day and the concentrations of vitellogenin in females, reflecting the decreasing E2 synthesis due to the inhibition of the ovarian aromatase activities. At 7 and 14 days, GFP intensities in ovaries were similar over the treatment groups but significantly increased after 21 days at 17.2 and 172.6 µg/L. A similar profile was observed for the endogenous *cyp19a1a* expression measured by qPCR thereby confirming the reliability of the GFP measurement for assessing aromatase gene expression. The overexpression of the *cyp19a1a* gene likely reflects a compensatory response to the inhibitory action of PCZ on aromatase enzymatic activities. These experiments showed that the physiological responses observed in control and PCZ-exposed *cyp19a1a-eGFP* transgenic zebrafish (E2, VTG, reproduction) are similar to those of wild-type zebrafish, both qualitatively and quantitatively. Besides, the *cyp19a1a-eGFP* model reliably informs on the time- and concentration-dependent effects of PCZ on ovarian aromatase through *in vivo* GFP fluorescence of the ovaries, thereby providing novel mechanistic information without increasing the number of animals needed. Overall, the *cyp19a1a-eGFP* transgenic zebrafish line proved to be a relevant model to study the effects of EDCs in fish, which might also be wisely used to refine mechanism-based assays such as OECD TG 229.

1.09.4
Recommendations on how to perform, report and evaluate hormonal and gross pathological endpoint for non-mammalian vertebrates for Endocrine Disruption (ED) assessment
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Within the EU, the approval of pesticides is regulated by Regulation (EC) No 1107/2009. An amendment to this Regulation (Regulation (EU) No 2018/605), which establishes the scientific criteria for the identification of pesticide active substances with endocrine disrupting (ED) properties, was published in April 2018. In June 2018, European Food Safety Authority (EFSA), the European Chemicals Agency (ECHA) and the Joint Research Centre (JRC) published a guidance document on the implementation of the scientific criteria, this publication primarily addresses the Estrogen, Androgen, Steroidogenesis and Thyroid, known as 'EATS', modalities in vertebrates. It specifically included recommendations on how to perform hormonal measurements in mammals (i.e. rodent models). However, when it comes to non-mammalian vertebrate test guidelines (TG), there are a number of 'gaps' which need to be filled to fully address assessment of 'EATS' modalities and mirror mammalian TGs. For example, in the OECD test models which do address endocrine specific endpoints (i.e. amphibians and fish) there are no specific recommendations on which hormones to measure, how or when to measure hormones, or how to interpret the results. To harmonise vertebrate OECD TGs, these additional endpoints need to be investigated. Reflecting the mammalian situation, some guidance on how to perform, report and evaluate these additional endpoints is required. The project approach combined systematic investigation of the literature, a semi-structured survey targeted at end-users (e.g. contract research laboratories) and expert experience, to deliver a guidance document on recommendations on how to perform, report and evaluate; • Hormonal measurements in fish, amphibians and birds, • Gross pathology examinations in birds, Here we present the project outcomes and key messages.

Novel Tools and Bioassays for the 21st Century Environmental Toxicology

1.10.1
Biomarker development for neonicotinoid exposure in the soil dwelling Folsomia candida
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Mechanistic data can be used to develop Adverse Outcome Pathways (AOP), which incorporates toxicity information on the molecular, organismal and population level into a tool for environmental risk assessment. To use mechanistic data in environmental risk assessment deep understanding of the molecular processes behind the Key Events (KE) that constitute an AOP is required. Moreover, molecular endpoints, biomarkers, have to be identified that are specific for the severity and type of toxic exposure. We study the mechanistic changes induced by pesticide exposure in *Folsomia candida* (springtail) in order to develop methods for soil quality assessment. The focus of the work is on neonicotinoids, the most widely applied class of insecticides. To generate candidate biomarkers, we generated time-resolved transcriptomic and proteomic data of animals exposed to Imidacloprid, a neonicotinoid. Moreover, we validate these candidates by inhibiting the main route of neonicotinoid detoxification, the biotransformation pathway. By measuring reproduction, we show that Piperonyl butoxide (PBO) can enhance the potency of two neonicotinoids, Imidacloprid (IMI) and Thiacloprid (THIA). Moreover, we show that two targets of PBO inhibition, Cytochrome P450s (CYPs), are upregulated during THIA, IMI and PBO exposure. These results contain putative neonicotinoid biomarkers and support PBO as a possible tool for validation. Future work will focus on generating neonicotinoid biomarkers in the context of pesticide mixtures and on agricultural soils. Ultimately, the results from the project can lay the foundation for rapid and cost-effective soil quality assessment.

1.10.2
Characterization of endocrine disruptors linked to metabolic related adverse outcome pathways using artificial intelligence and systems toxicology
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Exposure to endocrine disrupting compounds (EDC) represents one of the most critical public health threats nowadays. In line with the regulatory framework implemented within the European Union, new and effective methods for EDC testing are needed. The OBERON project, that is part of the EURION cluster, will build an integrated testing strategy (ITS) including advanced computational models to detect metabolic outcomes of EDCs. In addition, the HBM4EU project aims at developing bio-monitoring studies and at linking exposure to chemicals with health effects. Even if several metabolic diseases have now been associated with EDCs, biological mechanisms are not fully understood. Therefore, it is essential to have a better understanding of the biological pathways that suspected EDCs may perturb to identify their real impact on the human population and develop relevant tests. Adverse Outcome Pathways (AOP) consist in a linear or a network representation of mechanistic perturbations at different levels of the biological organization, and although AOPs are chemical-agnostic, they may help to link chemicals to biological events in order to gain insights on possible toxic outcomes. The aim of the present computational study was to identify in an automatic way, existing knowledge, i.e EDC-related metabolic outcomes and events, from the literature, and to map them to AOP and AOP networks. The approach uses a combination of a recently developed tool called AOP-helpFinder, based on artificial intelligence and graph theory to identify EDC-biological event linkages, and integrative systems biology. Using AOP-helpFinder, texts from scientific abstracts in the PubMed database were compiled, prepared (text classification) and screened (text mining) for specific EDCs and events (165 MIE, 704 KE and 125 AO), and findings were prioritized by scoring. The identified EDC-event associations combined with systems biology allowed to suggest AOP hypothesis.

1.10.3
Harvesting what we sow: exploiting the potential of bioassay data to support mechanistically oriented hazard and risk assessment
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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 project to 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 devloping computational approaches to harvest AOP-informed data and conduct quantitative evaluations for mechanisticaly oriented hazard and risk assessment are thus highly warranted. Efforts to develop new AOPs, quantitate the relationships of events across the AOP continuum (quantitative AOPs) and interpret these in terms of ecological relevance are thus quickly becoming bottlenecks in successful use of an ever increasing amount of effect 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 lacking. The present work demonstrates how AOP-informed exploitation of effect data from different bioassays and exposure information from monitoring studies can be used to identify suitable bioassays, toxicity pathways and endpoints, and predict the hazard potential of single

chemicals and complex mixtures. The study also demonstrates how site-specific exposure data can be used to characterise "risk" profiles and identify "risk" hot spots, susceptible species groups (taxa), risk drivers, and MoA of relevance for a given exposure scenario using bioassay data from different levels of biological organisation. The proposed approach is anticipated to assist bioassay development, exploit existing bioassay (effect) data, and support Integrated Approaches to Testing and Assessment (IATAs) and more mechanistically oriented hazard and risk assessment. Acknowledgements: Financing - RCN 268294 "Cumulative hazard and risk assessment of complex mixtures and multiple stressors (MixRisk)", and NCTP: NIVA's Computational Toxicology Program, NCTP (www.niva.no/nctp).

1.10.4
Poster spotlight: Multi-omics Analysis Reveals that Co-exposure to Phthalates and Metals Disturbs Urea Cycle and Choline Metabolism
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1.10.5
Poster spotlight: Could we offer a comprehensive assessment of chemical mixtures without previous knowledge of the individual components?
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One Health and the Environment

1.11.1
Antibiotic and resistance gene occurrence in cattle manure and digestate of anaerobic digestion plants fed with agrozootechnical waste
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Antibiotics are essential for maintaining public and animal health and overall quality of life. However, the presence of antibiotics in soil and water ecosystems causes particular concern for the development of multi-resistant bacteria and genes, which can be transferred in different ways to human and animals. For example, huge amounts of antibiotics are used in livestock farms and their persistence in the cattle manure can cause environmental contamination when it is used directly as a biofertilizer. An innovative practice of energetic biomass waste valorization is the use of manure in biogas plants. The possibility that the anaerobic digestion process can affect the antibiotic residue presence and antibiotic resistance gene abundances has not exhaustively investigated so far. In this context, the number of ARGs responsible for sulfamethoxazole (SMX) resistance (sul1, sul2), ciprofloxacin (CIP) resistance (qnrS, qepA, aac-(6')-lb-cr) and the mobile genetic elements intl1, together with antibiotic (SMX and CIP) concentrations were measured in the input and output samples of various digesters located in an agricultural area in Central Italy. The abundance of sul1 was comparable among all farms investigated, with an average frequency (ARG/16S) of 10-5. A similar frequency was found for the gene intI1, while sul2 showed a higher average frequency of 10-4. CIP resistance genes showed different patterns: qnrS gene was not detected, qepA was found with a frequency of 10-5 and aac-(6')-lb-cr with an average frequency of 10-2. Some digesters showed a strong seasonal influence, with a higher abundance of ARGs in summer samples, however this result was not found in all plants. Moreover, no correlation between ARGs and antibiotics was found, suggesting some co-selection events able to promote ARGs spreading.

1.11.2
Implications of stereochemistry in antibiotic resistance - the stereoselective metabolism of antibiotics by environmental bacteria
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Antibiotic resistance (ABR) is recognised as a serious global health threat that is most efficiently managed via a 'one health' approach which incorporates environmental risk assessments. The environmental dimension of ABR in the past has been largely overlooked, with recent studies underling the importance of non-clinical settings in the emergence and spread of ABR. Despite this, a number of research gaps remain in regard to the drivers of ABR, these include the development and fit-for-purpose environmental risk assessments for antibiotics. Here we explore the implications of stereochemistry on the fate of antibiotics within the environment and ABR. Molecular genetic approaches have greatly increased our understanding of the evolution and spread of antibiotic resistance, however there have been fewer studies on the dynamics of antibiotic-microbial (A-M) interactions, especially with respect to stereochemistry. In order to address this knowledge gap an interdisciplinary approach is required, along with the development of sensitive and selective analytical tools. In order to address this a novel approach for assaying bacterial resistance mechanisms in the context of A-M interactions was developed utilising a combination of traditional microbiology, whole genome sequencing and analytical chemistry. Chloramphenicol was used to provide a proof-of-concept to demonstrate stereoselective metabolism by resistant environmental bacteria. Our data confirms that chloramphenicol can be stereoselectively transformed by environmental bacteria, possibly due to due to the lack of historical exposure to one enantiomer.

1.11.3
Impact of personal history on health effects related to food contaminants
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Introduction: Classically, the risk for health due to dietary exposures is assessed from short-term measurements of food consumption and food contamination. At the individual level, this method does not allow us to consider either changes in eating habits with age, or the evolution of food contamination with regulations. In this study, a method to take these changes into account is proposed, based on individual lifelong profiles. Method: A tiered method was developed, allowing the consideration of certain behaviours or some demographic and socioeconomic characteristics. To this end, correlations between selected parameters and dietary exposures were studied. For significant variables, lifetime trajectories were simulated, from which exposure trajectories were predicted. The evolution of dietary exposure over time was also studied. Moreover, for bioaccumulative substances, the lifetime body burden was simulated using a physiologically based toxicokinetic model. Lastly, exposure and body burden trajectories were compared with external and internal health based guidance values (HBGVs), respectively. Results: On the examples of PCBs, cadmium and bisphenol A, dietary exposures greatly varied with the dietary pattern or according to the sociodemographic characteristics, as the region, the economic level or the marital status. Simulated lifetime trajectories indicated the existence of exceedances of the critical thresholds, reflecting a risk of adverse effect for individuals with a specific profile. However, conclusions in terms of risk assessment varied according to the selected HBGV and according to the approach (i.e. external exposure or body burden). This underlined the importance of considering the kinetics of the substance when calculating exposure and assessing the health risk. Discussion and conclusion: Lifetime trajectories highlight the impact of certain behaviours on dietary exposure and allow the identification of sub-populations more likely to develop adverse effects because of their profile. The method presented here can be extended to the study of other particular characteristics and to a large number of chemicals.

1.11.4
Effects of heavy metals to neurodevelopment in a mother-infant cohort study.
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The neurodevelopmental exposome paradigm was applied on a 178 mother-infant pairs cohort. Heavy metals prenatal exposure was determined to measure mercury in hair samples selected at birth, while cord blood and breast milk samples were analysed for mercury, cadmium, lead, and arsenic, as well as for essential elements (selenium, zinc, copper). Cognitive function, language, and motor development were assessed in children at the age of 18 months by the Bayley Scale for Infant Development (Bayley-III) development tool. The individual-level biological profiles were characterized using both nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) for the untargeted urinary and plasma metabolomics analysis. Integrated pathway analysis and exposome-wide association algorithms were used for the evaluation of the associations between in utero exposure to metals and metabolic pathway dysregulation, as well as between metabolic pathway perturbations and neurodevelopment. NMR and LC-MS/MS analysis of plasma samples, as well as the analysis of urine samples, pointed out the presence of oxoglutaric acid, oxalosuccinic acid, succinate, 2-oxoglutarate,

formate, isocitrate, oxoglutaric acid, glycerol, L-carnitine, glutathione, methionine, cysteine, pyruvate, N-acetylglutamic acid, β-alanine, serine, and arginine. Therefore, pathway analysis revealed that the most perturbed metabolic pathways from exposure to heavy metals were related to TCA cycle, purine, pyrimidine, phospholipids and carnitine metabolism, and glycolysis. The aforementioned results suggested major disturbances to cells biochemistry, which resulted in the impairment of antioxidant defence mechanisms leading to the clinically observed results in linguistic, motor development and cognitive capacity.

1.11.5
Developmental nephrotoxicity of low-level chemical mixtures: implications for chronic kidney disease of unknown etiology
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Chronic kidney disease of unknown etiology (CKDu) is a global health concern, potentially linked to childhood onset. Exposure to heavy metals, herbicides (e.g., glyphosate) and pesticides are thought to contribute to CKDu, but the precise role remain widely debated since these compounds are found at levels considered safe in affected regions. Notably, kidney developmental effects of these compounds at safe levels, especially when in mixtures, remains unknown. Here, using zebrafish *Danio rerio,* a toxicology and kidney disease model, we established effects of exposure to (i) arsenic, cadmium, vanadium, lead and glyphosate mixtures, and (ii) environmentally derived samples (e.g., from drinking water wells) from CKDu endemic and non-endemic regions in Sri Lanka – a country that is significantly affected. Histology studies and gene markers of kidney development and kidney injury, and mitochondrial toxicity analyses showed mixture specific developmental effects—e.g., kidney injury marker was increased with glyphosate, but was reversed when in mixture with arsenic. Our metal and non-targeted organic compound analyses also found nephrotoxic compounds at in drinking water wells from CKDu areas. Collectively, results indicate that metal-mixtures, even at safe levels, can impede kidney development, potentiating increased susceptibility to other nephrotoxic compounds in the drinking water. Furthermore, our study highlights that environmental chemistry analyses coupled with toxicology studies are critical to understanding human health impacts of chemical mixtures. Ongoing studies continue to focus on mitochondrial toxicity based effected directed analyses of drinking water as well as examining kidney morphology of indigenous fish species in CKDu affected regions.

Perspectives on Data Driven Biology: Applications and Safety Assessments

1.12.1
Ecotoxicogenomic fingerprints of neuronal- and growth -targeting insecticides in zebrafish embryos
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EU legislation requires environmental risk assessment (ERA) data for the registration of pesticides, biocides or pharmaceuticals. This inquires a large number of time consuming animal studies. Consequently, alternative approaches to screen for adverse effects are required. Novel tools such as omics, enable scientists to assess the responses of tens of thousands of genes and their products from a single sample, which bears a great potential for sensitive and informative chemical risk assessment.
Using transcriptomics, our study aims to identify specific ecotoxicogenomic fingerprints in the zebrafish embryo as aquatic vertebrate non-target model for two neuronal- (Abamectin and Imidacloprid) and two growth-targeting (Clofentezine and Spirotetramat) insecticides with known mode of action (MoA) in the target organism. In a modified version of the FET (OECD TG 236), embryos were exposed to different sublethal concentrations of the active compound. Subsequently, RNA-Seq and differential gene expression studies were performed and comparing high and low exposure conditions.
Although no visible physiological impairments or acute toxic effects were observed for any tested substance, significant alternations in transcriptomic profiles were prominent in all tested concentrations. High exposure (HE) induced far more differentially expressed genes (DEGs) (Independent hypothesis weighted Wald's t-test, $p.adj < 0.05$) than the low exposure (LE) condition. For all substances, both conditions showed a large common subset of DEGs, ranging from 85 to 96 % (with respect to LE). For the overlapping DEGs we found a clear correlation in their regulation comparing the HE and LE condition, suggesting a dose response relationship for those genes. Large sets of overlapping DEGs were exclusively found for all insecticides in comparison to each other, suggesting their suitability as molecular biomarkers.
The current study demonstrates that different insecticides with different MoAs show distinct transcriptomic profiles in non-target organisms, even at very low levels of exposure. If these profiles are MoA specific requires further investigation. However, these findings will help to further develop an ecotoxicogenomic signature-based molecular screening (e.g. in the zebrafish embryo model) for a time- and cost-efficient as well as informative ecotoxicological risk prediction to ensure the development of environmentally safe active substances.

1.12.2
Metabolomics to decipher phytoplankton responses to silver nanomaterials in aquatic environment
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The important advances in the omics- technologies opened novel avenues towards uncovering the possible contaminant-induced effects in complex environmental settings. Metabolomics, the youngest among -omics technologies, characterizes low-molecular-weight molecules involved in different biochemical reactions and provides an integrated assessment of the physiological state of an organism. This feature makes the metabolomics well suited to study organism – environment interactions. Nonetheless, very few studies use the metabolomics approach to explore the contaminant-induced metabolic perturbations in phytoplankton.In the present pilot study, the potential of the liquid chromatography - based metabolomics to uncover the effects of AgNMs and dissolved Ag on phytoplankton metabolism is explored. The particle-ingesting brown-yellow alga *Poterioochromonas malhamensis* was selected as a representative of freshwater phytoplankton. *P. malhamensis* was exposed for 24h to 20 nm citrate coated AgNMs and the responses compared with those induced by the dissolved Ag and unexposed controls. Total Ag and AgNMs uptake was also determined. AgNMs suspensions in the exposure medium were characterized in terms of stability and dissolution. Ninety primary metabolites were quantified by liquid chromatography – mass spectrometry, including the antioxidants, amines, organic acids/phenolics, nucleobase/side/tides, amino acids, sugar/sugar alcohols and fatty acids.The results revealed that even forming the agglomerates of 300 nm size, AgNMs accumulated in the vacuoles of *P. malhamensis*, disturbed its metabolism and induced oxidative stress; a significant fraction of AgNMs – induced perturbations originated from released dissolved Ag. Exposure to AgNMs and dissolved Ag significantly influenced the amino acids metabolism, citrate cycle, photosynthesis and photorespiration, as well as glutathione and fatty acid metabolism. The implications of the obtained results for assessment of the toxicity and tolerance responses in phytoplankton and for enabling a discovery of sensitive markers for early warning are discussed.

1.12.3
Metabolomics approach reveals disruption of metabolic pathways in the marine bivalve Mytilus galloprovincialis exposed to a WWTP effluent extract
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Conventional wastewater treatment plants (WWTP) discharge a highly diverse range of organic contaminants in aquatic environments. Because of the high demographic pressure, coastlines are affected by contaminant discharges through the development of WWTP sea outfalls, but also by contributions from catchment areas. The health of those important ecosystems could be threatened by contaminant release. In this context, new approaches can be helpful to assess the effects of multi-contamination on marine organisms without *a priori* knowledge. Environmental metabolomics could be one of those approaches since it is able to provide meaningful information on the biochemical response of organisms to a stress. The aim of the present study was to applicate metabolomics approach to highlight key metabolites disrupted by WWTP effluent exposure and then elucidate the biological effects of such exposure on Mediterranean mussels (*Mytilus galloprovincialis*). Mussels were exposed for 7 days in controlled laboratory conditions to a vehicle or to a WWTP effluent extract (WEE) corresponding to an environmental dilution of 5%. Metabolic fingerprints were generated by liquid chromatography combined with high resolution mass spectrometry. Moreover, to better understand these observed effects, the WWE

was characterised based on a suspect screening approach of 80 contaminants in an attempt to provide a first understanding of the relationship between observed effects and contaminants. Key molecular events triggered by the WEE exposure (modulation of amino acids, neurohormones, purine and pyrimidine metabolism, Krebs cycle, etc.) which could lead to adverse outcomes on individuals (reproduction, energy metabolism, osmoregulation, etc.) were highlighted. A sex-specific response was also demonstrated showing the importance to consider sex in the experimental design. Finally, 42 contaminants were detected in the WWTP effluent extract and could be related to the observed effects.

1.12.4

Exploiting the concept of functional extrapolation to identify conservation of toxicity pathways across species

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Regulatory accepted Environmental Risk Assessment methodologies in ecotoxicology have remained largely unchanged for decades. Traditional approaches rely on the availability of toxicity data covering taxonomic groups in relevant receiving environments. However, the limited availability of such data and the desire to move away from in vivo testing means there is a recognized increased need to rely on New Approach Methodologies (e.g. in silico, in vitro, read-across, etc.) to fill data gaps. Yet, these alternative approaches are still limited in both chemical and biological coverage. Opportunities for improvements now present themselves with the increasing availability of large in vitro and in silico (including molecular/genetic) data sets. In order to be able to further exploit these data and prioritize needs, it is critical to compare and discern physiological processes across species to better understand and ultimately predict responses and toxicity outcomes for certain Modes of Action (MoAs) in the environment. An increased understanding of cross-species conservation of pathways and toxicity effects would catalyse a tangible shift toward adopting Next-Generation Risk Assessment (NGRA) approaches and bridge the existent gap between Human Health and Environmental risk assessment.While genomes for a vast collection of different organisms are already publicly available, there is still an overall lack of reliable functional annotation, often inferred from sequence-homology. In this perspective, protein families inferred on structural domains may offer a more relevant metric of relative functional conservation within a given pathway, than the individual gene level.Overall, the goal of this project is to provide scientific evidence to the process of functional pathway re-construction, thus reducing the uncertainty inherent to cross-species extrapolation. Using a novel pipeline for functional extrapolation of toxicity effects across species, here we present case-studies that enable the identification of relevant toxicity pathway-conservation levels across-species. We foresee that this approach will contribute in adding weight-of-evidence to support risk-based decisions using functional conservation of pathways across species and improving the confidence in how we can use data from multiple sources.

Wildlife Ecotoxicology: From Sub-lethal Responses to Adverse Effects at the Individual and Population Level

1.13.1

At risk: Preying on seals pushes killer whales from Norway above pollution effects thresholds

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Killer whales that feed on high-trophic prey are at a higher risk of adverse health effects from biomagnifying pollutants, such as polychlorinated biphenyls (PCBs) and mercury (Hg), due to increased exposure. Previous toxicological risk assessments for the Norwegian killer whale population have assumed fish as the sole prey source, and assessed the population as below established effect thresholds. However, some individuals have been observed to feed on higher trophic prey such as seals across multiple years and seasons. The aim of our study was to model intra-population dietary preferences in the Norwegian killer whale population, quantify differences in pollutant levels, and compare levels to threshold values for possible toxic effects. Our study is the first to quantify pollutants in seal-eating killer whales from Norway, to measure Hg levels in the Norwegian killer whale population, and to measure Hg levels in the skin of the killer whales. We collected biopsy samples from 38 killer whales, throughout all seasons, in 2017 and 2018 in northern Norway. We quantified the stable isotope ratios of carbon and nitrogen ($\delta13C$ and $\delta15N$, respectively) in skin, and with corroboration from field observations assigned individuals as seal-eaters or fish-eaters. Pollutant exposure was quantified by measuring organohalogen contaminant (OHC) levels in blubber ($n = 31$), and total Hg levels in skin ($n = 38$). The seal and fish-eating dietary groups had disparate and non-overlapping isotopic niches. Higher $\delta15N$ values in the seal-eating group, irrespective of sampling season, indicated year-round predation on seals. We found higher levels of all pollutants in the seal-eating than fish-eating killer whales. ΣPCBs of seal-eaters ($n = 7$, mean = 56 µg/g l.w.) were three times higher than fish-eaters ($n = 24$, mean = 17 µg/g l.w), and double as high as the levels previously used in risk assessments. Total Hg levels in seal-eaters ($n = 10$, median = 3.95 µg/g d.w.) were twice as high as in fish-eaters ($n = 28$, median = 1.79 µg/g d.w.). The higher ΣPCB levels in the seal-eaters pushed all sampled individuals over the threshold value of 10 µg/g l.w established by the Arctic Monitoring and Assessment Programme (AMAP) for a risk of immune and hormone system health. In contrast, only 46% of the fish-eating whales exceeded this threshold. We show that by feeding on higher trophic prey, the Norwegian killer whale population is at higher risk of health effects from pollution than previously assumed.

1.13.2

Mercury modulated immune responses in Arctic Barnacle goslings (Branta leucopsis) with viral challenge

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Due to historical coal mining activities and global transport, mercury has been detected in soil and vegetation at Arctic sites including Svalbard. Recent studies have demonstrated that developing Barnacle goslings grazing in a former mining area around Ny Ålesund, Svalbard, showed significantly higher internal hepatic mercury levels with adverse effects on brain receptor levels, behaviour but not on baseline immunity. However, little is known about the potential effects of mercury exposure in arctic goslings on their immune response upon viral infection. In the current study, 18 newly hatched (0 days old) barnacle goslings were collected at Indre Breøyane, an uncontaminated island near Ny-Ålesund. and randomly assigned to different treatment groups: a control group (herded in a control area with Actric background exposure levels), a mining group (herded in the old mining site, environmentally exposed to elevated mercury levels) and the additional mercury group (herded in the old mining site with extra mercury-containing additional feeding). Activation with Poly I:C, to induce anti-viral immune responses, was conducted on day-19 via intraperitoneal (i.p.) injection, 24h before termination of the experiment. Immune indicators including leukocytes populations, natural antibodies and nitric oxide levels were found to be influenced by mercury exposure even at low, environmentally relevant levels. Natural antibody levels declined with increasing mercury exposure, while nitric oxide levels only lifted significantly in the mining group and went back to the baseline levels in extra mercury group. Our results suggested that even at low environmentally relevant levels, mercury exposure can modulate the anti-viral immune responses in Arctic Barnacle goslings. These adverse effects are related to the dose although not always uni-directional. More details on the dose-response relationships will be provided in the presentation, but results indicate that exposure to low Hg-levels may lead to an affected host defense to viral challenges in Arctic Barnacle goslings at the individual level, which may cause increased susceptibility of infections.

1.13.3

Embryotoxicity of diluted bitumen applied to eggshells of wild double-crested cormorants and domestic chickens

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Breeding birds that become oiled may return to incubate their eggs, but even small quantities (≥1 mg) of conventional crude oils are embryotoxic when applied to the exterior of the eggshell. Therefore, we investigated whether an unconventional Alberta oil sands product, diluted bitumen (dilbit), was toxic to embryos of two species, domestic chickens (*Gallus gallus*) and wild double-crested cormorants (*Phalacrocorax auritus*). We incubated embryos in artificial egg incubators and applied various doses of lightly weathered dilbit (Cold Lake blend) ranging from 1 to 20 mg to the eggshells during early or late embryonic development. In both species, eggshell oiling with dilbit caused no significant decrease in embryo survival at sampling (80-85% embryonic development), and we found few effects on physiological endpoints related to growth, relative liver size (liver somatic index) and aerobic capacity. To investigate more subtle effects on liver function and metabolism associated with embryotoxicity, qPCR gene expression analyses on embryonic livers are underway and will be presented. We will also describe the physicochemical properties of the dilbit used in this study and its toxicity compared to similar studies with conventional crude oils. To date, this study indicates that even considerable amounts of lightly weathered dilbit transferred to the avian eggshell by incubating parents (up to 10-20 mg, approximately 9-19 µl) is unlikely to cause pre-hatch mortality in embryos of a domestic and wild avian species, although effects on gene expression are still under investigation.

1.13.4

Experimental analysis of the impact of the seeds treated with triazoles fungicides on the physiology and reproduction of the Red Partridge

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The agricultural intensification that has occurred since the middle of the last century and the use of agrochemicals are among the main risks for wildlife associated with these environments. The treatment of seeds with pesticides is an extended practice in the current agriculture. In particular, triazole fungicides are the most widely used products to treat cereal seeds, so there is a high risk of expsoure of granivourous species such as red-legged partridge (*Alectoris rufa*) that can consume those seeds that remain on the surface during sowing. Triazole fungicides can act as estrogenic disruptors altering the synthesis of hormones and therefore reducing the reproductive capacity of the partridges, which in the long run can be translated into a decline of their populations. This work aims to determine the mechanism of action of these fungicides on the partridges by analyzing changes in gene expression involved in the synthesis of sterols and in the levels of steroid hormones in exposed animals. To this end, we conducted an exposure test of adult partridges to treated seeds with four different formulations containing triazoles as active ingredients (flutriafol, protioconazole, tebuconazole, and a mixture of the latter two).We also tracked reproduction, analyzing clutch sizes and rates of fertilization and hatching of eggs. The results show that the all treatments produced biochemical changes and an overexpression of different genes related to the synthesis of sterols and steroid hormones. We detected adverse effects on reproduction, including a decrease in estradiol in partridges exposed to tebuconazole and a delay in the beginning of laying season in those exposed to flutriafol. These results demonstrate that the seeds treated with triazole fungicides have effects on different physiological and reproductive parameters of the red-legged partridge and is a factor to consider in the decline of birds in agricultural environments to propose measures for habitat improvement and possible amendments in current protocols of environmental risk assessment of pesticides **Keywords:** treated seeds, estrogenic disruptors, fungicides triazoles, *Alectoris rufa*.

1.13.5

Stress response gene expression markers and their relation to metal concentrations in blood of São Tomé endangered green sea turtles (Chelonia mydas)

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Due to their complex life, sea turtles are susceptible to changes in the environment. Metals are persistent in the environment leading to bioaccumulation processes that can have harmful effects on marine organisms. Through ecotoxicological studies, combining chemical contaminant analysis with physiological effects on the organisms, improved conservation and management actions of endangered sea turtles can be expected, which are crucial to maintain and monitor the ecosystem quality. São Tomé island harbours important sea turtle nesting and feeding grounds, however no study of metal contamination has been made in the archipelago. Thus, the aims of this study were to assess metal contamination on the blood of green sea turtles (*Chelonia mydas*) of ST island, identify suitable biomarkers of stress response and metal transport and/or detoxification and infer about potential impacts of metals on their population. The results suggest that almost 80% of metallothionein (*mt*) expression is significantly related with the metal levels (e.g. Hg and Cu) proving its function in protection and conjugation under metal contamination. Between the tested antioxidants, the best General Linear Model (GLM) found were for thioredoxin interacting protein (*tdx*) and catalase (*cat*) expressions which indicate that these could be the first line of defense against oxidative stress in this species – 52% of their expression variability is explained by metal contamination (mostly Cd for *tdx* and Cd*Hg for *cat*). The remaining antioxidants (*sod*, *txrd*, *gclc* and *selp*) presented similar values of expression variability that could be attributed to the presence of metals – between 40-50% - suggesting that these genes might be working together in the protection against metal contamination. Moreover, GLM models showed that some genotoxic effect might be occurring on this species due to metal contamination, since the model indicates that the appearance of micronuclei can be explained in 65% by metal contamination (e.g. Cu, Mn and Hg). Consequently, this study suggests that green sea turtles from São Tomé Island are responding to the oxidative stress and damage induced by metals through the increased expression of genes coding for antioxidants and metal transport/detoxification proteins. This study also identified possible candidate biomarker genes to be used in future biomonitoring studies of the marine environment using *Chelonia mydas* as metal pollution sentinel.

Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment

2.01.1

Glyceria maxima: Development of an OECD Test Guideline

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In 2014, a Workgroup affiliated to the SETAC Plant Interest Group was formed with the objective of developing a test guideline for assessing the effects of chemicals on the rooted, emergent macrophyte Glyceria maxima in a water-sediment system. Since this time, the workgroup has performed two ring-tests. The first ring-test was completed by 13 laboratories during 2016 and 2017 with the herbicide, isoproturon, and the primary objective of establishing the necessary test duration (i.e. 14 or 21 days). Results from this ring-test were used to adapt the test protocol in terms of plant propagation recommendations, test system specification, test duration (14 days), assessment parameters and draft validity criteria. The revised protocol was used in a second ring-test with the herbicide, imazapyr in 11 laboratories during the Summer/Autumn of 2018. The primary objective of this ring-test was to determine the feasibility of assessing effects on root growth. Results of this ring-test showed that shoot assessment parameters are more reliable than root endpoints and hence, the protocol has been further revised to remove root assessments. In April 2019, this work was accepted as an OECD Test Guideline project and preparations are underway for a further ring-test in 2020. Meanwhile, a workshop is planned for Spring 2020 to allow participants to share best practice for plant propagation and test conduct. This presentation will summarize data generated to date and discuss progress towards delivery of an OECD Test Guideline.

2.01.2

Ecotoxicity Testing of Water Plants under Flow-Through Conditions

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The investigation of ecotoxicological effects of compounds, for which the concentrations cannot be kept constant during the test, is either carried under semi-static or flow-through conditions. While testing of fish toxicity under flow-through conditions is quite standard, and also for invertebrates (e.g. daphnids) suitable systems have been introduced, growth inhibition testing of aquatic plants under flow-through conditions is still challenging. EFSA Technical report 2015:EN-924 requires that a test compound is still present in at least quantifiable levels at test end. In case of compounds for which the concentrations decrease significantly during the testing period, the sensitivity of the analytical method has to be improved or, more appropriate, suitable measures have to be taken in order to renew the test water continuously during the test to maintain the concentrations at a stable level. This presentation describes the growth inhibition testing of *Lemna gibba* and *Myriophyllum spicatum*, as representatives of mono - and dicotyledonous aquatic plant species, under flow-through conditions. In the case of *Lemna gibba*, the volatility and hydrolytically instability of the test compound did not allow the testing under semi-static conditions. For *Myriophyllum spicatum*, a water soluble and hydrolytically stable test compound was tested, but the presence of the compound in the system promoted bacterial growth leading to further biodegradation of the test compound and the substances was suspected to be used as energy source for the plant itself as well. In both cases (*Lemna* sp. and *Myriophyllum* sp. test) continuous water renewal by a flow-through system maintained the concentrations at a stable level. The system is based on flexible mechanical components, which are already successfully in use for acute and chronic fish and daphnids toxicity testing under flow-through conditions. By introduction of specifically designed vessels, the system can now also be used to fulfil the validity criteria as described in OECD 221 (*Lemna gibba*) and OECD 238 (*Myriophyllum spicatum*) and to maintain the concentrations of these difficult test compounds in the range of 80% to 120% recovery. Details in terms of test design as well as biological and analytical results will be presented.

2.01.3

A question of complexity: Investigating regime shifts caused by agricultural run-off and climate warming between aquatic plants and microalgae in micro- and mesocosms

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Shallow aquatic systems are frequent in agricultural regions. They often suffer from enhanced anthropogenic eutrophication and from organic or inorganic pesticides. Their good ecological status is a clear water state dominated by submerged macrophytes. Turbid water due to phytoplankton blooms represents a bad ecolog-ical state. However, regime shifts between both states occur depending on the level of disturbance acting on the system. Our French-German project CLIMSHIFT investigates how agricultural run-off (ARO) composed of nitrates, copper and organic pesticides in combination with climate warming affects such systems. We use indoor microcosm and outdoor mesocosm approaches to investigate at different spatial and temporal scale the impact of ARO on the first two trophic levels in these ecosystems. The microcosms were inoculat-ed with selected strains of planktonic and benthic algae and three different species of macrophytes as pri-mary producers, and Daphnia magna, Dreissena polymorpha and Lymnaea stagnalis as primary consumers. The mesocosms received inoculates of phytoplankton, periphyton and zooplankton from a natural lake, and otherwise the same organisms as used in the microcosms. Microcosms ran for 4 weeks after ARO addition, mesocosms for 8 weeks. Temperature was 22 and 26°C for the microcosms, and ambient +3°C for the mesocosms. Microcosms received one high dose of ARO ('3X'), with or without nitrates, while we used a gradient approach between 0 and the high dose of ARO in the mesocosms. In the microcosms, ARO caused a strong algal bloom about 1 week after addition, and only towards the end of the experiment, Daphnia populations slowly developed. The size of L. stagnalis was also smaller in ARO treatments, causing less grazing on macrophytes and periphyton. Myriophyllum spicatum grew better in the controls than in the ARO treatment, and this effect was more pronounced at higher temperature. In the mesocosms, an effect of ARO was observed mostly in the ambient temperature treatments, where phytoplankton development increased with ARO level, while effects were random in the heated mesocosms. M. spicatum grew more slowly in the higher ARO treatments, and flowering was delayed. Snails were also smaller in the higher ARO treatments, both at ambient and heated conditions. In our presentation, we will outline the major results from both experiments and conclude on similarities and differences, and reasons for this due to upscaling from indoor microcosms to outdoor mesocosms.

2.01.4

Based on field data, is the current standard risk assessment for NTTPs at the EU-level sufficiently protective?

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The current European risk assessment (RA) for no-target terrestrial plants (NTTP) is based on two OECD test guidelines (208 and 227) (or the corresponding OCSPP guidelines) typically performed with ten plant species each. Under Regulation 1107/2009 the lowest ER50-endpoint of both tests and all test species is the basis for the RA, considering an assessment factor of 5. The procedures for testing and assessing Plant Protection Products (PPPs) on NTTPs are currently under discussion, and a number of fundamental changes are proposed that would lead to a more conservative initial RA, and consequently also to more frequent needs of higher-tier testing, including reproductive endpoints and field-testing. In order to address the question whether the current EU standard risk assessment for NTTPs is sufficiently protective, we matched existing higher-tier endpoints with the regulatory relevant rates (RARs) defined by the current European risk assessment procedure. Fundamentally, Risk quotients were calculated dividing the RAR by the field NOER endpoints, specifically including (but not restricted to) reproductive parameters. Any field NOER higher than the RAR indicates that the current standard RA was protective (RQ < 1). Based on the datapairs evaluated so far (work ongoing), the vast majority of cases indicate that, at exposure levels considered safe based on the current standard RA (the RARs), no effects were observed in the field. Often these test rates were even equivalent to "no effect concentrations" (i.e. EC0). There are a few exceedances, which will be discussed. Decreasing numbers of plant species have been recorded from many European edge-of-field habitats, and corresponding reductions in biodiversity can no longer be ignored. Many authors observerd a correlation with pesticide use, and have postulated a causal realtionship. This is however in contrast to the field data assessed here, where generally no adverse effects on populations were observed at exposure levels considered safe based on the current standard RA in the EU. The implications of these seemingly contradictory findings will be discussed.

Bees, Bugs and Beneficials in Environmental Risk Assessment and Testing

2.02.1

Effect of agricultural landscape structure on response of the two consecutive generations of the ground beetle Poecilus cupreus to Proteus 110 OD insecticide

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The widespread use of pesticides and changes in landscape structure leads to the loss of the biodiversity. During SETAC Europe 2019 we showed that field collected (F0 generation) beetles (*Poecilus cupreus*) from large oilseed rape monocultures exhibit increased resistance to Proteus 110 OD insecticide (active ingredients: Thiacloprid and Deltamethrin). This raised the question if the resistance is a result of short-term acclimation or adaptation. Here, we investigated the effect of agricultural landscape structure on the response of *P. cupreus* lab-bred F1 generation to Proteus 110 OD and compared the resistance in F0 and F1. The F0 beetles were collected from landscapes dominated by small- (S) or large-scale (L) farming. In both landscapes there were 3 areas with 3 sites each: with small oilseed rape coverage (ORC; 10-14%; SS), medium ORC (20-52%; SM) and reference with no ORC (S0) in S landscape and with medium ORC (28-33%; LM), large ORC (80-98%; LL) and no ORC (L0) in L landscape. Some beetles were used for F0 resistance test and the remaining were bred to obtain F1 generation. Individuals in each generation were exposed to a single topical application of 1 µl of the insecticide or control solution applied on scutellum with a Hamilton syringe with repeater. The pesticide commercial formulation was dissolved in acetone to obtain the concentration equivalent to 0.2 recommended dose for field use. Statistical analysis revealed significant differences in beetles survival ($p < 0.001$) both in F0 and F1. Median survival times (LT50 in days) in F0 populations from meadows S0 (LT50=7.3±4.42 SE) and L0 (10.3±1.97 SE) did not differ from each other ($p=0.5$). Similarly, no difference between S0 and L0 populations was found in F1 survival ($p=0.1$) but the LT50s were generally much shorter: S0 LT50=0.4±0.97 SE and L0 LT50=0.25±0.09 SE. However, F0 beetles from LM (23.3±1.64 SE) and LL (21.3±3.41 SE) and F1 beetles from LM (11.0±0.77 SE) and LL (8.0±1.37 SE) survived significantly better than any other group compared to reference habitats. Our results indicate that *P. cupreus* beetles from habitats with medium and high ORC exhibit some sort of resistance to the used insecticide, but only if they live in a large-scale agricultural landscape. To confirm that the resistance is inheritable, the test will be repeated on F2 generation. Studies supported by National Science Centre, Poland (2016/23/N/NZ8/01679) and Jagiellonian University in Kraków, Poland (DS/WBiNoZ/INoŚ/759/2018).

2.02.2

Can flower strips mitigate negative effects of a mixture of plant protection products to honey bees in a semi-field approach?

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Honey bee health has been reported to be affected by multiple factors including food resource quality, exposure to agrochemicals and pressure of pests and pathogens. Currently, concerns are growing about potential interactions between multiple stressors and their impacts on bee health. Maize pollen are considered to have low nutritional value for honey bees and is mostly collected by bees during late summer in some specific landscape according to shortage of other flowering plants. In the current study, the interaction between different nutritional pollen sources and/or exposure to plant protection products (PPPs) and their effects on honey bee health were investigated. A semi-field study with sixteen tents with different pollen quality for honey bees were realized: maize with less pollen quality and tents planted with 50% maize and 50% flower mixtures with diverse pollen qualities. Additionally, the variants were sprayed with a tank mixture of the fungicide Prochloraz and the neonicotinoid Thiacloprid. Various parameters were investigated such as mortality of adults, larvae and pupae, activity of detoxification enzymes, weight and longevity of newly emerged bees. Newly emerged bees from colonies foraged in tents with untreated maize and a diverse flowering strip show higher survival rate than bees with untreated monotonous maize diet. The application of a PPP mixture reduced the survival rate of emerged bees in both variants. There were no statistical differences in the longevity between bees from colonies in the untreated maize and bees from colonies foraged in treated tents contained maize and a flowering strip. Activity of the primary detoxification enzyme P450 reductase was higher in the abdomen of newly emerged bees compared to larvae. After application of a PPP mixture, bee larvae origined from tents contained flower strips in addition to maize showed higher enzyme activity than bees from tents contained only maize. Our study shows for the first time that a diverse diet due to flower strips may have positive effects (longevity and physiological) on honey bee colonies compared to a poor monotonous diet with maize under semi-field conditions. This effect can also be seen in colonies exposed to a mixture of the fungicide Prochloraz and the neonicotinoid Thiacloprid. Development of flowering resources in agricultural fields, especially in late summer, may be an effective approach to maintain bee health and their response to PPPs.

2.02.3

A feeding study with a sublethal neonicotinoid dosage: observed effects by

conventional assessment methods and a novel AI based visual monitoring technology.

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Feeding experiments are standard tools in pollinator risk assessment. The Oomen study design was developed to test insect growth regulators and herbicides. In recent years, the outline of the study design has been adapted, in order to allow the testing of different dosages in the same environment. With its help, insights into the cause-and-effect relationships of different concentrations can be used to determine the best dosage, thus reducing the uncertainty of risk to pollinators. The main objective of the presented experiment was to determine the real variability of forager losses of hives fed with a sublethal neonicotinoid concentration compared to an untreated control group. Additionally, the hive monitoring system assessed other observable sublethal effects as a result of neurotoxic exposure. Lastly, the correlation between the monitored forager loss and the manual hive development assessment was evaluated. The study setup consisted of a treated group of 4 hives and a control group of 4 hives. Over a ten-day period, the treated group was fed a 500 g sugar solution containing 200 µg of imidacloprid/kg. The control group was fed plain sugar solution. As is customary, dead and moribund bees were collected in dead bee traps to determine the number of bees killed. Meanwhile, the colony strength was estimated, changes in behaviour were observed and the hive weight was recorded. Aiming to evaluate the correlation between the actual total daily loss of bees and the results of the manual assessment, we used a novel AI (Artificial Intelligence) based visual bee monitoring system. The technology developed by apic.ai analysed video footage of the hive entrance to deduce the daily losses of foragers, the start and end times of foraging activities as well as temporal changes of activity. The latter function will be used to measure sublethal effects. Our results will display the data from the feeding period as well as the four months following exposure.

2.02.4

Fungicides, herbicides and effects on bees: where are the gaps?

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Plant protection products (PPPs), including insecticides, herbicides and fungicides, are used widely in modern agriculture. Although designed to deal with pest, disease and weed problems, they can also come into contact with beneficial organisms in the environment such as bees which may be providing pollination services to crops. In recent years, there has been a focus on the impacts of insecticide use on bees and although fungicides and herbicides are more widely used little is known about the potential impacts of these groups of PPPs on bees. Here, we review the research methods and approaches used in the existing literature on the potential impacts of fungicides and herbicides using a systematic review. We find that the majority of studies have been carried out in Europe and USA, with little from elsewhere globally. Must work has focused on mortality and sub-lethal effects in honeybees and investigation into effects on other bee species are lacking. We suggest a number of areas for further research to improve the knowledge base on potential effects of herbicide and fungicide use on bees, to inform sustainable use of these compounds and improve risk assessment. This work is part of the Irish PROTECTS project (Protecting terrestrial ecosystems through sustainable pesticide use),which will also be discussed in the context of ongoing work within this project.

2.02.5

A comparative analysis of active ingredient and commercial formulated glyphosate on the brain and gut proteome of the bumblebee Bombus terrestris.

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Pollinating insects provide vital services for food crop production and ecosystem functioning; however, many species are currently in decline in abundance and diversity around the globe. Drivers of this decline include disease, habitat loss and pesticide use. Although significant research on the impacts of insecticides exists little is known about the effects of non-insecticidal pesticides, such as herbicides and fungicides, on insect pollinators. To address these gaps, the PROTECTS (Protecting Terrestrial Ecosystems Through Sustainable Pesticide Use) project was established to provide baseline information on understanding and mitigating the effects of pesticide use on terrestrial ecosystem services, focusing on *Bombus terrestris* as a model insect pollinator. Using mass-spectrometry based proteomics, we characterized glyphosate effects on the neuronal and digestive systems of *B. terrestris*, to determine whether cellular and physiological processes are altered at the molecular levels. In addition, we investigated whether differences in effect occur when glyphosate was used alone (active ingredient; AI) or as part of a commercially sourced formulation (hereafter CF-A). Chronic exposure over 5 days to AI and CF-A resulted in changes in protein abundances for both brains and digestive tracts (DTs). Higher numbers of differentially abundant proteins were identified in brains compared to the DTs although the range of relative fold change was greatest in DTs. Both AI and CF-A resulted in the differential expression of core sets of proteins highlighting a glyphosate specific response/effect regardless of source. However, key differences were also identified suggesting that the adjuvants found in commercial formulations may also alter the bumblebee proteome. Gene Ontology analysis resolved proteins associated with metabolism that were affected by both AI and CF-A exposure in the brain. In the digestive tract, metabolism, cellular organization and stress-responses are most affected in AI-exposed bees whereas metabolism and some stress-responses were observed in CF-A exposed bees. This study demonstrates the potential for proteomics to assess pesticide effects on non-target organisms where no known mode of action has been established. Furthermore, we highlight potential issues with using AI pesticides alone when assessing potential effects, as many pesticides are applied as part of commercial formulations which contain other ingredients potentially toxic to insects.

2.02.6

A multibiomarker approach to evaluate the impact of anthropogenic contaminants on the ecotoxicological status of honey bees, Apis mellifera

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A rapid decline of Apis mellifera, a keystone pollinator of wild plant species and agricultural crops, was recorded worldwide in recent years. The massive use of insecticides and fungicides in agriculture associated with pollution generated by other human activities and presence of parasites can cause toxicological effects in bees including a decrease of the immune defences, leading to the collapse of the colonies. Effective assessment of the ecotoxicological impacts of anthropogenic contaminants requires an approach that combines different biomarkers that enable a more precise diagnosis of exposure to environmental stressors through a combination of different biological responses. The aim of this study was to develop and apply a set of biomarkers to study the ecotoxicological status of honey bees. In the first phase, we investigated in the laboratory the effects of EMS, cadmium and a commercial fungicide (azoxistrobin 18.2% and ciproconazole 7.3%) in adult honey bees, evaluating eventual variation in glutathione S-transferase (GST), carboxylesterase (CaE), acetylcholinesterase (AChE) activities, alkaline phosphatase (ALP), lysozyme, erythrocyte nuclear abnormalities (ENA) assay and differential haemocytes count (DHC). Genotoxic effects, as well as alteration of the immune system, were found in bees treated with EMS, cadmium or the fungicide. Cadmium and the fungicide also inhibited AChE and CaE activities, GST was induced by all the compounds investigated. In the second phase, adult honey bees were collected from apiaries located in four environments characterized by different chemical input: a wooded environment (low input), an urban site, an orchard and a cultivated countryside site. Honey bees from the urban site were also collected and analyzed before and after treatment for parasites. ENA assay showed that bees taken from the countryside and the orchard had a greater number of abnormalities compared to the forest, confirming the presence of genotoxic substances in agricultural environments compared to control environments. GST activity was induced in bees from the urban environment, AChE was inhibited in the countryside compared to the forest, suggesting the presence of substances with neurotoxic effect in this environment. ALP activity was induced in all sites in comparison to wooded one. The bees collected after the parasites treatment showed an increase for GST activity as well as AChE inhibition.

Contaminants in Highly Exposed Wildlife: Interactions of Contaminants, Climate Change, and Other Environmental Stressors

2.03.1

Landfills represent significant atmospheric sources of exposure to halogenated flame retardants for urban-adapted gulls

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A large suite of halogenated flame retardants (HFRs) including polybrominated diphenyl ethers (PBDEs) and certain emerging HFRs have been determined in tissues of ring-billed gulls (Larus delawarensis) nesting in the Montreal area (Quebec, Canada). More specifically, elevated concentrations of the highly hydrophobic DecaBDE were reported in ring-billed gull plasma, and spatial tracking showed that these concentrations in males were correlated with the time spent in specific foraging habitats such as landfills. Landfills are known hotspots of emissions for HFRs, which are efficiently transported in the atmosphere. Hence, gulls feeding in and around landfills may be exposed to these chemicals via dust and particle ingestion and inhalation. However, no method has yet been developed to measure this type of exposure. Therefore, we recently designed a miniature passive air sampler that can be carried by gulls and collect gas- and particle-phase HFRs in air. The present study aimed to a) quantify HFRs in air that ring-billed gulls are exposed to when breeding in the Montreal area, b) to model the contribution of landfill use on the daily sampling rates of HFRs in the samplers, and c) to localize the sites that contributed the most to the atmospheric exposure to HFRs at the regional scale. We equipped 67 nesting gulls with a miniature passive air sampler and a GPS datalogger in order to collect HFRs in air while monitoring their movements outside the colony during two weeks. HFRs were extracted from the sorbents of the samplers (polyurethane foam and glass fiber), and were identified and quantified using a gas chromatograph coupled to a single quadrupole mass spectrometer. The major PBDE congeners determined in the samplers were characteristic components of the commercial mixtures PentaBDE (BDE-47 and -99) and DecaBDE (BDE-209). Also, a few emerging HFRs (hexabromobenzene, Dechlorane-604 CB, and Dechlorane plus) were detected, although at lower levels. The daily sampling rates of PBDE mixtures were positively correlated with the presence probability of gulls in landfills, but not the emerging HFRs, which suggests alternative sources for these chemicals. This study showed that landfills represent major environmental sources of atmospheric exposure to PBDEs for birds and potentially other mobile wildlife that use these sites to forage on predictable energy-rich human food resources.

2.03.2
Herring gull and common eider as indicators of contaminants in an urban fjord
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Seabirds, such as the herring gull (Larus argentatus), are commonly used in environmental monitoring as indicators of contamination high in the food web. The herring gull is a generalist predator, which in urban areas feeds from terrestrial and anthropogenic sources in addition to the marine food web, possibly introducing a weakness in the use of herring gull as marine indicator species. In this context, we compared contaminant concentrations and dietary markers in herring gull and a seabird with a different ecology, the exclusively marine-feeding common eider (Somateria mollissima), in an urban area. The measured contaminants included legacy lipophilic organohalogen contaminants (OHCs), such as the polychlorinated biphenyls (PCBs) and polybrominated biphenyl ethers (PBDEs), emerging non-lipophilic OHCs such as the per-and polyfluorinated substances (PFASs), and the element mercury (Hg). Ecological nice was assessed using stable isotope (SI) ratios of carbon and nitrogen ($\delta 13C$ and $\delta 15N$). Our aim was to investigate how well blood and eggs of the two species function as indicators of contamination in the urban marine food web of the Inner Oslofjord, Norway. The hypothesis of common eider belonging to the marine food web, and herring gull using more terrestrial food sources was confirmed in the SI analysis. We found higher concentrations of lipophilic OHCs and Hg in blood of common eider than of herring gull, which contrasts studies from more remote areas. For the PFAS however, there was no clear species difference, possibly reflecting distribution in the urban environment. Egg samples gave opposite results; higher concentrations of lipophilic OHCs in herring gull than common eider, which we linked to fasting during breeding in the eider. We suggest that in urban areas, herring gull might not be suited as an indicator of contamination in the marine food web, but it can serve as an indicator for the urban ecosystem. Common eider can be used as a marine indicator, but because of the breeding strategy, results must be interpreted carefully.

2.03.3
Poster spotlight: Effect of agricultural management on males' reproductive status in Iberian hare (Lepus granatensis)
R. Mateo Soria, IREC (CSIC- UCLM) / Grupo de Toxicología de Fauna Silvestre
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2.03.4
Poster spotlight: Time trends of persistent organic pollutants and mercury in Canadian Arctic ringed seals and relationships with climate parameters
M. Houde, Environment and Climate Change Canada / Aquatic Contaminants Research Division
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Ecosystem Functions and Services: Understanding and Managing Anthropogenic Impact

2.04.1
Ecosystem functions and services of the land under municipal dumpsite impact
E. Kovaleva, M.V. Lomonosov Moscow State University / Department of Soil Science
The biosphere, especially soils plays a regulatory role and maintains the environmental parameters in a range, suitable for human survival. In terms of economic principles and natural resource management, environmental functions turn into goods and ecosystem services, giving multiple benefits to humans. The threat of a global crisis in ecosystems necessitates to work on development of strategies to establish optimal relation between human and nature. The aim of our work was to assess the quality of soils and associated aquatic environments in the area adjacent to the solid waste dumpsite, to analyze the ecological functions and ecosystem services in the area, to trace the relations between ecological functioning and risk assessment. We used sod-podzolic gley and peat soils, developing along the creek; water and sediments from the creek under impact of municipal solid waste dumpsite in Moscow region, Russia. Soil, water and sediment samples were studied by marker indicators in line with the filtrate composition of dumpsite. Coefficient Zc was calculated as the sum of the ratios of contaminants concentrations to their background. A battery of biological parameters was applied: phytotoxicity of garden cress and barley, E. foetida earthworm growth rate and mortality; enzyme activities soil respiration parameters. The toxicity of water was done using crustaceans and infusorians. The soil performs as an acting regulator of water quality, environmental functions - protective and buffer biogeocenosis barrier influencing the ecosystem services. Thus, the hydrospheric function provides the ecosystem service – regulating and provisioning at the same time for human by supplying clean soil and water. The contamination at Zc 40, is the level of drastic changes in hydrospheric and biocenotic functions in the soil studied. The risk for aquatic ecosystem was assessed as high, decreasing the quality of water in creek, supplying water to a main river. The municipal waste dumpsite impacting the environment, leads to changes in regulating functions that should be managed to maintain the environment biodiversity and physico-chemical state. Risk assessment could be one of the instruments allowing to determine the loss of ecological functions and services. The economic interpretation of the soil function and ecosystem services was done, based on the parameter assessment of the soil, water, sediment quality degradation, lost benefits and their recovery (US$ 10 078 ha-1).

2.04.2
Functional traits link anthropogenic impact and disturbance regimes driving ecosystem function in a floodplain wetland complex
N. Rideout, Canadian Rivers Institute / Biology; Z.G. Compson, Environment and Climate Change Canada / University of New Brunswick; W.A. Monk, Environment & Climate Change Canada / University of New Brunswick; M. Hajibabaei, University of Guelph / Centre for Biodiversity Genomics, Department of Integrative Biology; T. Porter, Natural Resources Canada / University of Guelph; M. Wright, University of Guelph / Centre for Biodiversity Genomics; D.J. Baird, Environment and Climate Change Canada / Canadian Rivers Institute
Floodplains are disturbance-driven ecosystems with high spatial and temporal habitat diversity, making them both highly productive and hosts to high biodiversity. The unpredictable timing of flood and drought years creates a mosaic of habitat patches at different stages of succession, while water level fluctuation directly influences macrophyte community dynamics, and thus habitat structure. This habitat complexity and diversity of disturbance regimes makes floodplains an ideal ecosystem in which to examine the links between biodiversity, traits and ecosystem function. With up to 90% of floodplains in North America and Europe altered to the point of functional extinction, it is particularly imperative to study and conserve those that remain intact, such as the Lower Saint John River and its associated floodplain, including the Grand Lake Meadows and Portobello Creek wetland complex. Despite the rise in trait-based science, taxonomic resolution has imposed limitations, especially in wetland and floodplain ecosystems where communities are vastly understudied compared to their riverine counterparts. Compared to traditional biomonitoring, DNA-based biomonitoring from high-throughput genomics sequencing methods is powerful in that it can reliably characterize community composition in unprecedented detail, allowing us to assess how disturbance and environmental filters interact with invertebrate traits and ecosystem function. Using structural equation analysis, we take a whole ecosystem approach to examine ecosystem health across a floodplain disturbance gradient. We focus chiefly on how anthropogenic alteration within watersheds affects downstream floodplain wetlands, how the resulting patch diversity shapes communities and, finally, how those communities influence ecosystem function through trait diversity metrics. We also examine and compare which traits are associated with crucial ecosystem gradients.

2.04.4
Disentangling multiple stressor pathways in a highly complex deltaic ecosystem
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Freshwater deltaic systems are highly dynamic, diverse ecosystems connecting

terrestrial, riparian, subsurface and aquatic habitats hosting high levels of biodiversity. As the world's largest inland delta wetland complex, the Peace-Athabasca Delta (PAD) is subject to encroaching development including upstream oil sands mining operations, hydroelectric development and associated infrastructure in addition to regional climate change. These potential drivers have triggered multiple science and monitoring projects led by provincial and federal governments, Indigenous Peoples, and academics to assess and quantify their potential impacts. Here we present an evidence-based framework to explore direct and indirect pathways within a novel multiple stressor framework within the PAD derived from the OECD Driver-Pressure-State-Impact-Response (DPSIR) framework. The mechanism-based framework is supported by ongoing high-resolution ecosystem observations paired with historical records, Indigenous knowledge and geospatial data. We clearly demonstrate the power of adopting a systematic framework to advance quantitative assessment of ecosystems affected by multiple drivers, pressures and stressors across multiple scales including identifying novel disturbance pathways within the PAD that could be used for further scientific inquiries. Our results also highlighted the broader applicability of adopting the proposed framework for assessing complex environments and the strength of the approach for supporting the development of targeted management and monitoring strategies.

2.04.5
Assessing chemical risk within an ecosystem services framework: proof of concept case studies
L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences; P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences; R. Brown, University of Exeter; S. Marshall, Consultant
Ecosystem services (ES) are the direct and indirect contributions that ecosystems make to human well-being. The ES concept is gaining broad interest in regulatory and policy groups for use in landscape management and ecological risk assessment (ERA). A recent multi-stakeholder evaluation of the use of an ES approach in guiding chemical environmental risk assessment has identified clear advantages of using the approach, but also highlighted a number of challenges related to its implementation [1,2]. Here we evaluate the feasibility of adopting an ES approach to chemical risk assessment using current knowledge and tools. Three regulatory-relevant proof of concept case studies have been undertaken, each co-designed by representatives of the chemical industry, policy/regulatory agencies and academia. (i) Prospective risk assessment for a plant protection product relevant to Regulation 1107/2009. Organophosphate insecticide applied to a apple orchard twice a year. Focus on ES provided within the orchard and immediate boundary area. Prioritized ES are soil quality, pest control, pollination and recreation. (ii) Prospective risk assessment for a general chemical relevant to REACH. Laundry surfactant disposed via domestic wastewater. Focus on freshwater ES provided by the receiving water. Prioritized ES are water quality, recreational fishing, observing nature. (iii) Retrospective risk assessment relevant to the Water Framework Directive. Metal-contaminated water body. Focus on freshwater ES and prioritized ES are water quality, recreational fishing, observing nature. Population and food web models have been used to extrapolate data from single species laboratory toxicity tests to more ecosystem service relevant endpoints (i.e. population abundance, activity). Evidence-based logic chains have been developed to link chemical-induced changes in abundance/activity to changes in ecosystem service provision. Ecological production functions have been reviewed and applied where appropriate. The outcome of ERA within an ES framework has been compared to those based on current regulatory ERA frameworks. [1] Maltby et al (2018) Advantages and challenges associated with implementing an ecosystem services approach to ecological risk assessment for chemicals. Sci Tot Environ 621: 1342-1351 [2] Faber et al (2019) Priorities and opportunities in the application of the ecosystem services concept in risk assessment for chemicals in the environment. Sci Tot Environ 651: 1067-1077

2.04.6
Towards a holistic method deriving specific protection goals based on ecosystem services
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According to Regulation 1107/2009, the agronomic efficacy of plant protection product (PPP) must be demonstrated, while demonstrating through risk assessment (RA) that PPP's can be used with no unacceptable side effects on non-target organisms. The purpose of the regulation is to balance two competing objectives: an appropriate high level of protection for humans, animals and the environment, and enabling products enhancing agricultural productivity to be placed on the market. This inevitably creates numerous trade-offs. To address at least some of these, the PPP Regulation came along with the release of the Sustainable Use Directive (DIR 2009/128/EC). General aspects of future agricultural strategies are laid out in the Common Agricultural Policy (CAP). Unfortunately, this complexity tends to abet confusion about the scopes and purposes of the different legislations and policies in scientific and public discussions. Currently, there is activity at EU level to develop a method that allows identifying specific protection goals (SPG) for RA in the PPP registration process. In 2016, EFSA guidance was published on the development of SPG, relating them to ecosystem services (ES) and biodiversity. EFSA is legally restricted to the RA perspective, therefore their proposal was not able consider the agronomic context in which the SPG's should become operational. However, for compliance with the overall purpose of 1107, i.e. balance of agronomy and environment, it is crucial to embed the SPG development process into the agronomic context. Here we present and discuss a method proposal to expand the initial EFSA approach to integrate the agronomic perspective with examples from non-target terrestrial plants. Basically, we propose to include 2 additional steps. First, identification of trade-offs and resilience factors regarding the identified ES and their service providing units. Second, the output of the trade-off discussion should be ranked according to ecological, agronomical and legislative relevance. The latter considers also the wider agricultural policy beyond the pure PPP Regulation as well as conservation frameworks. This approach facilitates developing SPG's for the PPP registration process paralleled by identification of the relevant legislative frameworks for those ecologically important aspects that are not in scope of 1107. This paralleled approach may strengthen future improvements of a sustainable and productive European Agriculture.

Embedding Ecological Concepts in Ecotoxicology: Processes, Populations and Communities

2.05.1
Responses of invertebrates in streams to pesticides: review of field studies and conclusions for risk assessment
R. Schaefer, University Koblenz Landau / Institute for Environmental Sciences
Pesticides encompass a variety of chemicals that are deliberately released into the environment to control agricultural pests. Before pesticides are placed on the market, they undergo a complex authorisation procedure that aims to prevent unacceptable effects on ecosystems. The authorisation largely relies on data from laboratory and semi-field studies. In the first part of my presentation, I review field studies on the effects of pesticides on macroinvertebrates in freshwater ecosystems. Field studies were the key in establishing evidence that despite a complex pesticide authorisation procedure, effects of agricultural pesticide use (mainly insecticides and fungicides) on macroinvertebrates are widespread. Of 13 field studies in different regions of the world in the last 15 years, nine found a clear or likely relationship between pesticide toxicity or concentrations and biotic responses such as community composition or different indices. I discuss the problem of establishing causality based on field studies and governmental monitoring data. In the second part, I provide an overview of ecological processes that moderate the action of pesticides in (linked) ecosystems that need consideration if the aim is to prevent biodiversity loss in these systems.

2.05.2
Hot, dry and deadly: Climate warming and imidacloprid pulses determine macroinvertebrate community dynamics in experimental streams
S.J. Macaulay, University of Otago / Department of Zoology; K.J. Hageman, Utah State University / Chemistry & Biochemistry; J.J. Piggott, Trinity College Dublin / Department of Zoology; N. Juvigny-Khenafou, Xi'an Jiaotong-Liverpool University / Department of Environmental Science; C.D. Matthaei, University of Otago / Department of Zoology
Sustainable management of insecticide use in the context of climate change is an important issue for environmental managers to address. Mesocosm experiments are a valuable tool for assessing the impacts of contaminants in realistic experimental systems, especially in combination with other stressors that are not easily manipulated *in situ* such as those associated with climate change. Further, by including biotic interactions and providing the opportunity to investigate effects at the community and ecosystem-levels, mesocosms represent an ideal method for integrating ecological principles into ecotoxicological research. We performed a seven-week streamside channel experiment using 128 flow-through circular mesocosms to test the effects of pulsed imidacloprid exposure (at four environmentally relevant levels between 0-4.6 µg/L) and raised water temperature (ambient and 3°C above) on stream macroinvertebrate communities representative of fast and slow-flowing microhabitats. Invertebrate drift and insect emergence were monitored during three pesticide pulses (ten days apart) and during the 48 hours immediately following the first two pulses. Benthic invertebrate communities in the entire mesocosms were sampled after 24 days of heating and pesticide manipulations. All three manipulated factors strongly affected invertebrate drift community composition, with the first pulse of imidacloprid and increased temperature having a greater impact on communities in fast-flowing channels. Heating and imidacloprid exposure both generally resulted in increased emigration by drift. Increased temperature was the most pervasive stressor for the benthic invertebrate community, negatively affecting 80% of response variables. The combined effect of stressor manipulations and an intense, natural heatwave drastically reduced relative abundances of EPT (mayfly, stonefly and caddisfly) and insects overall and caused a shift to oligochaete, crustacean and gastropod-dominated communities. These findings demonstrate the potential impacts of heatwaves on freshwater macroinvertebrate communities under future climate scenarios and reveal which invertebrate taxa will be most at risk from the

combined effects of reduced flows, raised water temperatures and imidacloprid contamination. By allowing the assessment of contaminant effects at the community and ecosystem-levels, this study shows how mesocosm experiments can be a valuable tool for integrating ecological concepts into ecotoxicology.

2.05.3

Integrating field indicators of contamination in structural equation modelling to understand the effects of multi-pressures in freshwater

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Nowadays ecotoxicological indicators and ecological concepts are more frequently associated into one study, providing a quantification of multiple pressures effects, including chemicals, and an evaluation of communities' state. For instance, in Pressure-Impact approaches, used in ecology, ecotoxicological pressures are considered. However, these measures of chemical pressures do not always consider the bioavailable fraction of a contaminant (for example, measurements in water or sediment matrices). Active biomonitoring, based on caging transplanted organisms in freshwater sites, takes into account the exposure of organisms. Amphipods like gammarids, enabled a standardised measure of bioavailable contamination. To determine the state of communities, we need further researches on the effects of multiple pressures (including bioavailable contamination) on freshwater communities. Therefore, the links between chemical contamination and its determinants should be quantified in order to study the effects of multiple pressures on communities. A dataset with 196 samplings of metallic contamination (for eight substances) in gammarids was established in watercourses at the national scale of France. Each sampling of contamination was geographically associated to its determinants (including pressures). A Structural Equation Model (SEM) was applied. SEM enables to confirm causality between processes after emitting hypotheses of effects. These hypotheses were validated by the quantification of the effects of determinants on metallic contamination. Direct, indirect and total effects were investigated to determine the main contributors to metallic contamination. This approach shows how to integrate bioavailable contamination in a multiple pressure context. The outputs also present the major effects of pressures like anthropic land cover, industries and Wastewater Treatment Plants. The tested concepts and field measures used in this approach could serve as a first base to study the links between multiple pressures and freshwater communities.

2.05.4

Mainstreaming biodiversity in European field crop production

A. Dollacker, Bayer Ag / Regulatory Policy

Biodiversity change and loss has led to increased attention and efforts to protect and conserve biodiversity. In Europe, the Nature Directives protect habitat and species (e.g., birds), while e.g., the EU Common Agricultural Policy (CAP) addresses biodiversity conservation on cropland. More recently technology regulations include biodiversity policy protection goals. To conserve biodiversity on cropland the EU CAP's Agri-Environmental Schemes (AES) support the application of good agricultural practices (GAPs), and the provision of non-crop habitat. The integrated application of the above efforts are decisive for the conservation of biodiversity as they support the establishment of broader networks of habitat areas at landscape level. However, biodiversity still declines and the outcomes of the AES have been questioned critically. In an attempt to better understand the benefits that can be derived from these two practical approaches, this paper links GAPs and non-crop habitat creation to agro-ecosystem services. While GAPs have been applied for centuries as a crop management tool, their benefits for functional or associated biodiversity have not been widely elaborated on in terms of ecosystem services language. Similarly, while the benefits of non-crop habitat creation for biodiversity within fields have been well documented, their benefits to crop management and crop profitability have been poorly explored in the EU. We suggest that the enhanced application of GAPs and the provision of non-crop habitats constitute practical implementation tools to mainstreaming biodiversity, while contributing to achieving more resilient crop production over time and scale. With regard to habitat creation we analyse which aspects could be improved to increase their implementation and suggest that key prerequisites need to be addressed to yield more benefits. These include: (a) increased research in identifying the best suited preferably multi-species, non-crop habitat/measures for use within fields (subfield areas and field edges) (b) increased research to highlight the benefits of non-crop habitat to crop production, and (c) the identification of priority landscape types (e.g., simplified landscapes) for the creation of multi-species, non-crop habitats. In addition to these practical implementation approaches we agree with other authors that practical biodiversity mainstreaming in crop production has to be supported by congruent regulations and policies, which need to support the broader landscape management plan and with it the broader nature protection agenda called for by society and policy makers worldwide.

2.05.5

Predictive Modelling of Metal Mixture Toxicity to Daphnia magna Populations

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Current practice of environmental risk assessment lacks ecological realism, because it depends mostly on toxicity of single substances to individual organisms. It is desirable to develop mechanistic, predictive models that take mixture toxicity on higher levels of organization into account. We conducted a population experiment with *Daphnia magna* exposed to Cu-Ni-Zn mixtures and the single metals, in order to calibrate a Dynamic Energy Budget Individual-Based Model (DEB-IBM) with single-metal population data and generate blind predictions on mixture toxicity. Metals with different physiological modes of action (PMoA) can be implemented independently in the DEB-IBM, without making further assumptions concerning mixture toxicity. For metals with the same PMoA, we assume no interactions between metals.We first explored approaches to calibrate a DEB-IBM with population-level data, which imposes constraints on parameter estimation as compared to conventional DEB-IBM calibration with individual-level data.We further evaluated the predictive capacity of the DEB-based approach in comparison with common reference models IA and Concentration Addition (CA). While the performance of CA and IA was concentration-dependent, the DEB-IBM has the capacity to capture such trends, because mixture toxicity is an emergent property and interactions between organisms can be taken into account. We conclude that an approach based on DEB-IBMs is a promising way forward to generate predictive models and enhance understanding of mixture toxicity at higher levels of biological organization.

2.05.6

Excellent growth of fish in German rivers as a consequence of re-oligotrophication?

C. Wesch, Trier University / Biogeographie; R. Klein, M. Paulus, D. Teubner, Trier University / Biogeography

Length and weight of fish are reliable indicators for long-term changes of fish health and imply long-term changes of environmental quality. Biometric monitoring data of the German Environmental Specimen Bank show an extreme increase of growth of common bream (*Abramis brama*) in the last 20-27 years in German rivers. The increment of bream with a defined age (8 years ±1 year) is up to 400% for weight and up to 60% for length. To explain this phenomenon, we assessed potential drivers of fish growth in rivers. We hypothesise that a decreased fish density caused by re-oligotrophication is the key driver behind that phenomenon, which is described for the first time for rivers. This process is probably supported by improved chemical water quality and neobiota. The combination of all these drivers seem to explain the drastic growth of bream in German rivers. Our results confirm the high value of biometric parameters for wildlife biota monitoring to detect long-term changes in the quality of aquatic ecosystems.

2.05.7

Assessing the impact of wastewater exposure on macroinvertebrate populations in England

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Wastewater contains a vast range of household chemicals including pharmaceuticals and personal care products, pesticides, metals and plastic residues. Aquatic macroinvertebrates are used across the world as indicators of the ecological health of rivers. The vast range of these different groups, families and species of molluscs, worms, insects, and crustaceans could be sensitive indicators of chemical impacts on their populations. A 4-year NERC-funded study was launched in 2018 to identify whether links between chemical exposure and wildlife population impacts exist based on a review of historical monitoring data. The Midlands Region, in central England, was selected as a trial area to test out some of the approaches that will be used across the whole of England. The Midlands part of England has many urban centres and moderate rainfall resulting in a generally low dilution factor for its rivers. Thus, following the wastewater prediction exercise using the LF2000WQX model it was found that 4% by length of the reaches are >50% wastewater and 32% by length of reaches are >25%

wastewater based on the mean. A review of general water quality, using data from the Environment Agency Harmonised monitoring scheme sites was possible to give an indication of trends. The mean ammonia dropped from 0.6 to 0.2 mg/L; the mean biological oxygen demand (BOD) dropped from 3.6 to 1.8 mg/L; the mean PO_4 dropped from 1.9 to 0.6 mg/L; whilst NO_3 remained unchanged around 35 mg/L over the period 1980 to 2014. The BMWP (biological monitoring working party) approach gives a single metric based on weighted scores awarded to macroinvertebrate families. The score can be expressed in terms of the average score per taxon (ASPT). In this case, an overall sustained improvement in these scores occurred from the early 1990s across the whole of the Midlands Region. Perhaps the general improvements in water quality (with the exception of nitrate) have made the environment more habitable for macroinvertebrates but this has yet to be tested statistically. The trial of the methods to be used across the project for England in ChemPop for macroinvertebrates has proved useful. Initial observations appear to confirm that the aquatic habitat for macroinvertebrates, particularly with respect to sites with high wastewater exposure, is improving. However, the full suite of statistical tests of the hypotheses have yet to be carried out.

From Lab to Field: Relevance of Effects Observed in Lab Studies for Non-target Local Populations and Communities and their Habitat Selection

2.06.1
Endocrine disruption of fish growth and reproduction in DEBtox
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A major challenge in ecological risk assessment is to quantitatively link suborganismal level endpoints to ecological level outcomes. Dynamic Energy Budget (DEB) models can play an important role in this. A DEB model describes the rates at which an individual organism acquires resources from its environment and use the energy and nutrients therein for universal processes supporting its survival and reproduction, and thereby serves as a hub between suborganismal metabolism and population dynamics. In the application of DEB models to ecotoxicological problems, commonly referred to as DEBtox, toxicant effects on the reproductive performance of an organism are the result of changes in one or more model parameters, including those governing the rates of energy acquisition and subsequent expenditure on maintenance, maturation and production processes. However, the impact of endocrine disrupting compounds (EDC) have been largely ignored in the DEBtox framework. This presentation is built on simulation studies designed to explore how EDCs with different modes of action in the DEBtox framework affect commonly measured organismal endpoints in medaka. Obesogens may increase or decrease growth and reproduction, depending whether these compounds affect reserve utilization or feeding and assimilation rates. Compounds that enhance growth also cause reproduction to increase (at abundant food conditions), as bigger fish tend to reproduce at a higher rate. Compounds that increase the allocation of reserve to reproduction at the expense of growth have a diminishing stimulatory impact on reproduction. The results will be used to evaluate the capability of the OECD Medaka Extended One Generation Test (MEOGRT) to discriminate among EDC modes of action.

2.06.2
Development of a neurobehavior adverse outcome pathway in larval fish to facilitate cross-species extrapolations and assess population risk
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To assess the impacts of chemical exposure on threatened or endangered species usually requires making assumptions about animals where there is limited information on which to base risk assessment. Our lab is developing methods to improve cross-species extrapolations so that we can make decisions on data poor species. We report on progress for an approach that we believe has the potential to improve cross-species extrapolations and to bridge essential data gaps so that we can predict effects from molecular/ cellular level to population and community level responses on many species from experiments conducted on laboratory animals. We base our work on adverse outcome pathway (AOP) framework because this framework is a useful organizational for collecting data in a systematic way to facilitate cross- species extrapolations. We are developing a neurobehavior AOP in the zebrafish exposed to PCB 126 and methylmercury, and we are extrapolating it to two other species of ecological importance, the fathead minnow, and the yellow perch. We collected in depth data on zebrafish, yellow perch, and fathead minnow that allow us to predict from molecular initiating evens to cohort survival and growth - endpoints that are critical for ecological risk assessments. With these data sets we assess the feasibility of cross-species extrapolations – we are able to determine what information is useful for cross-species comparisons and what information is lost when a model species is used instead of the target organism and the amount of error associated with cross-species assumptions. We also incorporate uncertainty in our cross-species and cross-biological level of organization extrapolations into our estimates of population risk.

2.06.3
To be or not to be a rat? Translating toxicology data for use in wild mammal risk assessment.
N. Hallmark, Bayer AG - Crop Science Division / Environmental Safety - Ecotoxicology; J. Hahne, Bayer AG Crop Science Division / Environment Safety; A. Weyers, Bayer AG - Crop Science Division / EnSa. Ecotoxicology
Mammalian toxicity data in rats, mice, rabbits and dogs are generated to predict potential human health effects as part of the standard data package for the EU registration of pesticides. These data are also used to predict potential long-term or reproductive effects on wild mammals, including rodents and lagomorphs. However, to do this 'translation', careful interpretation of the toxicology data by an eco-toxicologist is required. Specific EU regulatory guidance on the derivation of ecotoxicologically relevant endpoints for mammals is pending; meanwhile general principles can be derived from available EFSA documents. We have developed a systematic approach for the translation of toxicology data into an ecotoxicology relevant assessment, including consideration of the different protection goals. The derived ecotoxicologically relevant endpoint should be protective against effects on survival, growth, development and reproductive performance of wild mammals that may result from exposure to the chemical under assessment. This approach can lead to the selection of different endpoint(s) for use in environmental risk assessment compared to human risk assessment. A case study will demonstrate the methodology using a 4-step approach: 1. Data gathering: collate relevant toxicology data from regulatory studies, publications, etc. 2. Data assessment: review of each study against pre-defined inclusion criteria including: regulatory acceptability, study quality; dose-response, severity, concordance, statistical vs biological significance, etc. 3. Ecotoxicological (population) relevance: review each study against pre-defined relevance criteria. There are several possibilities why an endpoint may be of non-relevance for the environment (NRE) eg: · adaptive changes e.g. in liver or blood parameters · slight histological changes without impact on growth and reproduction · statistical vs. biological significance · transient changes, e.g. bodyweight changes that are quickly compensated · low frequency or late-onset effects, not representative for environmental conditions · consequences of avoidance of food or water containing the test chemical 4. Conclusion: identify the ecotoxicologically relevant endpoint for mammal long-term (reproductive) risk assessment This framework is ready for use to derive an ecotoxicologically relevant endpoint for mammal long-term (reproductive) risk assessment and is flexible so can be adapted to incorporate future alternative data types.

2.06.4
Spatial avoidance of the shrimp Palaemon varians exposed to a contaminant gradient of galaxolide and tonalide in seawater
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Environmental risk assessments of galaxolide (HHCB) and tonalide (AHTN) have been performed using data from lethal and/or sublethal responses under forced exposures without considering the behavioural responses, such as spatial avoidance. Our goal was to assess the potential of HHCB and AHTN to elicit avoidance response in shrimps (*Palaemon varians*) and to predict the population immediate decline (PID; by integrating both avoidance and lethality responses) of *P. varians* when exposed to HHCB and AHTN. The non-forced approach was used to measure the avoidance response by employing a non-forced multi-compartmented static system, in which shrimps could move freely among the concentrations. Shrimps were exposed to a gradient (0, 0.005, 0.05, 0.5, 5 and 50 µg/L) of each substance and their positions were checked at every 20 min for 3 h period. The results from 24-h forced exposure showed no dose-response relationship, with the highest percentage mortality being 17% for HHCB at 0.005 and 0.5 µg/L. In the 3-h non-forced exposure to gradient of HHCB and AHTN, significant concentration-dependent avoidance was observed for both substances. The shrimps avoided the least concentration of HHCB and AHTN (0.005 µg/L) by 14.6 % and 16.2%, increasing significantly ($p < 0.005$) to 60.5% and 57.1%, respectively, in the highest concentration (50 µg/L). The shrimps' population immediate decline at local scale might be driven by the avoidance behaviour rather than mortality, which might have serious consequences for their habitat selection processes.

2.06.5
When the environmental stress due to contamination is beyond the traditional toxic effects: Changing the paradigm of confined exposure to a habitat selection approach

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Up to date, the traditional approach used in ecotoxicological studies is fundamental to identify how contaminants might (potentially) cause environmental disturbance, from subcellular levels to physiological homeostasis. However, if the spatial perspective of toxicity tests is conceptually expanded, two central questions related to the effects of contamination on organisms arise: (i) are organisms able to escape from contaminants thus avoiding their toxic effects? (ii) what is the role of contamination on the organisms' habitat selection processes? When the prediction of the risks is based on a mandatory forced exposure to contaminants, it is assumed that physiological mechanisms, such as detoxification, is the means used by organisms to minimize the effects of contamination. However, recent studies using a non-forced exposure approach, in which organisms can freely move within a (heterogeneous) gradient of contamination, have shown that mobile organisms tend, under some conditions, to avoid the continuous exposure to adverse contaminant levels by escaping to favorable areas. The aim of this review is i) to present the different non-forced, multi-compartmented exposure systems already used in ecotoxicity assays, ii) to discuss the methodological and conceptual advances in effects assessment that such approach can provide, and iii) to show data demonstrating how the integration of this approach could complement the environmental risk assessment process by helping to understand the contamination-caused environmental stress from a novel perspective based on the habitat selection response. Concepts like spatial avoidance, preference, colonization, habitat connectivity (meta-ecosystem), chemical fragmentation of habitats and metapopulation bring more of ecology to ecotoxicology under the umbrella of Stress Ecology.

Impact and Trophic Transfer of Chemical Pollutants in Food Webs within and across Ecosystem Boundaries

2.07.1

Synthesis: Predicting the Dark Side of Ecological Subsidies

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Aquatic ecosystems are particularly vulnerable to chemical pollution because of their topographically low position in the landscape. Food web linkages between aquatic and terrestrial ecosystems can also be disrupted by these pollutants with dire consequences for consumers. Understanding the effects of contaminants on these linkages and implications for consumers is an area of active research. Here, we review multiple frameworks useful for predicting the effects of chemical contaminants on cross-ecosystem flux of resources (i.e., ecological subsidies) at the land-water interface. We focus on effects of contaminants on emergence of animals with complex life histories from aquatic habitats, use and quality of terrestrial detritus to aquatic ecosystems, and the flux of contaminants through food webs both within and across ecosystem boundaries. We then present a synthetic framework that pulls together many of the factors and processes reviewed, including landscape, contaminant, ecotoxicological, and ecological factors. Finally, we use this framework to identify 1) patterns of contaminant effects on ecosystems; and 2) data gaps and areas for future research. We use Monte Carlo simulations to illustrate how identifying the factors driving effects of contaminants on cross-ecosystem linkages within their study system can help researchers effectively target areas for study and predict risk of effects due to chemical pollution.

2.07.2

Transcending borders? Effects of heavy metals on the development and emergence of Chironomus riparius

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Structure and functions in freshwater ecosystems can be affected by anthropogenic stressors altering resource fluxes (subsidies) to adjacent terrestrial ecosystems. In this context, heavy metals might be of particular concern as they can accumulate in sediments and benthic organisms. This may reduce, delay or modify the nutritious quality (i.e., lowered nutritious value or export of accumulated contaminants) of insect emergence. In addition, heavy metals may interact with other stressors elevating the magnitude of effects. Therefore, we examined the interacting effects of essential (copper, Cu or nickel, Ni) or non-essential (cadmium, Cd) metals and short-term, recurrent water temperature increases on *Chironomus riparius* (Diptera: Chironomidae) using a spiked sediment test. Furthermore, nanoparticles (TiO_2) were included in the Ni experiment as they may influence heavy metal bioavailability. Chironomids' development (i.e., number of recovered larvae and larval weight) and emergence (i.e., success, timing and weight of emerging adults) was monitored, employing a two- or three-factorial test design for 28 days. All metals significantly and adversely affected the larval development of *C. riparius* with reductions of larval survival (up to 30%, Cu; 64%, Ni; 82%, Cd) and weight (up to 56%, Cu; 91%, Ni; 93%, Cd) at concentrations of 200 mg Cu/kg, 540 mg Ni/kg and 18 mg Cd/kg, respectively. Presumably, toxic stress requiring energy to be utilized for repair or detoxification processes may have resulted in higher maintenance costs and an altered energy allocation, ultimately causing significant effects on emergence success (reduction up to 45%, Cu; 100% Ni; 95% Cd), emergence time (delay up to 7 days, Cd) and adult biomass (reduction up to 14%, Cu). Effects of temperature fluctuation were less pronounced and varied depending on the developmental stage, heavy metal and observed endpoint. Nanoparticles did not significantly influence any endpoint. Based on our results, heavy metals may profoundly affect merolimnic insects, which, in turn may cause effects on riparian food webs. To address the impact of heavy metals on the quality of the subsidy (the emerged adults), preserved individuals are currently analysed for their heavy metal concentrations as well as their fatty and amino acid profiles.

2.07.3

Cross-boundary transport of nanoparticles via merolimnic insects compromises aquatic subsidy quality

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Knowledge on how and to which extent contaminants are transported from the aquatic environment to terrestrial ecosystems is still scarce. The emergence of merolimnic aquatic insects may constitute one potential pathway for contaminants directly into terrestrial food webs. At the same time, contaminants may cause adverse effects on the temporal emergence pattern and nutritional quality of aquatic insects. Consequently, both the trophic transfer of contaminants by and shifts in the availability and quality of aquatic subsidies can be of serious concern for the well-being of terrestrial predators. To address this issue, we exposed caddisfly larvae (*Chaetopteryx villosa*) towards nanoparticles (NPs; 4 and 400 µg $nTiO_2$/L; 6.5 µg nAu/L) as model contaminants for 140 d using a laboratory flow-through stream microcosm system. In addition, the photocatalytic effects of NPs on the response of *Chaetopteryx* were assessed by additional exposure to ultraviolet irradiation at ambient intensities. Consumption of leaf material, survival, emergence rate and its temporal pattern, energy reserves (=lipids), and concentration of NPs in adult (emerged) organisms were monitored. NPs delayed *Chaetopteryx* emergence by ≤30 days and reduced lipid reserves by ≤40%. Furthermore, emerging *Chaetopteryx* carried substantial concentrations of $nTiO_2$ (up to 2.68 ng/mg) and nAu (1.51 ng/mg). These results underline the potential for contaminant transfer from the aquatic to the terrestrial ecosystem via the emergence of merolimnic aquatic insects. Assuming that median internal NP concentrations measured here are comparable among merolimnic insect species and the total annual biomass of emerging merolimnic insects from streams measured elsewhere, NP-fluxes from the aquatic to the terrestrial environment can potentially be as high as 2.4-56.5 mg $nTiO_2/m^2$ and 1.4-31.8 mg nAu/m^2. These data raise concern about the implications for higher trophic levels in riparian systems associated with the transfer of contaminants from the aquatic environment into the terrestrial food web. However, even though recent studies on the transport of contaminants (e.g., microplastics, metals, NPs, and pharmaceuticals) suggest effects on the aquatic-terrestrial subsidy, this area of research is still in its infancy and requires further scrutiny.

2.07.4

Food web controls on mercury flux and fate in the Colorado River, Grand Canyon (USA)

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Mercury (Hg) biomagnification in aquatic food webs is a global concern, yet pathways of Hg flux — and species traits and biotic interactions promoting these fluxes — remain poorly understood. To address this data gap, we measured animal secondary production and consumption rates to develop organic matter flow food webs that quantify important pathways of energy flows as well as the key trophic interactions dictating these flows (i.e., 'the trophic basis of production"). We then coupled these food web models with traditional ecotoxicological survey data for Hg concentrations across different organic matter sources (detritus and biofilms), invertebrates, and fish to track Hg fluxes within food webs and to determine Hg fate between linked aquatic and riparian food webs (i.e., Hg retained in aquatic food webs vs. exported to land via adult insect emergence). We developed these Hg-based food webs for 6 sites spanning 370 river km and over two years. Annual sampling events occurred before and after a large experimental flood on the river, allowing us to evaluate how ecosystem disturbance may also alter Hg flux in food webs. We found that only a few pathways dominated Hg flux in the Colorado River despite large spatial differences in food web complexity, and these fluxes were largely mediated by one critical invertebrate trait, resistance to predation. Food web properties, i.e., the match/mismatch between insect production and fish consumption, governed amounts of Hg retained in the river versus exported to land. An experimental flood redistributed Hg fluxes in the relatively simple, tailwater food web just downstream of a large dam, but not in more complex, downstream food webs. Recognition that species traits, ecological interactions, and disturbance regimes mediate contaminant exposure can improve risk management of linked aquatic-terrestrial ecosystems.

2.07.5

Aquatic and terrestrial contaminants in Pied flycatcher nestlings - effect of diet on metal uptake

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One major exposure route for metal exposed insectivorous birds are through dietary uptake, which mean that exposure depends on uptake by the prey and their proportion in birdsâ_ diet. Invertebrates differ in the uptake of contaminants, where invertebrates in close contact to the contaminated medium (soil or sediment living) often accumulates more contaminants. In addition, for insectivorous birds living near lakes and streams, metals can be transported from the aquatic system by emerging aquatic insects. In this study, we estimated how having access to different composition of prey resources, using stable isotope analysis (δ13C, δ15N), affected lead (Pb) exposure for the insectivorous bird Pied flycatcher (Ficedula hypoleuca) nestlings. The study was conducted in the vicinity of a closed Pb/zinc (Zn) mine in northern Sweden, using sites close to lakes (< 300 meters) or sites located with distance from larger streams or lakes in order to reduce aquatic insect availability. Nest-boxes was established in 2017, and 12-14-day old nestlings was monitored and sampled for blood for metal exposure and stable isotope signal. Simultaneously with monitoring were insects captured for Pb concentration and isotopic signal. Nestlings near lakes had significantly higher proportion of aquatic insects in their diet. No apparent pattern in Pb concentration between insect groups was observed, indicating different bioavailability from contamination sources between sites. Nestling with higher proportion of aquatic insects had lower Pb concentration in blood, which was not connected the metal concentration in emerging aquatic insect. The study shows that exposure to metals is affected by the type of prey in the diet and that insectivorous birds can be benefit from feeding on aquatic food sources in contaminated environments. It is therefore important to consider the type of available insects when estimating the exposure risks of metals for insectivorous predators.

2.07.6

Trophic transfer of isotopically labelled 65CuO NPs from sediment to worms (Tubifex tubifex) to fish (three-spined stickleback)

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Nanoparticles (NPs) released into the aquatic environment will likely accumulate in sediment and be available for uptake in sediment-dwelling invertebrates that serve as food for higher trophic level organisms, such as fish. The aim of the present study was to investigate trophic transfer of CuO NPs and dissolved Cu ($CuCl_2$) along an experimental freshwater food chain including natural sediment, the sediment-dwelling worm *Tubifex tubifex* and the predatory fish *Gasterosteus aculeatus* (three-spined stickleback). Worms were first exposed to sediment spiked with environmentally relevant concentrations (100 µg g^{-1} dw sed) of CuO NPs and $CuCl_2$ for 7 days. After exposure, they were collected and used as live feed for dietary exposure of fish (7 day-exposure). Both CuO NPs and $CuCl_2$ used in this study were enriched in the stable copper isotope ^{65}Cu to enhance detection and facilitate discrimination of newly added/assimilated Cu from background levels. ^{65}Cu concentrations in sediment, worms and fish tissues (intestine, liver, carcass) were measured by ICP-MS. Biological effects of dietary Cu exposure in fish were assessed measuring expression of genes related to Cu transport, detoxification and oxidative stress (*ctr1, mta, gcl, gr, gpx, sod, cat, zo-1*) using RT-qPCR. Worms accumulated ^{65}Cu from both $^{65}CuCl_2$ and ^{65}CuO NP-spiked sediment. Weight-specific ^{65}Cu body burdens in worms were low and not significantly different between Cu forms (0.7 and 1.1 µg ^{65}Cu g^{-1} dw tissue, respectively). Dietary accumulation of ^{65}Cu in fish feeding on $^{65}CuCl_2$ and ^{65}CuO NP-exposed worms for 7 days was generally low and limited to intestinal tissue (80 and 65 ng g^{-1} dw tissue, respectively; no significant difference). ^{65}Cu transfer to internal tissues/organs was marginal. No significant effects of dietary Cu exposure on the investigated genes was observed, except for *gpx*, which was significantly upregulated in intestinal tissue of fish feeding on $^{65}CuCl_2$-exposed worms. Altogether, our results suggest that CuO NPs (NP-derived Cu) may enter freshwater food webs by transfer from sediment to worms and subsequently to fish. Yet, their dietary bioaccumulation and trophic transfer potential at environmentally realistic doses appears to be low and not different from that of dissolved Cu.

2.07.7

Mercury biomagnification in a Southern Ocean food web

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The biomagnification of mercury was determined for the Scotia Sea food web, using the stable isotopes of nitrogen (□15N) and carbon (□13C) as proxy for trophic level and habitat use. Here mercury and stable isotopes were measured for 12 species of zooplankton, 2 species of squid, 7 species of mesopelagic myctophid fish 6 species of notothenioidei fish and 8 species of seabirds chicks in two, 10 years apart, sampling years (2007/08 and 2016/17). All the trophic groups had similar □13C, except seabirds, but this differences were expected as feather (inert tissues) are more enriched in □13C than muscle or other organs (live tissues). □15N showed the expected increasing trend over trophic levels zooplankton < squid < myctophids fish < notothenioidei fish < seabirds, there were also significant differences within the predators groups, as different species feed on different trophic levels. Mercury also increased along the trophic level, with lower higher values in seabirds chicks (3.88 ± 2.41 µg g-1 in Stercorarius antarcticus). Again in the predators as possible to see differences in mercury levels depending on the species diet, with species with higher □15N reflection also higher mercury levels due to the ingestion of more contaminated prey. Looking into the mercury levels between the two sampling years, it was possible to see a decreasing trend on mercury concentration along the last decade in the mid trophic web groups (squid and fish), that can be explained by a decline of the anthropogenic emission of mercury. With mercury decreasing in mid trophic levels it would be expect that top predators would also have lower concentrations, although, an opposite trend was found in top predators, with seabirds from 2016/17 having higher levels than the ones from 2007/08. With the This puzzling increase of mercury in seabirds may be explain by a shift in the food web. Antarctic krill is a central species in the Southern Ocean food web, in years with low abundance of Antarctic Krill species are forced to feed on higher trophic level prey. This was the case on 2016/17, so predators had to feed more in myctophids, that have higher mercury burden than Antarctic krill, while in 2007/08 there was an high abundance of Antarctic krill. This results reinforce the importance of Antarctic krill in the Southern Ocean, as the main prey for several species, but also as a food with lower mercury uptake.

2.07.8

Can stable isotopes in consumers improve interpretation of long-term contaminant data?

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One of the overall objectives of contaminant monitoring programmes is to evaluate the rate of change in hazardous substances when assessing measures taken to reduce contaminant loads. Temporal trend monitoring in combination with compliance monitoring (if contaminants are below or above a specified threshold) provides a reliable status assessment because the between-year variability is considered. However, to improve the statistical power of detecting changes it is critical to reduce both the within- and between-year variability by adjusting the data for relevant confounding variables. One such potentially important confounding variable is the trophic position (TP) of organisms commonly derived using stable nitrogen isotopes ($\delta^{15}N$). Diet is generally the main route of exposure to organic contaminants and therefore TP of organisms is a major driver of bioaccumulation. The current EU recommendation regarding human fish consumption is to adjust the contaminant monitoring data from lower trophic positions to represent a TP of 3.8 before comparing to Environmental quality standards in biota. However, as the $\delta^{15}N$ is not only indicative of TP but also reflects the physiological status of a consumer its value for reducing uncertainty in time series data require investigation. In the Swedish National Monitoring Programme for Contaminants in Marine Biota, blue mussels (*Mytilus edulis*) and herring (*Clupea harengus*) are, among other species, sampled and analysed for a wide array of contaminants each year. In this study, we evaluate the highly variable temporal trends of various contaminants with regard to $\delta^{15}N$ and TP in herring, the latter using blue mussels as an isotope baseline, in 35 years time series from the central Baltic Sea. Results demonstrate that the $\delta^{15}N$ of herring better explain the Hg concentrations than the TP of herring, using ANCOVA analyses. We discuss potential causes for this discrepancy between $\delta^{15}N$ and TP such as a shifting biogeochemical baseline affecting blue mussels more than herring. We conclude that adjusting for $\delta^{15}N$ but not TP can improve the statistical power of the highly variable temporal contaminant trends in herring from the Baltic Sea. Ongoing work aims at comparing TP analysed using a baseline organism as described above with TP calculated from specific amino acids in herring only.

Microbial Community Ecotoxicology under Multiple Stressors Scenarios

2.08.1
Biofilm interactions can modulate microbial decomposer responses to antibiotics
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Sulfonamide antibiotics are emerging contaminants often detected in surface waters because of their frequent use in veterinary medicine, their high excretion rates and their important chemical stability in the terrestrial and aquatic environments. Using a microcosm approach, the separate impact of two sulfonamides (sulfamethoxazole (SMX) and sulfamethazine (SMZ)) on the ability of aquatic microbial heterotrophs to decompose alder leaves was investigated. More precisely, we determined whether sulfonamide impact (5 µg L-1) on microbial decomposer activity varied when microbial communities from leaves and periphyton were mixed in the same microcosm. As expected, sulfonamides decreased the biomass of bacteria accumulated in alder leaves but did not modify that of fungi. Despite these differences in microbial biomass, sulfonamides did not significantly modify decomposition rates of leaves mediated by microbes (kcontrol = 0.054 +/- 0.001, kSMX = 0.058 +/- 0.011, kSMZ = 0.060 +/- 0.013). Only a significant enhancement of b-glucosidase activity in the presence of sulfonamides was observed and attributed to a stress response set up by bacteria to face antibiotics. The mix of leaves and periphyton in the same microcosm changed water physico-chemical characteristics. Through primary production, the presence of periphyton increased dissolved organic carbon (DOC) concentration and decreased SUVA254 organic matter recalcitrance index comparing to microcosms containing leaves alone. The greater DOC availability and its greater biodegradability in these microcosms significantly reduced alder decomposition rates (kleaves = 0.057 +/- 0.003, kleaves+periphyton = 0.042 +/- 0.002) suggesting a decoupling of microbial decomposition activity from particulate organic matter (leaves) to dissolved organic matter (water). The presence of periphyton did not emphasize sulfonamide impact on bacterial biomass from leaves; however, it tended to soften the response of bacterial b-glucosidase activity after exposure to antibiotics. This study highlights the importance of biofilm interaction when assessing antibiotic risk in aquatic microbial communities.

2.08.2
Microbial community structure in a chemically stressed freshwater ecosystem. Do chemical mixtures play a role?
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Anthropogenically impacted freshwater ecosystems are characterised by complex chemical mixtures from local to regional scale [1]. Agriculture, mining operations, and wastewater treatment plant (WWTP) discharges are considered as the main sources of chemicals into the aquatic ecosystems, with profound impacts at different ecosystem levels. Microbes play a key role in organic matter and nutrient recicling in freshwater ecosystems. It has been propossed that the structure of microbial assemblies changes in response to physical and geochemical features. However, the chemical pressure from chemical mixtures, as a shaping drivers, has been widely neglected. WWTP effluents and run-off together with spray drift have been reported to be the main processes transporting these chemicals into the aquatic environment. Therefore, the pollution produced by these anthropogenic activities may jeopardise aquatic organisms and ultimately human health. In this study, we aimed to: i) characterise a freshwater ecosystem from a physico and geochemical perspective, ii) identify and quantify complex chemical mixtures, and iii) assess if complex chemical mixture affect structural patterns of the microbial community through the River Aconcagua ecosystem in Chile.

2.08.3
Looking beneath the surface: are fungicide effects in sediments comparable to those in the benthic zone?
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Leaf litter decomposition is a microbially driven ecosystem process, that is crucial for energy and nutrient fluxes in streams and can be negatively affected by fungicides. Although most of the organic matter is stored in sediments, information about fungicide effects in the hyporheic zone is missing. In a full-factorial laboratory experiment (n=7), the effects of a fungicide mixture on the structure (fungal biomass and sporulation as well as phospholipid fatty acid fingerprint) and functioning (leaf decomposition) of leaf-associated microbial communities in the hyporheic and benthic zone was studied. Substrate-dependence of fungicide effects was tested by using two grain sizes. In the hyporheic zone leaf decomposition, fungal biomass, fungal sporulation and general microbial abundance were lower than in the benthic zone, while the importance of microbes other than fungi increased. This effect is most likely triggered by differences in oxygen conditions or hydraulic conductivity. In line with previous studies targeting fungicide effects, benthic fungal biomass and fungal community composition were affected. However, in the fine-grained hyporheic zone fungicide effects on leaf decomposition showed a two-fold greater effect size relative to the benthic zone. This might be caused by a lower hydraulic conductivity of the fine sediment, which influences the dispersal of microorganisms as well as oxygen and nutrient fluxes across the sediment-water-interface and within the sediment. Functional effects, especially in the hyporheic zone, were not fully explicable by the analyzed structural endpoints. We can nevertheless conclude, that fungicide effects can be even more severe in the hyporheic zone, requiring further assessments to improve risk analyses.

2.08.4
Long-term effects of copper nanopesticides on soil and sediment microbiome diversity in two outdoor mesocosm experiments
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The use of novel pesticides containing nanomaterials (nanopesticides) is growing and is considered a promising approach to reduce the impacts of agriculture on the environment and human health. However, the environmental effects of these novel agrochemicals are not fully characterized, and more research is needed to determine the benefits and risks they confer. Here, we assessed the impacts of repeated exposures to a Cu(OH)2 nanopesticide on the soil and sediment biodiversity of target (terrestrial) and non-target (wetland) ecosystems by performing long-term outdoor mesocosm experiments. As pesticides are often used concomitantly with other agrochemicals, we also tested for interactive effects between nanopesticide exposure and fertilization treatments in both ecosystems. We used high-throughput sequencing on three marker genes to characterize effects on bacterial, fungal and total eukaryotic community structure and diversity. Interestingly, we found limited effects of nanopesticide exposure on the terrestrial soil communities. Conversely, we found significant shifts in the sediment communities of the wetland mesocosms, especially for eukaryotes (protists, fungi, algae). In the absence of fertilization, fungal and total eukaryotic community compositions exposed to nanopesticides for long periods of time were distinct from unexposed communities. We identified 60 taxa that were significantly affected by nanopesticide exposure, most of which were micro-eukaryotes affiliated to Cercozoans, Gastrotricha or unicellular algal taxa. Our study suggests that this nanopesticide has limited effects on the soil biodiversity of a target terrestrial agroecosystem, while non-target aquatic communities are more sensitive, particularly among protists which are not targeted by this bactericide/fungicide.

2.08.5
Microbial communities and hydrocarbon biodegradation in land sea continuum
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Microbial communities inhabiting coastal marine sediments play a key role in the carbon cycle and particurlaly in the degradation of hydrocarbons (HC) resulting from anthropogenic activities highly concentrated on the coasts. Terrigenous inputs from the leaching of soil drive the functioning of the coastal ecosystems as in estuaries with the transfer of organic matter and pollutants. However, information about the transfer of microorganisms at the community level and the link with the fate of HC are still missing. The study propose to estimate the impact of the terrigenous inputs on the relation structure/function of the microbial

communities in coastal marine sediments exposed to HC. An original experimental device was set up to mimic the terrigenous inputs, by leaching of the soil, to the marine coastal sediments for 39 days. Different conditions were applied, with the addition or not of deuterated hexadecane and phenanthrene in soil and sediment and planting or not the soil with a grass, *Catapodium marineum*. The diversity and functional potential of microbial communities from the different soils, leachates and sediments, were analysed by 16S and 18S rRNA gene sequencing (Illumina MiSeq) combined with functional DNA arrays (Geochip). The HC removal and the metabolites production were characterized by GC/MS and FTICR. The phenanthrene was removed in soils and sediments while hexadecane removal was observed only in soil. The metabolite composition was different between soils and sediments, and among sediments with or without leaching (control). The terrigenous inputs shape microbial communities in sediments by providing mixing of microorganisms and/or even ecosystems indicating a coalescence. The functional gene composition, particularly genes involved in HC transformation, changed according to the terrigenous inputs and the presence of plants. In addition, taxonomic and functional patterns were congruent regarding hydrocarbon degradation genes, suggesting a specialization of the microbial communities. Finally, microbial indicators (ASVs and functional genes) were identified in sediments receiving inputs from soil under HC contamination. Such information provide new insights to develop microbial indicators that will be helpful to manage hydrocarbon polluted marine coastal sediments.

2.08.6

Crossing obstacles: fungal transport of bacteria in PAHs contaminated marine sediments

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Polycyclic aromatic hydrocarbons (PAHs) are persistent ubiquitous organic pollutants that often accumulate in marine sediments. Driven by their hydrophobicity they tend to be non-uniformly distributed in sediments and, hence, become differently accessible for microbial degradation. Studies in soil environments have shown that fungal mycelia provide networks (“fungal highways”) for efficient bacterial dispersal that enable increased bacterial activity and efficient PAH-degradation in heterogeneous environments. In contrast to soils, sediments are water-filled environments with hydrophobic patches in presence of contaminants resulting in different challenges to bacterial transport by fungal highways. The aim of our work to assess (i) the ability of bacteria to disperse along marine fungal mycelia in heterogeneous sediment environments and (ii) the role of fungi in the selection of specific PAH-resistant bacterial communities. To that pourpose, eight PAH-degrading bacteria and two marine fungi (*Alternaria* sp. and *Fusarium* sp., with high and low PAH-removal capacity, respectively) were isolated from a heavily PAH contaminated coastal sediment (Etang de Berre, France). We test the fungal hyphae as translocation network for marine bacteria through a gap filled either with air, sterile wet sand, sterile sediment, or wax mimicking spatially distinct and heterogeneous sediment conditions. Also the diversity of translocated bacterial communities was analized by Illumina Miseq. Our results showed that bacterial motility was crucial for the dispersion since we observed both bacterial transport along the fungal hyphae and abiotic glass fibers. Fungal mycelia cross and transported bacteria through all type of gaps showing better performance in hydrophobic gaps (wax and contaminated sediment). This observation highlight the importance of fungal mycelia in the dispersion of bacteria in PAH polluted sediment. We also showed that the bacterial community transported by fungi is not selected by the fungal hyphae (in tested conditions) being *Pseudoalteromonas* the most abundant genus. This is the first study showing the effect of fungal hyphae on the dispersal of PAH degrading marine bacteria in sediments. Nevertheless, deeper analysis are required in order to understand the importance of bacterial dispersion and fungi/bacteria interactions in PAHs degradation in marine sediments.

2.08.7

Nanoscale micronutrients to enhance crop disease resistance: Unintended consequences in the rhizosphere?

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Achieving and sustaining global food security will become increasingly difficult as a changing climate increases crop loss due to greater pest and pathogen activity. Nano-enabled agrichemical delivery platforms offer a unique potential to manage pathogens and increase productivity with reduced negative environmental consequences. We have focused on the use of nanoscale micronutrients to suppress crop diseases through modulation of plant nutrition. The potential of several Cu nanomaterials to enhance the nutrition and growth of tomato, eggplant, watermelon, and soybean upon fungal infection was evaluated. The particles were foliarly applied once (100-500 mg/L; 1-2 mL dose) to seedlings prior to 30-40 days of growth in the greenhouse or full life cycle in field studies. Fungal infection reduced plant growth by up to 65% across all species but foliar amendment with nanoscale Cu significantly reduced disease. For example, disease was reduced by 31-40% in tomato, resulting in significantly greater plant mass. Similar findings were reported for field studies with tomato, eggplant and watermelon. In tomato and soybean, the time-dependent expression of genes integral to plant defense was shown to be uniquely modulated by nanoscale Cu and Si amendment. However, any application of nanotechnology in agriculture must include a thorough understanding of the potential implications of this strategy. For example, work from our group has shown that certain nanoscale materials may offer some benefit to the plant but may also cause negative changes in the microbial community in the plant rhizosphere. We analyzed the metatranscriptome of the maize rhizosphere and observed multiple unintended effects of 117-d exposure to 100 mg/kg nanosilver. The *Archaea* population was reduced by 30%, and as such, their involvement in nitrogen cycling was compromised. In addition, certain potentially phytopathogenic fungal groups showed significantly increased abundance (up to 5-fold), likely due to the negative effects of nanosilver on bacteria that exert natural biocontrol against these fungi as indicated by negative interactions in a network analysis. Collectively, these results highlight the potential of nano-enabled strategies to increase food production but also highlight the importance of gaining a thorough understanding of the relevant mechanisms of action so as to ensure the safety and sustainability of these approaches.

2.08.8

The effect of oxytetracycline long-term exposure on zebrafish and water microbiome in a post-exposure scenario

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Due to food demand, aquaculture industry has been growing fast. Thus, to supply all the necessities, fish are usually grown in stressfully conditions, allowing the easy dissemination of diseases. Due to its broad-spectrum, oxytetracycline (OTC) is one of the most used antibiotics in the aquaculture industry to prevent economic loss. It is known that this compound is poorly absorbed by fish and 70-90% of this compound is excreted, reaching the aquatic environments and affecting non-target organisms. Moreover, in their natural environment, fish are not always exposed to this chemical stress and a post-exposure scenario may occur. Thus, our work intended to study the effect of OTC long-term exposure on zebrafish and water microbiome in a post-exposure condition. Zebrafish adults were exposed to two concentrations of OTC (0 and 10000 µg/L) via water exposure for two months. After this period, organisms were transferred to clean water for one month. Samples were collected at 5 days and 2 months of exposure (5dE and 2mE) and at 5 days and 1 month of post-exposure (5dPE and 1mPE) period. DNA was extracted from fish gut and water samples in triplicate. Then, V3-V4 region of the bacterial 16S rRNA gene was sequenced using Illumina technology. Changes in alpha and beta diversity were evaluated. Results revealed that, at OTU (Operational Taxonomic Unit) level, OTC exposure had an impact in the community structure after 5dE for both water and gut microbiome sharing low similarity with the control group (28 % and 36 %). In addition, the same tendency was observed after 2mE although not so evident in fish gut microbiome (56% of similarity). Regarding the post-exposure period (5dPE and 1mPE), exposed and non-exposed communities shared higher similarity levels than during exposure indicating a recovery of microbiome structures. Diversity indexes revealed no differences in fish gut microbiome. On the other hand, in water microbiome, a significant decrease in species richness was observed after 2mE and an increase after 1mPE. Concluding, our results indicate that OTC affects zebrafish gut and water microbiomes and the impact is detectable after 5dE and 2mE. Nevertheless, when the exposure ceased, bacterial communities appear to recover, becoming more similar to control communities. Therefore, to better understand the real ecological impact of antibiotic exposure, future works should consider more realistic scenarios including post-exposure impacts.

2.08.9

Connecting gut microbiome changes and fish metabolism in oil exposure studies

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Recent studies show that the exposure of fish species to contaminants, including polycyclic aromatic hydrocarbons (PAHs) can influence the diversity of the intestinal microbial community. The relationship between gut microbiome changes and the fish gut metabolism is, however, less understood. Two exposure studies were carried out using crude oil. In the first experiment, juvenile Atlantic cod (Gadus morhua) were exposed to dispersed crude oil (0.05 ppm) for 1, 3, 7 and 28 days. In the second treatment, adult polar cod (Boreogadus saida) were exposed to the water-soluble fraction of crude oil (0.0125 ppm) for 47 and 131 days during polar night, an important season for polar cod reproduction. The general health condition of each fish was evaluated by calculating the condition index and hepatosomatic index. The adverse effects of oil exposure was monitored by supporting data such as the PAHs metabolites in juvenile Atlantic cod and the measurements of cytochrome P450 (cyp1a) in liver. Metagenomic analyses were performed to characterize the species composition, richness and diversity of the gut microbial community. In addition, RNA-sequencing was also conducted to provide gene expression information within the gut microbiome, giving insight in functional changes of the microbiome and potential interactions with the host. An Illumina MiSeq platform was used for metabarcoding (V4-V5 region of the 16S rRNA gene) and for mRNA sequencing. The 16S rRNA sequencing results were examined with the software- QIIME2 and R packages. The taxonomy study revealed that the most abundant orders in the juvenile Atlantic cod gut were Vibrionales, Mycoplasmatales, Actinobacteridae, Alteromonadales, Rhodobacterales and Pseudomonadales. The connection between the changes in the functional profile of the gut microbial community (metatranscriptome) and the fish gut metabolism (host transcriptome) were studied through differential expression analysis. This approach is an important step forward from metabarcoding and metagenomics studies toward better understanding of the interaction between toxicants and microbiome and between microbiome and host.

2.08.10

Gut microbiota and host-related responses of Xenopus laevis tadpoles exposed to nanoparticles

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The worldwide increase of nanoparticle (NPs) uses and production raises the question of the potential consequences of their release in the environment, especially in the aquatic environment in which it is likely to accumulate. Among these NPs, boron nitride nanotubes (BNNT) or graphene family materials such as graphene oxide (GO) are triggering high expectations for the development of new applications. Thus, there is a need to evaluate their biological risk. Gut microbiota constitute a compartment of crucial importance in regulation of host homeostasis. However, most of ecotoxicological studies neglect its role in toxicity-related responses of host organisms. Thus, the aim of this work is to assess the effects of exposure to these NPs toward the gut microbial communities of *X. laevis* tadpoles to determine the consequences of these changes on host physiological responses. For this purpose, growth of larva exposed to NPs was monitored and total DNA from intestines was extracted. The structure of microbiota was assessed by high-throughput sequencing, targeting the V4-V5 region of the bacterial 16S rRNA gene (Illumina MiSeq). Opposite effects were observed on host physiology, resulting in a dose-dependent increase or decrease of larval growth rate after exposure to BNNT or GO respectively. The results obtained from gut microbiota survey indicated that microbial communities were modified by exposure to both of the tested NPs, leading to a decrease of diversity index at high BNNT concentration and at low GO concentration. In both cases, relative abundance of the major phyla constituting the gut microbiota was modified after NP exposure. Statistical analysis suggested a strong implication of the phylum Bacteroidetes in the host response related to growth parameters. Studying gut microbia composition in response to nanoparticle exposure constitute a good marker of metabolic changes in host.

Soil Ecotoxicology: New Methods and Novel Applications in Environmental Risk Assessment

2.09.1

Identifying structural responses of bacterial, fungal and eukaryote communities to trace-metals using multi-marker metabarcoding

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The pollution of soils by multiple metals released from mining and smelting activities is one of the most pervasive and intractable pollution issues faced today. To understand the effects of metal pollution on ecosystem services, we need to better understand how metals impact on the often unseen soil biodiversity that contributes to key soil processes, such as the bacteria fungi and micro-eukaryotes. The Avonmouth smelter located in the UK was, during its operational life (1928-2003), the largest primary Cd/Pb/Zn smelter in Europe and also one of the largest point pollution sources on the continent. Sampling of 92 soils from sites in the area around the smelter revealed prevailing metal concentrations resulting from aerial deposition from this source ranging from grossly polluted e.g. Cd, Pb, Zn of 300 mg/kg, 1000 mg/kg and 30,000 mg/kg respectively to near background at >12 km distance. These measured concentration were used to calculate multi-substance potentially affected fractions (msPAF) for all soils to indicate the potential metal stress experienced by the soil community. The structure of the bacterial, fungal and eukaryotic communities inhabiting the sampled site soils were also characterised using 16S rDNA, ITS and 18S rDNA marker gene metabarcoding. This complex multi-dimensional data-sets was then interrogated to identify the main drivers of soil community change with the aim of identifying metal stress to community structure relationships and identifying characteristic indicators of the metal pollution load. Rank order tests were used to correlate entropies with variables to identify primary driver-diversity relationship. Trace metal concentration and soil pH were identified as driving variable of community structure, however, relationships against overall diversity metrics were relatively weak. Cluster analysis was used to identify the minimum number of community types (i.e. sets of related species) present in the region. The distribution of these clusters could be clearly related to soil pH and also the total concentration of metals and bioavailable concentrations in the site soils. Finally, AI algorithms were used to build a models that predict the minimal set of indicator species needed to correctly categorize soils according to their prevailing levels of both soil metal stress and pH.

2.09.2

Proteomic profiling to unravel the mechanisms of Ag NM300K in Enchytraeus crypticus - novel approach to understand risks

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Silver nanomaterials (Ag NMs) are one of the most investigated and explored nanotechnology-derived nanostructures. Despite their remarkable advantages in the industrial and medical applications, their toxicity became an issue of intense study. The ecotoxicological soil model, *Enchytraeus crypticus* already demonstrated altered molecular responses (e.g. gene expressions and DNA damage) to Ag NM300K, a reference NM. In order to have a mechanistic interpretation of its toxicity, a proteomic approach was applied using high-throughput (HTP) tools. Untargeted proteomics was performed using HPLC coupled to high-resolution mass spectrometry. Reversed-phase (RP) in combination with tandem mass tags was used for quantification of protein expression levels. EC20 and EC50 reproduction effect concentrations of Ag NM300K and $AgNO_3$ were tested after 14 days. A delay in toxicity was observed across both Ag forms. Generally, Ag NM300K caused increased protein expression, whereas $AgNO_3$ caused inhibiting effects. Diverse common proteins were linked to the same biological processes, namely, energy metabolism, cytoskeletal structural role, glycosylation, and S-adenosyl methionine cycle Ag forms. On the other hand, fewer unique processes related to oxidative stress are associated with $AgNO_3$, while Ag NM300K (EC20) was linked to stress metabolism. Since a shift in the regulation of most protein was found, the affected biochemical pathways (despite being the same) may cause dissimilar physiological outputs in *E. crypticus,* and therefore, different apical effects. Hence, these signatures can be used to reveal mainly metabolism-mediated toxicity mechanisms from Ag forms in *E. crypticus*.

2.09.3

Applying the EFSA Scientific Draft Opinions on standard testing in terrestrial ecotoxicology: Current state, challenges and outlook

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In the recently published EFSA draft opinions for Non-Target Arthropods (2015), Non-Target Terrestrial Plants (NTTP) (2014) and in-soil organisms (2017), EFSA outlined several gaps in the current environmental risk assessment and proposed e.g. the testing of herbivorous lepidopteran larvae to cover the oral exposure route, the replacement of artificial soil by representative natural agricultural soil as intermediate tier, to use alternative species of in-soil organisms in laboratory standard testing, and the testing of generative endpoints of crop and non-crop plant species. Additionally with regard to NTTP, the technical report of EFSA (2019) concluded that the ERx for visual injuries should be reported and used for the risk assessment. In the past years experiments with herbivorous lepidopteran larvae, study designs with different natural soils and the endogeic earthworm Aporrectodea caliginosa or the epigeic springtail species Sinella curviseta were conducted to evaluate the feasibility of the potential new requitements. For NTTP greenhouse studies to assess generative endpoints of crop and non-crop species were also conducted. In addition we evaluated visual injury in NTTP studies where where this endpoint was more sensitive than the usually measureable variables. Key results of these experiments are summarized, feasibility and benefits as standard testing discussed and challenges as well as further research required are debated.

2.09.4
Uptake, toxicity, and maternal transfer of cadmium in the oribatid soil mite, Oppia nitens: Implication in the risk assessment of cadmium to soil invertebrates
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Cadmium (Cd) is a heavy metal of concern in contaminated sites because of its high toxicity to soil biota and humans. Typically, Cd exposure is thought to be dominated by dissolved Cd in soil pore water and, thus, dermal uptake. In this study, we investigated the uptake, toxicity, and maternal transfer of Cd in a standard soil invertebrate, the oribatid mite (Oppia nitens), which is common to boreal and temperate ecozones. We found total soil Cd predicted Cd uptake in adult and juvenile O. nitens with no significant uptake from pore water by juvenile mites. Cadmium significantly inhibited juvenile production and recruitment as well as reduced adult fecundity. Adult O. nitens maternally transferred 39 to 52 % of their Cd body burden to juveniles (tritonymphs) while the maternally-acquired Cd accounted for 41 % of the juvenile internal Cd load. Our results suggest that dermal adsorption of metal ions is not important for O. nitens and that maternal transfer of Cd in soil invertebrates has ecological and toxicological implications for populations of soil invertebrates. Maternal transfer should be incorporated as a criterion in setting environmental soil quality guidelines (SQGE) for cadmium and other non-essential heavy metals.

2.09.5
Can the Avoidance tests work as a tool to evaluate the influence of soil pH in earthworm's re-colonization after a fumigant application?
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Dazomet is a fumigant used in agriculture to control fungal diseases and nematode pests and its efficiency may vary with soil pH due to the mode of action of its active ingredient (dazomet). In consequence of this chemical behaviour, the toxic effects induced by dazomet to non-targeted organisms are also dependent on soil pH. Fumigants are often weakly persistent in soil (DT50 of 1 to 2 days) and, because of that, their effects are usually severe immediately after application but tend to disappear with time. Thus, soil recolonization may be possible few days after the fumigant application. However, the period of time needed for a recolonization may also depend on soil pH. This aspect is highly relevant since, as advocated by EFSA, the acceptability of the magnitude of effects of a product in the environment depends on the recolonization time that must be assured in a relevant time frame. Thus, the evaluation of the impact of dazomet to the environment under different soil pH values should encompass not only the assessment of its immediate toxic effects to non-target organisms but also the time needed to occur the recolonization of the soil system after its application. Avoidance tests with soil invertebrates have been used to evaluate soil contamination, but these tests may also assist in the definition of the time frame needed for a recolonization after a negative impact. This work aimed to assess the influence of soil pH in the toxicity of dazomet to the earthworm Eisenia andrei. For this, the following tests were performed with soils with different pHs to achieve two specific goals: (1) earthworm reproduction tests to evaluate the immediate effect of dazomet in E. andrei and (2) avoidance tests with soils contaminated with dazomet differently aged to define the time period needed to expect a recolonization of the impacted soil. Reproduction tests suggested that toxicity of dazomet is not pH dependent, however, EC50 values estimated for the three soil pHs were always lower than the dazomet recommended doses. This indicates that the recommended dose is critically dangerous to soil organisms. Avoidance tests with different aged soils showed that higher soil pH values promote faster dissipation of fumigant indicating that, in high-pH soils, recolonization may occur earlier. The present data evidences that avoidance tests may be an important tool to predict the time period needed to occur a recolonization of the system after a negative impact.

2.09.6
Focal and target species in ecology, nature conservation and risk assessment, a sometimes confusing matter of definition
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In conservation ecology, keystone species as well as umbrella and flagship species are coined for their special position and roles that they play in the community of which they are part. A keystone species will typically have large effects on their habitat despite its abundance and plays a critical role in the structure of the ecological community of which they are part of. In comparison, a flagship species is the poster child which is typically used in conservation campaigns and sometimes are also referred to as umbrella species. In The Netherlands and European nature conservation policy there is an important role for focal or target species: target for conservation and recovery management. These species are selected because they are rare, significantly declining or predominantly present in the Netherlands. In the EU pesticide approval regulations, focal species are important for tier 2 and 3 assessments. These species represent specific functional groups in the food web (like seed eating birds) in order to be able to calculate the bioaccumulation in these focal species and the resulting bioconcentration in the food chain. ECHA biocide regulations have a special category for target species (PT 18) in which species are targeted to be eradicated by the application of pesticides, whereas PT19 comprises compounds that work as repellent. In ecotoxicological assessment procedures used in EU- and US environmental standard setting, species are treated as statistical entities with different sensitivities which show a normal distribution as total group. One of the tail parts of that distribution is extremely sensitive while the other tail part is extremely resistent/unsensitive. Looking at circumscriptions of the term focal or target species in these fields, it is clear that they don't have the same meaning, an origin for misunderstanding and mismanagement. This paper intends to describe these differences, clarify misunderstanding and misinterpretation, and provide some solutions.

Soil Function and Biodiversity: Impacts and Resilience under Stressed Environments

2.10.1
Soil Biodiversity: Current status and further developments (Results of the SETAC Europe Special Science Symposium 14)
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Protecting the structure and functioning of soil ecosystems is one of the central aims of current regulations on chemicals in soil. This is, for instance, shown by the emphasis on the protection of key drivers and ecosystem services as proposed as protection goal options by the European Food Safety Authority (EFSA) and by the United Nations Food and Agriculture Organization (FAO). Safeguarding soil biodiversity is key to protecting ecosystem services – a fact which is also getting acknowledged by the general public. This requires insight into soil biodiversity, its role in the functioning of ecosystems and the way it responds to stress. It also asks for tools and methodologies for properly assessing biodiversity. The central aim of the SETAC Europe 14th Special Science Symposium (SESSS) was, therefore, to provide a state-of-the-art overview on soil biodiversity and soil functions, with respect to protection goal options as well as the effects of chemicals and other stressors. It was discussed how different approaches could contribute to informing future risk assessment of chemicals in the regulatory context of protecting soil organisms and soil ecosystems. Based on the contributions presented, it was concluded that current single-species testing for chemical compounds has its value. However, there should be more emphasis on multi-species interactions in the soil ecosystem, both in higher-tier testing and modelling. Widespread interest was noted in going beyond testing of single chemicals and environmental realism should be realized by accounting for the interaction of multiple stressors. Testing should acknowledge the effect of time in ecological processes, long-term exposures, development of exposure levels, multiple generation effects, delayed effects etc. Assessing biodiversity should account for regional differences in climatic factors, soil properties or land management practices. Recent developments in modelling could help with all the above aspects and should be included early in the process of risk assessment. Finally, post-registration monitoring was felt necessary to help inform on the impact of chemical and non-chemical stressors in the field, something which cannot easily be incorporated in pre-registration. It became clear that this issue in general and the implication of the recommendations made into daily practice of risk assessment of contaminants as well as protecting soils in general will require further efforts (e.g. another workshop).

2.10.2
Can metals mixtures negatively impact soil functioning in the absence of structural effects? - A TME experiment
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Soils are habitat to a variety of flora and fauna, a heavily linked ecosystem that provides essential ecosystem services. Metals, as a result of anthropogenic activities can accumulate at high concentrations in soil, leading to toxic effects threatening the ecosystem and the services it provides. Current regulatory guidelines are based on single species responses to individual metals ignoring indirect effects inherent of the inter-linked nature of ecosystems and the effects of

contaminant on ecosystem functioning. In this study we tested three mixture ratios (ARL, CSQG, SUD) of five metal oxide (lead, copper, nickel, zinc, cobalt) at three dose level (Low, Med, High) in a terrestrial model ecosystem experiment, to determine if changes to soil invertebrate community implies changes to soil functioning and/or *vice-versa*. Metal mixtures did not affect invertebrate community structure, with only slight effects detected for invertebrate traits. Despite the lack of effects on structural endpoints, mixtures produced significant reductions in organic matter decomposition and feeding activity, although this parameter recovered over time. Soil enzymes DHA and Nitrification were severely affected with a reduction in activity below 50% of control levels, in all dosed treatments. These data demonstrate that ecosystem functioning might not be directly linked to commonly measured structural parameters, and at least metal contamination enzyme activity should be followed closely. For the adequate risk assessment of metal contaminated sites, holistic site-specific approaches consider both ecosystem structure and function should be pursued if the goal is adequate environmental protection.

2.10.3

Nanofertilizers as sustainable innovative agroproducts? Let soil organisms decide!

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Current global food demands pose huge challenges for sustainable food production, putting more pressure to terrestrial ecosystems. The current negligent use of plant protection products and mineral fertilizers is one of the major causes of ecosystem services deterioration. So, it is highlighted the need to improve sustainable agricultural methods in order to meet productivity demands, without compromising environmental integrity or public health. Innovative agroproducts, such as layered double hydroxides (LDHs), have received increasing attention due to their ability to achieve a more efficient use of resources, matching crop demands with controlled nutrient supply. It is a great scientific challenge to verify if these smart nanomaterials are capable of increasing agricultural productivity without compromising functional biodiversity. Therefore, this study aimed at investigating the effects of two different types of LDHs - ZnAl-LDH and MgAl-LDH - on the avoidance behaviour and reproductive output of the soil invertebrate species *Enchytraeus crypticus*. Tests were performed with fresh and aged LUFA 2.2 soil spiked with ZnAl-LDH and MgAl-LDH (nominal concentrations ranging from 100 to 9766 mg product kg-1 soil were selected) to evaluate the influence of LDHs on the soil habitat function throughout time. The avoidance behaviour tests showed that different LDHs types induced different strategies. When confronted with ZnAl-LDH treated soils, organisms avoided at concentrations above 1563 mg kg-1, suggesting some lack of ability to deal with this constraint by preferring clean soil. As for MgAl-LDH, when soil was treated with this nanomaterial, enchytraeids preferred the treated soils, except in the highest concentration (9766 mg kg-1). Overall, avoidance behaviour were corroborated by reproduction experiments, suggesting it as a cost-effective tool for time-course assessments of habitat suitability. Enchytraeids were able to detect both LDHs types in soil due to the presence of sensory receptors and, while in ZnAl-LDH treated soil, enchytraeids considered it as harmful and moved to more favorable place to escape, in MgAl-LDH treated soil, enchytraeids considered it as more favorable. Depending on the nanomaterial type, LDHs application in soils may lead to a loss of soil habitat function due to organisms' behaviour of escaping to a more favourable place, preventing organisms of performing specific functions and potentially impairing soil ecosystem functioning.

2.10.4

Long-term effects of Cu(OH)2 nanopesticide on soil microbiome

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Cu-based nanopesticides, with nanosized active ingredient-$Cu(OH)_2$, are commonly used in vineyards, to protect crops from fungal and bacterial diseases. However, their antimicrobial broad-spectrum may result in unintended consequences on soil microbiome (SM). Thus, our study aims to evaluate the long-term effects of a $Cu(OH)_2$ nanopesticide [Kocide®3000] on the SM, simulating applications on vineyard areas. Moreover, *Porcellionides pruinosus* (isopods) was included in this experiment, as one of the key organisms that exist in farming-based systems. Microcosms were setup with 5 replicates per condition including: unexposed soil (CT), soil spiked with $Cu(OH)_2$ nanopesticide (K), or ionic $Cu(OH)_2$–as a ionic control (IC). Copper concentrations tested were 0.45 mg(Cu).kg^{-1} soil (single application) and 19.86 mg(Cu).kg^{-1} soil (season application), according to the manufacturer recommendations. After 90 days of exposure, structural effects (by denaturing gradient gel electrophoresis of both bacterial and fungal communities) and functional effects (by β-glucosidase (BG) activity and community level physiological profiling-CLPP) were assessed. Copper dissolution was quantified by soil porewater measurements (by flame Atomic Absorption Spectrometry). In parallel, microcosms were setup in the same conditions but with the isopod (n=3 individuals per replicate). After 90 days, only the porewater obtained from spiked soils with K (season application) revealed a significantly higher copper level, regardless of isopods presence. The soil bacterial community exposed to both K or IC presented a significant richness decrease, and diversity and evenness increase, compared to the CT. Fungal communities were mainly affected by the K, resulting in a significant lower diversity and richness at season application. In the presence of isopods significant effects were not observed. At functional level, a significant decrease on BG activity and an increase on carbon consumption (CLPP) were observed for communities exposed to the K. The effects on CLPP were not observed in soils with isopods, suggesting an attenuation of the effect of the K. In the presence of isopods, the BG activity showed to be significantly lower in soils spiked with K. This study demonstrates that $Cu(OH)_2$ nanopesticide significantly impact the SM, possibly affecting its ecological role. On the other hand, invertebrates may attenuate this effect, perhaps due to microbial growth stimulus resulting from soil oxygenation.

2.10.5

PESTICIDES IMPACT ON SOIL MICROBIAL COMMUNITY COMPOSITION AND ACTIVITY

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Assessment of the effect of crop protection agents on the composition and activity of the microorganisms is a classical problem of microbiology. Although the impact of pesticides is considered to be negative, the scope of this phenomenon, presence and duration of its chronic effects, consequences of regular pesticide application for a microbial community are not determined. These problems can be solved only with a help of modern molecular-genetic methods in conjunction with the traditional methods of soil microbiology. The impact of three types of pesticides (herbicide metribuzin, insecticide imidalocloprid, and fungicide benomyl) has been assessed using the method of next-generation sequencing (NGS). The pesticides have been applied both separately and together at one- and ten-fold rates on the structure of microbial complexes and indicators of biological activity of Albic Retisols (the Moscow region). It has been discovered that pesticides have a greater impact on a fungal community compared with a prokaryotic one where only the changes of phyla of actinobacteria and proteobacteria occurred. The research of fungal communities based on the results of molecular-genetic analysis has revealed two dominant divisions of fungi in all soil samples - *Ascomycota* (72.6 ± 8.0%) and *Basidiomycota* (26.0 ± 7.7%). Moreover, the samples with ten-fold pesticide application rate (both in a mixture and separately) have been revealed to have the amplification of the *Basidiomycota* division. Pesticide application has a short-term stimulating effect on the carbon content of microbial biomass. It has been shown that insecticide imidalocloprid stimulates nitrogen fixation whereas other types of pesticides do not affect this factor. The study was funded by RFBR according to the research projects No 18-316-00054 and No 18-016-00130.

2.10.6

Agroecosystems' soil quality under long-term exposure to pesticides

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Pesticides and their metabolites are pollutants that tend to accumulate in agroecosystems due to extensive land use. In the western state of Paraná in Brazil, the extensive use of pesticides on farms has been identified as the biggest problem that affects farms' sustainability and soil biodiversity. The region of Santa Tereza do Oeste was selected as a study area since it presents the ideal conditions for impact assessment of different land uses and occupations, divided into urban and agricultural areas and the forest from the National Park of Iguazu (along with its edge). The objective of this study was to identify the impact of long-term use of pesticides on agroecosystems and their effects on soil quality and microbiome. Soil samples were collected at 12 points from each area. We evaluated soil's physical-chemical properties (e.g fertility, pH, CEC, SOC, bulk density, porosity, humidity), pesticides residues, soil enzymatic activities and the microbiome community composition (metabarcoding - 16S). The physical-chemical results show higher concentrations of nutrients and soil organic carbon (SOC) in soils from forest and its edge and better physical structure as well, indicating degradation in soils from rural and urban area. Similar concentrations of pesticides' residues were identified in all the areas. Arylsulfatase and alkaline phosphatase activities presented higher values in soil from forest and its edge, while the β-glycosidase activity was higher in soils from the urban area and the

acid phosphatase shows higher activity in soils from rural areas. The long-term pesticides use in agroecosystem decreased the diversity of the bacterial community. The results indicate that some key factors associated with pesticides long-term use impacts soil quality by changes in nutrient availability, enzymatic activities and physical degradation. This project was supported using seed funding from Cardiff University's GCRF QR Funding from the HEFCl for Wales. Dr Nuno Ferreira show by a MSC COFUND Fellowship (H2020-COFUND-SIRCIW>MINT-512202) through EU, Welsh Government and Cardiff University. Natalia Pereira was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES).

Advances in Bioavailability Science and Application to Chemical Regulation

3.01.1
BIAVAILABILITY OF ORGANIC CHEMICALS IN SOILS AND SEDIMENTS: POTENTIAL REGULATORY ASPECTS?

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The bioavailability of organic chemicals is an important area of scientific investigation for environmental scientists, although this area of study remains only partially recognized by regulators and industries working in the environmental sector. Regulators have recently started to consider bioavailability within retrospective risk assessment (rRA) frameworks for organic chemicals; by doing so, realistic decision-making with regard to polluted environments can be achieved, rather than relying on the traditional approach of using total-extractable concentrations. However, implementation into prospective risk assessment (pRA) remains difficult because scientific developments on bioavailability are not always translated into ready-to-use approaches for regulators. The presentation provides an overview of existing bioavailability concepts and methods for organic chemicals, options for their innovative application and standardization, as well as pathways for the justifiable implementation into RA and regulation. The presentation will build on a book, edited by Springer (Bioavailability of Organic Chemicals in Soil and Sediment, The Handbook of Environmental Chemistry, no. 698), that will published in late 2019/early 2020, with individual chapters from main actors in bioavailability science and regulation, and edited by J.J. Ortega-Calvo and J.R. Parsons (UvA, The Netherlands). Over the last 30 years, numerous publications have discussed the concepts and definitions of bioavailability of organic chemicals. Still with a general consensus at the scientific community on the relevant *bioavailability processes*, the main schools of thought considered *bioavailability* (focusing on the aqueous or dissolved contaminant), *bioaccessibility* (incorporating the rapidly desorbing contaminant in the exposure), *chemical activity* (determining the potential of the dissolved contaminant for biological effects) and *non-extractable residues* (NER). These concepts are the basis for different methodologies, ready to be used in regulation (desorption extraction, passive sampling, NER fractionation and biological tests) and mechanistic studies that consider the different processes that are involved (contaminant soil/sediment interactions, transport and passage across cell membrane, and biological processing towards toxic effects or biodegradation).

3.01.2
Bioavailability as A Determinant in Assessing Need for Remediation

A.R. Taylor, University of California, Riverside / Environmental Sciences; J. Wang, D. Schlenk, J. Gan, University of California, Riverside / Environmental Science

Numerous soil and sediment sites in the world are contaminated with metals and hydrophobic organic contaminants (HOCs). At most of such sites, contamination usually occurred years or decades ago. Due to aging and other biogeochemical processes, the bioavailability of such contaminants has likely diminished substantially over time, as compared to a fresh contamination scenario. It is crucial to consider bioavailability when assessing the need for and predicting the effectiveness of remediation treatments. Here we used a historically contaminated sediment as an example to illustrate the importance of incorporating bioavailability in black carbon-based remediation. Black carbon, such as activated carbon and biochars are known for their strong sorption capacities for HOCs such as PCBs and DDTs. A marine sediment collected from a Superfund site off the coast of Los Angeles contained high levels of DDT and metabolites. The sediment was amended with two forms of activated carbon (powdered and granular) and a biochar. The amended sediments were incubated for up to one year in seawater, and both the freely dissolved concentration C_{free}, and the biota-sediment accumulation factors (BSAFs) were followed to assess the influence of black carbon amendment on sequestration of the DDTs at different time points. C_{free} decreased with time in all treatments compared to controls, and was closely correlated with body residue (C_b) in a marine benthic invertebrate ($R^2 = 0.66$, $p < 0.0001$). BSAFs also decreased over time compared to controls. However, both C_{free} and BSAFs were very small, even in the unamended control sediments, ranging from nd to 1.34 ng/L and from nd to 0.024, respectively. Surprisingly, addition of black carbon materials, at even 2% (w/w), did not lead to significantly enhanced sequestration of these HOCs, as compared to the unamended sediment control. The limited effectiveness of black carbon amendment was attributed to the very low HOC bioavailability in the sediment. The findings suggest that at historically contaminated soil or sediment sites, the bioavailability of the target contaminants are likely already low due to aging. Remediation inventions, such as amendment of a strong sorbent, may have limited effectiveness for such sites. Therefore, bioavailability of a given site should be quantitatively determined through chemically and biologically based assays when assessing the need for or benefits of remediation.

3.01.3
Improving modelling of chemical bioavailability in terrestrial and aquatic ecosystems: Novel approaches estimating association of neutral and ionizable organic chemicals to dissolved organic carbon

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Dissolved organic carbon (DOC) plays a fundamental role in influencing bioavailability of organic chemicals in terrestrial and aquatic ecosystems. DOC fluxes can enhance chemical mobility, determining chemical loadings toward surface waters and groundwaters and reduce bioavailable concentrations in aquatic phases. The correct prediction of the partition (K_{DOC}) and distribution (D_{DOC}) coefficients between chemicals and DOC is therefore crucial in estimating freely dissolved bioavailable concentrations in soils, sediments and aquatic ecosystems and therefore in assessing actual chemical exposure and potential risks for organisms. In the present study, quality criteria for reliable K_{DOC} and D_{DOC} measurements were established to assemble a robust dataset of K_{DOC} and D_{DOC} values and novel predictive regressions for K_{DOC} and D_{DOC} estimation of neutral and ionizable chemicals were proposed. The different DOC origin (soil, surface water, wastewater and Aldrich humic acid (HA)) was accounted for. Data suggest that proposed regressions have a good predictive capability with RMSD (root mean square deviation) generally smaller than 0.3 log units and could be a useful tool to be implemented in research and regulatory models to better account for the role of DOC in affecting chemical bioavailability in various environmental media.

3.01.4
Regulation of pollutants bioavailability in soil with the help of natural adsorbents

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Currently, there is an increasing tendency in process of soil pollution with various pollutants. Their bioavailability is one of the most important factors that regulate the pollutants behavior in the environment. The use of natural adsorbents can significantly reduce the contamination of soil and adjacent. We demonstrate in this presentation that application of natural adsorbents (mineral, organic and carbonic) may accelerate biodegradation of "degradable" pollutants and/or reduce bioavailability of highly persistent (slightly degradable) and nondegradable pollutants. Introduction of granular activated carbon (GAC) could significantly accelerate the bioremediation of various soils highly contaminated with crude oil and products (5-15% w/w) by reducing the toxicity of contaminated soils due to mostly reversible adsorption of petroleum hydrocarbons. In addition, the GAC amendment sharply reduced migration of highly toxic intermediate oxidized petroleum products to groundwater. At the same time, only insignificant amounts of 5-nuclear PAHs (including benz(a)pyrene) were accumulated in the soil: no more than 20% compared to the unamended control, but those remained practically unavailable to plants and soil biota. In the other experiments, it was proved that the introduction of GAC into the soil historically contaminated with PCBs (near Capacitor plant, Serpukhov) accelerated the biodegradation of low chlorinated (3-4 Cl) congeners and simultaneously reduced the availability of highly chlorinated (5-8 Cl) congeners of PCBs to plants and biota. Similar results were observed in soils sampled near the burial site of prohibited pesticides (DDT and HCCH) in the Rostov Region. In the GAC or biochar amended soils, degradation of some DDT metabolites were accelerated, while the accumulation of those pesticides in plants were sharply reduced. It was also shown that the use of a relatively expensive GAC can be replaced by a cheap adsorbent "biocoke" - a pyrolyzed sewage sludge. Our experiments showed that heavy metals presented in the biocoke remained inaccessible to biota due to some increase in soil pH because of presence of alkaline-earth metals. This gives the possibility to use the biocoke as a soil ameliorant and a source of biogenic elements (mainly phosphorus and potassium) to increase soil fertility, as well as to use this very chip adsorbent for the restoration of technogenic contaminated soils. This was supported with RFBRmk 19-29-05265

Advances in Exposure Modelling: Bridging the Gap Between Research and Application

3.02.1
Bridging the Gap between Research and Application with EASE Suite

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Toxicology; A. Sangion, University of Toronto Scarborough and ARC Arnot Research and Consulting Inc. / Department of Physical and Environmental Sciences; L. Li, University of Nevada, Reno / Community Health Sciences; J.M. Armitage, AES Armitage Environmental Sciences, Inc / Physical and Environmental Sciences; L. Toose, ARC Arnot Research and Consulting. Inc; T.N. Brown, TNB Research / .
Thousands of chemicals require chemical assessment. For most of these chemicals the required assessment information is quite limited. In some cases, there is limited time to make decisions and the pace of scientific advancements outpaces knowledge transfer to industry and regulatory scientists. These issues pose scientific and regulatory challenges towards the implementation of "state of the science" techniques for practical applications in chemical assessment and management. Here we introduce the Exposure And Safety Estimation (EASE) Suite platform to bridge the gap between evolving scientific research and regulatory assessment challenges. EASE Suite is comprised of curated empirical databases, quantitative structure activity relationships (QSARs), and tools to aid chemical assessment decision-making. EASE Suite includes multi-media mass-balance environmental fate, bioaccumulation and exposure models such as RAIDAR and RAIDAR-ICE as well as the CiP-CAFE tool for estimating life cycle chemical mode-of-entry and emission rates. Using only chemical structure as an input parameter EASE Suite provides overall persistence (POV) and bioaccumulation metrics, such as the biomagnification factor (BMF) in a range of aquatic and air-breathing organisms. Large numbers of chemicals can be screened and evaluated for Persistence and Bioaccumulation as well as exposure potential based on chemical structure alone. Including a chemical production volume estimate provides exposure estimates in a range of ecological receptors and humans. Effect thresholds can estimate margin of safety and potential risk of adverse effects. Here we apply EASE Suite as a case study for screening-level ecological assessment of 144 organic chemicals, comprising a range of chemistries and use scenarios, using only chemical structure and production volume estimates as system input parameters. The POV for these chemicals in the RAIDAR environment ranges from about 10 to 1,000,000 h. The maximum BMFs in representative RAIDAR vertebrates range from about 0.002 to 100. The maximum exposures span 12 orders of magnitude demonstrating the capacity to quickly screen and differentiate chemicals for exposures to ecological receptors. EASE Suite provides opportunities to address regulatory challenges for new and existing chemical assessments for ecological and human health objectives. Further expansion of the databases and tools in the EASE Suite platform are briefly outlined.

3.02.2

The new EUSES for REACH and Biocides environmental exposure assessment: implementation plan and challenges

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Both the REACH Regulation (EC) No 1907/2006 and Biocidal Products Regulation ((EU) 528/2012) require that the chemicals addressed by the respective legislations are used safely. EUSES (the European Union System for the Evaluation of Substances) is a the tool enabling the environmental exposure assessments. The tool was last updated in 2004 and therefore ECHA decided to develop the it to improve its usability and to include the most relevant scientific updates emerged over last 20 years. Possible updates were identified, discussed and prioritised in a dedicated workshop with stakeholders (2018) and a pre-study performed to better analyse the IT development, define a plan and get an overview of the associated costs for developing the "new" EUSES tool (2019). The outcome of process was a decision to develop, over a period of 2 years (2020-2021), a new tool, containing a guided workflow to perform both Biocides and REACH relevant assessments, while maintaining the full flexibility of the current EUSES tool. The release scenarios and the fate and distribution modules (including their connection) are in the focus of the update process. Following topics are included: a) harmonisation (where possible) of release scenarios between REACH and Biocides; b) complementing the release scenarios covered by EUSES, for example including the direct release to agricultural soil at the local scale; c) implementation of SimpleTreat (4.0); d) updating man via environment to better estimate transfer from environmental compartments to crops for organic substances and to support the assessment of metals in the food chain by means of (measured) transfer factors (e.g. soil to crops); e) improvement of regional contribution, e.g. by introducing the non-stationary version of SimpleBox in the tool; f) introduction of the possibility to perform more site specific assessment, e.g. by specifying characteristics of the source of air emission. In this presentation, we discuss in more detail: a) general description of the new tool; b) actual plan for EUSES update; c) description of the main topics that will be implemented (update of existing modules and introduction of new modules); d) outlook for the future.

3.02.3

Advances in Exposure Modelling - Spatial and Temporal Refinements in Receiving Water Modelling in Australia

C.I. Lee-Steere, Australian Environment Agency Pty Ltd

Screening level methodology for estimating environmental concentrations of pesticides arising from runoff involves a "one size fits all" approach that applies conservative default values. A risk deemed to be acceptable at this level does not require further assessment. Unfortunately, where the ability to refine modelling of exposure estimates does not exist, the screening assessment results in large numbers of substances being identified as "risky" with either rejection of registration, or imposition of risk mitigation measures that do not reflect reality. This essentially reflects a lack of context in terms of geographic location or time, and runoff risk is therefore assumed to be equal regardless of where in the country application occurs, or at what time of the year. A large body of real world data exist that enable such refinements to be undertaken in exposure modelling. These include use of GIS based systems to obtain knowledge of soil types and slopes within growing regions and such data allow spatial considerations to be included in the exposure modelling. Additional use of available data such as rainfall and stream flow rates further refine the spatial scale of assessments and allow assessment on a temporal scale. Such a method has been developed in Australia and at the highest tier of assessment, distributions of theoretical receiving water concentrations in given regions are developed allowing risk to be described in terms of probability of exceeding allowable concentrations. The problem facing regulators with the use of such data is the size of data sets and the complex relationships that require development. In Australia, high tier runoff assessments are undertaken based on developed relationships underpinned by millions of data points. These assessments can only be performed in a practical manner through the development of bespoke software due to the complexity of modelling exposure in different regions and time periods, and the high propensity for human error. A software tool has been developed to address the need for rapid, robust and contextual environmental risk assessments in Australia. The software and its output have been relied on in Australia for regulatory decisions and applied by industry in preparing their submissions. The process is adaptable to other countries and regions.

3.02.4

Multimedia modelling of nano- and microplastics in the environment

J.T. Quik, RIVM / DMG; J.A. Meesters, National Institute for Public Health and the Environment (RIVM) / VSP; A.A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group

Persistence of nano- and microplastics in the environment is a concern. Recently several field studies have detected the presence of microplastics in remote areas, indicating their high transport potential. However, quantitative estimates of exposure concentrations to nano- and microplastics is lacking, due to fragmented field campaign's, focussing on a single compartment, e.g. soil or water and specific locations. Furthermore, the detection methods applied are generally not applicable to nanoplastics, making it a big data gap in knowledge on fate and occurrence in the environment. A commonly applied tool in assessing fate and exposure to chemicals and particles is the use of multimedia fate models. Here, we present the first such model at the screening level for nano- and microplastics (NMPs) based on SimpleBox4nano [1]. SimpleBox solves simultaneous mass balance equations using simple matrix algebra. These link all concentrations and transfer processes using first-order rate constants for all processes known to be relevant for NMPs. The first-order rate constants are obtained from the literature. The model accounts for emissions to air, soil, water and atmosphere. To illustrate the applicability of the model and the need for a fate model specific for nano and microplastics it is applied to microbeads based on available emissions data. So called Predicted Exposure Concentrations (PECs) are presented for air, soil, water and sediment compartments at the regional, continental and global scales. Uncertainty in the PECs is due to the emission estimates and input parameters describing fate of the NMPs. Particle size, density and the fragmentation or degradatation rate constant explain most of the variation in PECs. As expected a large effect is found based on density, with low density NMPs largely remaining in the water fase in the fresh water compartment and transported to sea. PECs for sea water show a great sensitivity to the fragmentation or degradation rate, where unrealisticly high PECs are calculated when underestimating this process. This multimedia fate model for nano and microplastics provides a convenient tool for assessing exposure to different types of nano and microplastics at a screening level. Specifically, for nanoplastics this model provides an invaluable asset in future risk assessment studies as there is currently no adequate detection method. [1] www.rivm.nl/simplebox4nano

3.02.5

Poster spotlight: Bottom up exposure modelling of engineered nanoparticles in urban environments: tools for deciphering spatial and temporal trends

M. Domercq, Stockholm University, ACES

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3.02.6

Poster spotlight: How to make one's (spatiotemporal multimedia nanomaterial) exposure model as extensible, usable and useful as possible

S. Harrison, UK Centre for Ecology & Hydrology / Environmental Contaminants

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3.02.7

Poster spotlight: Fate and transport models for nano- and microplastics
A. Praetorius, University of Amsterdam/IBED Institute / Department of Environmental Science and Analytical Chemistry ACES
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Advancing our Understanding of Contaminants of Emerging Concerns Associated with Plastic and Microplastics: Identification, Analysis, Occurrence and Effects

3.03.1
A Multihyphenated TGA-FTIR-GC-MS Approach For Characterisation Of Polymer Type In Microplastic Research
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A major hurdle in microplastic research is the reliable identification of specific polymers found in the environment. To date, many studies rely on visual identification with little or no polymer characterisation or identification. Methods such as optical spectrometers (i.e. Fourier Transform InfraRed or Raman) can be subjective or suffer from poor sensitivity. Thermogravimetric analysis Fourier transformed Infrared - gas chromatography - mass spectrometry (TGA-FTIR-GC-MS) offers a unique characterisation platform that provides both physical and chemical properties of the analyte and as such generates a diverse array of data that can be used to assign polymer identity to plastics samples collected from the environment. In this work a library of 10 polymers was generated using virgin and post-consumer polymers. This library comprises of the TGA inflection points and mass of remaining residue following pyrolysis which in some cases can be indicative of the polymer type. FTIR analysis of the pyrolate for each polymer was able to differentiate between all but the polypropylene and polyethylene polymers. Finally, GC-MS was able to differentiate between the unique chemical fingerprints of the pyrolate for each polymer type in the library. This library was then used to characterise a range of mesoplastics collected from a beach in the U.K and microplastics from 3 beaches in South Africa. In the case of the mesoplastics, unambiguous identifications of the polymer type was possible for all samples with polyethylene being the most frequently identified polymer. The microplastics from South Africa could also be identified based either upon the TGA or the GC-MS data. This study demonstrates the suitability of TGA-FTIR-GC-MS to identify polymers in meso and microplastics based upon their physical and chemical properties across 3 different hyphenated platforms using a single sample, thus greatly improving the chances of identifying synthetic polymers present in the environment.

3.03.2
Chemical composition and ecotoxicity of plastic and car tire rubber leachates to aquatic organisms
A. Booth, SINTEF Ocean / Environmental Technology; L. Sørensen, SINTEF Ocean / Environment and New Resources; K. Jayasena, E. Fabbri, University of Bologna / Department of Biological Geological and Environmental Sciences; M. Capolupo, University of Bologna / CIRSA via S Alberto Ravenna
Most thermoplastics contain a variety of additive chemicals that can represent a high percentage of the final plastic materials (up to 50%). The current study characterised the chemical content of freshwater and marine leachates produced from CTR, PP, PET, PS and PVC, and assessed their adverse effects on freshwater (*Raphidocelis subcapitata*) and marine (*Skeletonema costatum*) microalgae. PP, PET, PS, PVC (cryomilled, 1000 µm sieved fraction) and car tire rubber (CTR)-derived granulate (1-2 mm) were subjected to a non-target screening for organic additive chemicals by solvent extraction and analysis by GC-MS. Leachates of material were generated in seawater, marine algae growth media and freshwater algae growth media. Metal leachates were characterized by ICP-MS and organic additives by solvent extraction followed by GC-MS. Leachate toxicity towards *R. subcapitata* and *S. costatum* was determined as inhibition of growth over 72 h. A range of organic additive chemicals were identified in the polymer materials and used as a basis for studying the additive compounds in the leachates. The highest number of organic compounds was found in PP (19), followed by CTR (17), PVC (9), PS (9) and PET (1). The concentrations of the different additives were material-specific and varied from tens of ng to hundreds of µg/L. The trends in organic additive concentrations were similar between freshwater and marine media for organic compounds, but more varied for inorganics. Leachate toxicity varied between the different polymers and there were observable differences between the two algal species for some leachates. CTR produced the most toxic leachate, with EC_{50} values of 0.5% and 19% of total leachate towards the freshwater and marine algae, respectively. PP exhibited a similar toxicity towards marine algae to that observed for CRT (EC_{50} 18%), but showed the lowest toxicity towards freshwater algae (EC_{50} 64%). PVC had high toxicity towards freshwater algae (EC_{50} 1.6% of total leachate), but the second lowest toxicity towards marine algae (EC_{50} 35%). The higher toxicity of CTR and PVC leachates was directly linked to the higher additive chemical content of these leachates. This study provides new knowledge on the chemical characterisation and ecotoxicological effects of chemical additive leachates from plastic and rubber materials under freshwater and marine conditions.

3.03.3
In vitro toxicity and chemical composition of plastic consumer products - What is in and what is getting out?
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It is well established that chemicals in plastics migrate from consumer products during and after use and, thus, represent a source of exposure to wildlife and humans. In our previous study, we extracted 34 everyday plastic products with methanol and found that three-third contained chemicals inducing *in vitro* toxicity. Out of the >1,000 chemicals detected with gas chromatography–mass spectrometry, 80 % remained unknown. Because not all of these chemicals will migrate under more realistic conditions, our current study aims at investigating the toxicological and chemical profiles of the chemical mixture *leaching from* plastics into water and comparing them with the compounds *extracted with* methanol. Based on our previous work, we selected 24 products available on the German market made of high-density polyethylene (HDPE, 1 item), low-density PE (LDPE, 4), polystyrene (PS, 4), polypropylene (PP, 2), polyethylene terephthalate (PET, 1), polyvinyl chloride (PVC, 4), polyurethane (PUR, 4) and polylactic acid (PLA, 4) and performed migration experiments for 10 d at 40 °C in water. We analysed the *in vitro* toxicity of the migrates regarding baseline toxicity, oxidative stress response as well as estrogenicitiy and antiandrogenicity and characterised the chemical composition by non-target high resolution mass spectrometry (LC-QTOF-MS/MS). We compared the chemical and toxicological profiles of the migrates and the extracts. All products released chemicals into water that triggering baseline toxicity and 22 samples also activated an oxidative stress response. While 13 samples inhibited the human androgen receptor, only one PVC sample released chemicals activating the human estrogen receptor alpha. Overall, the *in vitro* toxicity of these migrates (chemicals released in water) was less pronounced than of extracts (chemicals released in methanol) but was also dependent on the endpoint and product analysed. We detected over 22,000 chemical features (peaks) in the 24 migrates, with 18 common features in >9 samples. Our findings indicate that plastic products not only contain but also release a wide variety of chemicals which trigger toxicity, including unspecific toxicity, oxidative stress and endocrine activity.The on-going chemical analysis will reveal which of the chemicals in plastics will leach in water and which in methanol.

3.03.4
Bioconcentration of organic contaminants in fish in the presence of microplastics: Is the "Trojan horse" effect a matter of concern?
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The role of microplastics (MPs) as vectors for the uptake of hydrophobic organic chemicals in aquatic organisms, a phenomenon commonly termed as “Trojan Horse” effect, is still under discussion. Therefore, this study aimed to compare the bioconcentration of two hydrophobic organic chemicals (i.e. chlorpyrifos and hexachlorobenzene) in freshwater fish exposed to the chemicals in absence and presence of contaminated MPs. Two 14-days semi-static experiments were carried out with adult *Danio rerio*, each consisting of (1) a control treatment, (2) a chemical treatment (water spiked with 15.5 µg/L chlorpyrifos or 4.2 µg/L hexachlorobenzene) and (3) a treatment in which fish were exposed to a mixture of contaminated water (15.5 µg/L chlorpyrifos or 0.6 µg/L hexachlorobenzene) and contaminated MPs. For this, 100 mg polyethylene MPs/L (mean particles size 150 µm, Sigma-Aldrich) with a concentration of 45 µg/g chlorpyrifos or 16 µg/g hexachlorobenzene, were added. Each test unit consisted of a 3 L beaker with dechlorinated tap water containing six adult Danio rerio (n=6). The test medium was renewed at days 4, 8 and 11 and fish, water and MP samples were taken on these days as well as after 14 days. The concentration of chlorpyrifos and hexachlorobenzene in the samples was determined using gas chromatography (Agilent 7890A) coupled with mass spectrometry (Agilent 7000 GC/MS Triple Quad). The results show, that the chlorpyrifos bioconcentration factor (BCF) was higher in the absence of MPs compared to their presence. This difference was significant after 14 days with a log BCF of 4.62±0.09 L/kg for chlorpyrifos in the absence of MPs compared to a log BCF of 4.15±0.13 L/kg in the presence of MPs. The hexachlorobenzene BCF was significantly higher without MPs (log BCF between 4.19±0.24 and 4.86±0.46) as compared to their presence (log BCF between 3.08±0.2 and 3.64±0.27) after all exposure periods (i.e. 4, 7, 10 and 14 days). The study shows that polyethylene MPs are not enhancing the bioconcentration of the tested hydrophobic organic chemicals in freshwater fish. This is in agreement with the findings of recent modelling studies and suggests that MP pollution is not likely to increase the aquatic risks of contaminants that sorb to plastic.

3.03.5
Desorption of organic pollutant from microplastics under simulated physiological conditions
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Many studies have shown the presence of microplastics (MP) in different compartment of the enviroment and ingested by biota. In addition to the deleterious physical risks of such exposure, high concentrations of hydrophobic organic pollutants have also been reported to be associated with these particles and the possibility of transferring these pollutants from MP to organisms by ingesting them has been the subject of studies, as they are still scarce conclusive works on this subjected. In this aspect, the goals of this study were to evaluate the adsorption potential of high density polyethylene (HDPE) MP by the pesticide contaminant 14C-chlorpyrifos in freshwater condition and the desorption in simulated physiological condition of the gastric fluid. MP samples were prepared from pellets HDPE were crushed on knife mills and sifted by size from 100-700 µm and this samples were analyzed by elemental carbon analysis and scanning electron microscopyy. The adsorption and desorption process was performed in equilibrium batch and the adsorbed amount of chlorpyrifos was quantified in the adsorbent phase (MP) and by the mass balance in the aqueous phase by liquid scintillation couting. The results indicated that the adsorption equilibrium was achieved between 96 and 168 h and at that moment, the total of 44.77 ± 1.72% of the applied product was adsorbed to the MP particles, presenting coefficient of adsorption linear to Kd=4,451.99 ± 397.82 L Kg-1. The desorption result in simulated physiological system, considering three different digestion times, 4, 8 and 12 h, indicated that, respectively, 16.93 ± 4.44, 17.55 ± 0.58 and 22.45 ± 1.73% of the product contaminated the gastric fluid solution, presenting linear desorption coefficient (Kd), respectively, 13,476.35 ± 4,593.53, 11,786.54 ± 481.36 and 8,707.21 ± 802,11 L.Kg-1. However, when comparing this concentration of desorbed contaminant in gastric fluid by the concentration of contaminant present in river water (36.09%) after sorption equilibrium, these results indicate that the transfer of chlorpyrifos is low. Nevertheless, bearing in mind the growing environmental contamination by MP and pesticides on freshwater, as well as reports of ingestion of this particles by innumerous species, the dynamic of adsorption and desorption of these emerging contaminants from MP to the aquatic biota still deserves to be investigated with more details, considering exposing trials on organisms and also simulated intestinal fluids. The authors thank the National Council for Scientific and Technological Development (CNPq) (process number 141021/ 2019-5) and Foundation for Research Support of the State of São Paulo (FAPESP) (process number 2018 / 21733-0) for the financial support

3.03.6
From Beakers to Basins: How experimental data help to assess the contaminant transfer potential of microplastics in the field
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Microplastic particles are ubiquitously detected in all environmental compartments. Despite intensive public and scientific discussions, their potential to transport contaminants in rivers and oceans is still under assessment. To consider such particle facilitated transport, this project aims to quantify the underlying sorption mechanisms and to develop a comprehensive mechanistic model with parameter values derived from experimental data. The developed models consider material characteristics, substance properties and different types of sorption isotherms. The sorption kinetics was modeled as a combination of external mass transfer governed by diffusion through an aqueous boundary layer and intraparticle diffusion within the plastic particles. Which of these processes controls the kinetics depends on the sorption strength, particle size, diffusion coefficients, and time. To model coupled mass transfer, a semi-analytical model was developed in case a linear sorption isotherm was determined and a numerical approach was applied for the case of non-linear sorption. Both model types were successfully validated for several plastic types, allowing to accurately describe the measured kinetics. To broaden the scope and environmental relevance of the work, further experiments were performed. It was revealed that the presence of additional natural sorbents significantly influenced both equilibrium partitioning and desorption kinetics. Due to the combination of experimental and mechanistic modelling tools, it was possible to elucidate coupled mass transfer processes for different experimental and field settings. Eventually, it was ascertained that time scales observed under experimental conditions may not be transferred to field conditions without an appropriate mechanistic model accounting for coupled mass transfer and the specific boundary conditions. Appropriate hydrodynamic relationships coupled to a thorough mass transfer analysis can serve to assess the vector function of pollutant-loaded particles and to evaluate whether microplastics rather act as a passive sampler or show potential to facilitate long-range contaminant transport. Moreover, as the theoretical mass transfer considerations also apply to other suspended particles, well-defined microplastic particles are ideally suited to perform in-depth mass transfer studies and to act as surrogates for particles occurring in the environment, including microplastics in urban runoff and contaminated sediment.

3.03.7
TRACKING OF PLASTIC EMISSIONS FROM AQUACULTURE INDUSTRY
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Demands to further intensify aquaculture production is increasing to meet the growing needs of the market. In Norway, farmed salmon has become a significant source of national income with an excess of 1 million tons produced every year [1]. The expansion of the industry and the diversity of materials used to build and maintain the aquaculture systems have paralleled the development of synthetic polymers over the last decades. Synthetic fibre ropes offer greater strength, durability and easier handling at a cheaper price than those using natural fibres. Plastic materials within aquaculture sites are typically maintained and controlled for chemical degradation, biofouling and corrosion, and are regularly inspected to ensure strength and stability. Sources of plastic emission from aquaculture include lost gear, broken and fragmented equipment and fine fragment loss through abrasion from within feed and fish transporting equipment [2, 3]. However, the extent of these plastic losses has yet to be estimated and this knowledge gap needs to be filled to fully address the potential impact such losses may have on the environment [4, 5]. It is therefore crucial to look at the challenges and solutions related to the release of microplastics from the aquaculture industry. The aims of the present project are : a) to identify the sources of emissions of plastic and micro-plastic in the sea from aquaculture facilities; b) to identify and quantify the contribution of aquaculture operations to plastic found in the immediate vicinity of sea farms; c) identify processes within the seafood production largely responsible for plastic discharge and suggest measures to reduce eventual emissions; d) encourage active exchange of information and discussion between academia, industry and stakeholders for a joint solution towards marine plastic pollution.

3.03.8
Neat or on the rocks? Comparing the effects of virgin and contaminated polystyrene nanobeads on zebrafish larvae
C. Parenti, University of Milan / Biosciences; A. Ghilardi, University of Milan / Department of Biosciences; C. Della Torre, University of Milan / Biosciences; G. Caorsi, S. Magni, L. Del Giacco, University of Milan; A. Binelli, University of Milan / Department of Biosciences
The physicochemical properties of nanoplastics (NPs) make them very suitable for the transfer of chemical contaminants, potentially enhancing their risk for the aquatic organisms. Nevertheless, few controversial evidences exist on the toxic effects caused by NP adsorption of pollutants.Triclosan (TCS) is an antimicrobial agent included in several personal care products (PCPs) and is considered as a contaminant of emerging concern for aquatic ecosystem. The adsorption capacity of TCS on the most used plastics polymers, including polystyrene (PS), was previously demonstrated. Therefore, we contaminated a commercial PS powder with TCS and we performed a 7-days exposure to TCS, virgin and treated NPs on larvae of *Danio rerio*. Ingestion of NPs was assessed with advanced microscopy, and sub-individual and individual effects were investigated. Moreover, to investigate if TCS, virgin and contaminated NP play a different role in the modulation of protein expression in *D. rerio*, we applied a functional proteomics analysis. Imaging observations showed that NPs can be ingested and that they mainly accumulate inside the gastrointestinal tract. The accurate analysis of microscopy section revealed also the translocation of NPs to other body tissues and organs, such as gills, neuromasts and eyes. Anyway, NPs seem to not substantially affect fish defence system, since we did not find any significant variation in analysed biomarkers. The most significant effects were related to the TCS exposure. Behaviour test underlined an opposite effect of TCS alone and combined with NPs on fish locomotor activity, since the distance moved was significantly decreased by the TCS exposure, but significantly increased after the exposure to contaminated particles. Moreover, we found a considerable quantity of modulated proteins in the treated groups, respect to control. The purpose of this study was to open a new perspective on investigating the risk associated to the presence of NPs as carrier of environmental pollutants. The overall results showed that virgin NPs do not have significant effects on fish and that chemicals alone remain the most hazardous pollutants. Test on locomotor behaviour showed a change of TCS toxicity target when adsorbed on NPs, which needs further investigations. Finally, proteomics provided the identification of potential novel biomarkers to clarify the toxicity targets of NPs.

3.03.9
Poster spotlight: The global environmental footprint of indigo denim fibers
S. Athey, University of Toronto / Earth Sciences
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3.03.10

Poster spotlight: Biofouling on solid litter: what do they prefer?
L. Pinheiro, FURG- Universidade Federal do Rio Grande / Instituto de Oceanografia
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Analysis of Microplastics and Nanoplastics - From Harmonisable Protocols and Data Treatment to the Peculiarities of the Environmental Nanofraction

3.04.1
Detection of microplastics in the water treatment process: Considerations for sampling, processing, analytical methods and reporting microplastic occurrence in increasingly complex media
R. Cross, Centre for Ecology and Hydrology; A.A. Horton, National Oceanography Centre / pollution; A.C. Johnson, UKCEH / Wallingford; M. Jurgens, Centre of Ecology and Hydrology; D.S. Read, Centre for Ecology and Hydrology / Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford,; H. Ball, Lancaster University; C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology
Microplastics may be considered as plastic anywhere between 1 nm and 5 mm, presenting an analytical challenge for the detection and quantification of microplastics in complex environmental samples. No single method can enumerate all polymers, across the full size range under consideration, particularly if the size distribution or a number based concentration is desired. Reporting on microplastic quantification in environmental samples must be clear about the operational limits that define "microplastics" in any given sample so that results may be compared between studies. At each stage of the workflow from sampling through to analysis, consideration should be made as to how this influences quantification of microplastics in the sample. Sampling strategies often require filtration of liquids, introducing a minimum size for microplastics that must be recognised when reporting. Similarly, imaging approaches such as Raman or infra-red techniques can resolve microplastics to different sizes. The choice of analytical method (and increased size resolution that follows) must be balanced by the requirements of the study. Practical considerations of cost and time balanced against gains in precision are not trivial when large numbers of samples are required. We present our experiences in sampling, method development, and analysis as part of a campaign monitoring 8 water treatment works and 8 waste water treatment works over a 7 month period. This campaign (UK Water Industry Research Ltd, "Sink to River- River to Tap") surveyed the occurrence and enumerated the number and polymer identity of microplastics in raw water, potable water, influent, effluent and treatment residues. This presentation will focus on the practical recommendations, with particular reference to the use of blanks, spike recoveries and replication, in the context of controlling sources of contamination throughout the sampling and extraction processes and the "patchiness" inherent in inhomogeneous samples. Efforts are underway to harmonise the methods and analytical approaches to quantifying microplastics in the environment. However, there is much value in the meantime in increasing the longevity of the data currently generated in this highly productive field of research. By necessity data in the field arise from a plethora of different sampling, processing and analytical strategies. Sharing experiences from the lab and improving the transparency in reporting can go some way to allowing the data generated today to retain its value as the field develops.

3.04.2
Tackling the Challenge of Extracting Microplastics from Soils: A Protocol to Purify Soil Samples before µ-FTIR Analysis
J. Moeller, University of Bayreuth / Department for Animal Ecology I; C. Laforsch, University of Bayreuth; M. Loeder, University of Bayreuth / Animal Ecology I and BayCEER; A. Satzger, I. Heisel, University of Bayreuth / Animal Ecology I
In recent years, research on microplastic has expanded from exclusively the marine environment to nearly all environmental compartments, including the terrestrial environment. Soils are expected to be significant long-term sinks for microplastic particles and have been shown to have the potential for changing certain soil characteristics 1. However, little is known about the extent of microplastic pollution in soils and the influence of microplastic sizes and shapes on the soil ecosystem. Furthermore, analytical tools for the extraction and identification of small microplastic particles are lacking. To date the only analytical methods to characterise microplastic particles in terms of particle numbers, plastic type, size and shape are µ-FTIR spectroscopy and µ-Raman spectroscopy, both requiring a high measure of sample purification prior to the measurements. As soils are composed of a highly complex matrix containing varying proportions of soil organic matter and mineral components that can form stable aggregates, sample purification to an adequate degree without destroying the microplastic particles in the process poses a significant challenge. In our current research we have derived a method to isolate the smaller microplastic particles from the soil matrix in order to analyse them via a µ-FTIR spectroscopy. In a first step aggregate dispersion and wet sieving serve to obtain the < 500 µm fraction. Larger particles can, usually be picked out manually and measured with ATR-FTIR spectroscopy. For the purification of the smaller fraction, we combined a density separation step using a highly dense zinc chloride solution with a sequential enzymatic digestion. Here the enzymatic sequence mimics that of plant degrading fungi to break down the stable structures of soil organic matter. The protocol results in sample mass reduction rates of over 99 % and high microplastic recovery rates for the most common polymer types (preliminary results). The recovery rates of the biodegradable microplastic types tested (polylactide and polylactide-polybutylene adipate terephthalate blend) were below average (preliminary results). The reason for the lower recovery rates is still to be determined and may be remediated by May 2020. The method will allow more in-depth understanding of the extent and characteristics of microplastic pollution in soils and may allow conclusions on how the accumulation of introduced microplastic species may affect the soil environment and plant growth.

3.04.3
Automated Analysis of Microplastics in Environmental Samples with a Combination of Optical Microscopy with FTIR and Raman Spectroscopy
D. Fischer, Leibniz-Institut f. Polymerforschung Dresden / Analytics; F. Fischer, J. Brandt, Leibniz-Institut f. Polymerforschung Dresden / Analytics Deprtment; L. Bittrich, E. Kanaki, Leibniz-Institut f. Polymerforschung Dresden; K. Eichhorn, Leibniz Institute of Polymer Research Dresden; A. Käppler, SGS Institut Fresenius Dresden; R. Lenz, K. Enders, Leibniz Institut for Baltic Sea Research Warnemuende; M. Labrenz, Leibniz Institute of Baltic Sea Research Warnemuende
Polymers as microplastics (MP) have become an environmental threat with ubiquitous distribution. Microplastic pollutions have negative effects on the aquatic environment and probably on human health. After being released in the aquatic and terrestrial environment, polymers undergo biofouling and weathering processes, e.g. by mechanical abrasion and UV-light. That leads to smaller and smaller fragmentation into MP. Determining MP in environmental samples in the wide range from 1 µm to 5 mm nearly quantitatively, quickly and reliably is a challenging task. We present an analytical approach with a largely automated combination of optical particle analysis, FTIR and Raman microscopy and spectral database search. This approach can determine particle sizes, particle size distributions and the type of polymer including color and shape. The presentation describes the approach to identify and quantify MP in environmental samples in MP-free clean rooms with the use of blank samples in all steps. First procedure after purification of the sample is a three-step vacuum filtration to divide the particles in four fractions, above 500 µm, from 500 to 50 µm, from 50 to 10 µm and below 10 µm. After filtration, the particles of the different fractions are on IR transparent silicon filters. For all following analyses, we use the in-house developed open-source software GEPARD. GEPARD starts with an optical particle identification, which determines all particles in shape and dimension and stores their coordinates for the subsequent measurements with FTIR and/or Raman microscopy. Both methods identify the MP on the filter automatically using spectral databases. We focus in the presentation on the identification of MP particles < 500 µm in water and sediment/soil samples. The most frequently found MP particles are the bulk plastics PP, PE, PET, PMMA and PS. The presentation will show further results of the investigation of MP in different environmental compartments like wastewater, sediment, soil from agriculture, and beaches. For samples > 2 mm it is possible to measure MP directly on a boat or a ship on the sea with a hand-held NIR spectrometer. We will discuss the possibilities and limits of this NIR measurement by a comparison of these NIR measurements with laboratory FTIR measurements. A comparison between FTIR and Raman measurements for the identification of MP will also be discussed.

3.04.4
Abundance and distribution of microplastics in the German bight - A first try of source tracking
C. Dipke, Carl von Ossietzky University of Oldenburg / Institute for Chemistry and Biology of the Marine Environment ICBM; M. Fischer, Carl von Ossietzky University Oldenburg / Institute for Chemistry and Biology of the Marine Environment ICBM; B. Scholz-Boettcher, University of Oldenburg, ICBM / Institute for Chemistry and Biology of the Marine Environment ICBM
In the environment microplastics (MP, particles from 1 µm to 5 mm) have become one of the most urgent topics of the recent decade. They are ubiquitous pollutants in the marine environment. Its bioavailability increases with decreasing particles size. Accordingly particle number and size related data are highly relevant for ecotoxicological studies and a common measure with spectroscopic methods. Complementary mass related data provided by via thermal methods enable polymer specific geospatial and/or temporal studies independent of any particle appearance or size. Data acquisition in defined regions gives insight into general horizontal and vertical qualitative and mass quantitative MP composition and changes. Potential sources can be tracked and located within the area of interest. So far, MP data for the North Sea are restricted to few particle count related studies. In this first mass related survey of MP in surface waters of the German Bight, North Sea. The spatial MP load as well as its potential sources and temporal variation were comprehensively examined. For this purpose the most relevant 10 consumer plastics were analyzed using Py-GCMS/thermochemolysis as an excellent method for qualitative and trace level polymer specific mass

quantitative analysis [1, 2]. The overall MP concentration in surface waters of the German Bight ranges between 2 and 1400 µg m^{-3} (ppt to lower ppb level). Neither MP concentration nor type distribution is homogenous. Besides meteorological and oceanographic conditions (i.e. currents, different density related discontinuity layers) that seem to have substantial influence on spatial and temporal mass loads, this variability is attributed to the particulate nature of MP as well. The overall concentration range is comparable to that of other environmental pollutants. On a spatial scale the qualitative MP composition could be related to different sources mainly packaging, shipping traffic and combined estuary/urban pattern. [1] Fischer M, Scholz-Böttcher BM. 2017. Simultaneous Trace Identification and Quantification of Common Types of Microplastics in Environmental Samples by Pyrolysis-Gas Chromatography-Mass Spectrometry. ES&T, 51, 5052–5060.[2] Fischer M, Scholz-Böttcher BM. 2019. Microplastics analysis in environmental samples – recent pyrolysis-gas chromatography–mass spectrometry method improvements to increase the reliability of mass-related data. Anal Meth, 11, 2489-2497

3.04.5
Investigation into Recovery Rates of Microfibres from Water Samples Using a Forensic Fibre Tape Lifting System
C. Gwinnett, A. Osborne, A. Jackson, Staffordshire University / Criminal Justice and Forensic Science
· Microplastics (MPs) (< 5mm in size) are ubiquitous in aquatic environments and are currently an emerging pollutant of interest in environmental research studies. Attempts to fully capture the microplastic content in water samples require standardised techniques with high recovery rates. Methods to recover microplastics from water samples are varied with limited information about recovery rates known. Filtration methods using Buchner and glass frit apparatus and filter papers are widely used but their efficacy in recovering microplastics is unknown. This paper concerns the application of specific methods and technologies developed for use in forensic science to the recovery of MPs in pollution monitoring. Although not a panacea, if adopted in this new setting, it is believed that these methods and technologies could save time, decrease the opportunity for contamination and loss, and facilitate the development of largely automated methods of MP characterisation, classification and quantification. This paper describes these methods and technologies and explains why we believe that they are of value in MP pollution monitoring. The findings of a quantitative simulation experiment in which these methods and technologies have been applied to the isolation of MP fibres from tap water are also presented, as is a preliminary account of their performance during field trials. This experiment reveals variables that can be adjusted to optimise the capture of MP fibres for subsequent analysis. This paper will discuss the link of forensic fibre analysis to MP work and explain why the use of a tapelifting method is beneficial for the recovery of microfibres from filter papers in water sampling studies.

3.04.6
Poster spotlight: Characterization of chemical and morphological changes in microplastics during bacterial degradation at environmentally relevant conditions
L. Göpfert, Institute of Hydrochemistry - Technical University of Munich / Institute of Hydrochemistry
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3.04.7
Poster spotlight: Suspected screening of nano- and microplastics composition in the Ebro Delta area
M. Farre, IDAEA- CSIC / Department of Environmental Chemistry
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3.04.8
First lab-scale feasibility study on the enrichment of nanoplastic particles from water samples by continuous flow centrifugation using metal-doped nanoplastics and ICP-MS/MS detection
L.M. Hildebrandt, T. Zimmermann, Helmholtz-Zentrum Geesthacht / Marine Bioanalytical Chemistry; D.M. Mitrano, Eawag - Swiss federal Institute of Aquatic Science and Technology / Process Engineering; D. Pröfrock, Helmholtz-Zentrum Geesthacht / Marine Bioanalytical Chemistry
In the last 20 years, environmental pollution with particulate plastics has considerably raised concern in society and science. Currently, plastic particles < 1 µm remain mainly unexplored due to the related analytical challenges. There is, in particular, a lack of appropriate sampling and sample processing methods for such kinds of particles. Moreover, spectroscopic methods do not provide the necessary lateral resolution, whereas thermoanalytical approaches require immense preconcentration of the submicro- and nanoplastic fraction in order to meet the required detection limits. Special centrifuge systems, that can process a continuous suspension stream, are a promising tool to achieve nanoplastic enrichment from natural waters. A lab-scale study has recently shown that continuous flow centrifugation is capable of retaining small microplastic particles (MP) down to a few microns. As a proof-of-concept, the instrument has also been tested in the field to sample MP contained in suspended particulate matter. Afterwards, the idea came up to test the device's retention capacity with respect to nanoplastic particles. Utilizing nanoplastics containing chemically entrapped metals as a tracer enables accurate and straightforward quantification in complex media as well as the investigation of the capabilities of the setup to recover nanoplastics from the water phase for subsequent analysis. In this study, recently synthesized Pd-doped nanoplastics with a diameter of approximately 160 nm (Mitrano et al. 2019) were used as a model to evaluate the potential of continuous flow centrifugation for nanoplastic enrichment. First experiments yielded a retention efficiency of 60.4% ± 3.0% (1 SD (n = 3)).

3.04.9
Capabilities of transmission electron microscopy to detect and identify nanoplastic particles
R. Kaegi, Eawag - Swiss federal Institute of Aquatic Science and Technology / Process Engineering
Microplastic particles have been detected in the most remote places all over the globe and also have also been reported from in all kinds of water resources. This is to a large extent owed to the recent advances in analytical developments, most importantly µ-FT-IR and µ-RAMAN. Recently, it has been speculated that even a higher number of nanoplastic particles may be present in our environment, but due to analytical challenges, these particles may have been overlooked so far. With its excellent spatial resolution in combination with analytical capabilities at the nanoscale, transmission electron microscopy (TEM) is a very promising candidate to address the challenge of detecting particulate nanoplastics. We used polystyrene nanoparticles with sizes range from 30 – 100 nm and silica particles of comparable sizes to evaluate the potential of TEM to detect and characterize nanoscale plastic particles and to distinguish them from other (inorganic), nanoscale particles. Most commonly, carbon based substrates (e.g. carbon coated Cu or Ni grids) are used for TEM analyses. However, these substrates interfere with the analysis analyses of the polymers. Thus, TEM grids with other coatings were evaluated for their suitability to investigate nanoplastic particles and best results were obtained from 20 nm thick Si3N4 membranes. Particles were deposited on the TEM grids by either on-grid centrifugation or by drop deposition. Although the centrifugation method yields a very even distribution of the particles on the TEM grid, the brittle Si3N4 membranes were often damaged after the centrifugation procedure. The use of electron energy loss spectroscopy (EELS) provided significant advantages over the energy dispersive x-ray (EDX) analysis. Whereas the EDX spectra contained a few tens of x-ray counts of the carbon K edge, the respective EELS signal was orders of magnitudes more intense. On the one hand, this related to the low x-ray yield of light elements (such as carbon and nitrogen) and on the other hand to the higher collection efficiency of the mainly forward scattered electrons. In addition to this, the EELS of the carbon K edge contain characteristic features which may even allow an identification of individual polymer types. This presentation highlights the potential of electron microscopy analyses, which, especially in combination with recently presented TEM automation routines, may shed more light into the field of nanoplastic analyses in the near future.

3.04.10
Panel discussion
M. Meyns, Alfred-Wegener-Institute, Helmholtz Centre for Polar and Marine Research / Shelf Sea System Ecology; A. Lusher, NIVA Norwegian Institute of Water Research / Environmental Contaminants; B. Scholz-Boettcher, University of Oldenburg, ICBM / Institute for Chemistry and Biology of the Marine Environment ICBM; J. Gigault, CNRS UMR 6118
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Assessment of the Exposure and Effects of Contaminants of Emerging Concern in Drinking and Wastewater Systems and Assays to Evaluate their Removal

3.05.1
Assessing the impact of water reuse strategies using an integrated modelling approach
R. Delli Compagni, Politecnico di Milano; F. Scana, A. Turolla, Politecnico di Milano / Department of Civil and Environmental Engineering; F. Polesel, DHI / -; L. Vezzaro, DTU (Technical University of Denmark) / DTU Environment; M. Antonelli, Politecnico di Milano / Department of Civil and Environmental Engineering
The so-called "smart cities" aim at implementing innovative resource management strategies to efficiently (re)use their available resources (e.g. water, energy, etc.). For example, treated wastewater is more and more used for agricultural purposes to limit the freshwater demand. Also, the energy sector is moving towards more distributed and efficient uses of heat sources across urban areas, looking at energy recovery systems, as groundwater heat pumps. However, within this context, there is a high potential of cross-contaminations of hazardous substances, such as contaminants of emerging concern (CECs), which are persistent in the environment and likely to negatively affect the metabolism of a living being. Consequently, holistic approaches are needed to assess the impact on the

environment and human health of innovative resource management strategies before implementation. In this work, an integrated model was developed and adapted to a real case-study to simulate the environmental fate of a series of recalcitrant CECs (e.g. carbamazepine) under different water reuse scenarios (i.e. energy recovery through groundwater heat pumps, withdrawing water from the unconfined polluted aquifer and discharging in waterways from which water is subsequently reused for irrigation of edible plants). Results highlighted that implementation of groundwater heat pumps does not pose an additional environmental risk (risk quotient < 0.01), leading to predicted concentrations of carbamazepine in waterways lower than current situation (i.e. no energy recovery) due to a dilution effect. Also, human health risk shown a reduction of 30% with respect to the current situation. The developed model is a useful tool for assessing the fate of CECs and the impact of different water reuse practices in complex urban and peri-urban areas. The model can support decision makers in evaluating future water management strategies and the related risk on the environment and human health.

3.05.2

Biological responses in rainbow trout to wastewater treated by catalytic ozonation

V.V. Yargeau, McGill University / Chemical Enineering; F. Chassaing, McGill University / Department of Chemical Engineering; C.D. Metcalfe, Trent University / Water Quality Centre

Recently, there has been interest in applying catalytic ozonation for the treatment of wastewater to enhance molecular ozone reactions in water. The objective of the present study was to evaluate the residual toxicity of wastewater treated by catalytic ozonation by measuring biological effects in rainbow trout exposed *in vivo* to extracts from treated and untreated wastewater. Treatment of wastewater was conducted using a pilot unit installed at a treatment plant serving a population of about 95,000 inhabitants that receives 65,000 m^3/day of wastewater from both residential and industrial sources (at a 50/50 ratio). An Al_2O_3 based catalyst (AL-1010S) was supplied by BASF through Air Liquide Canada, the industrial partner on the project. Samples collected were extracted by solid-phase extraction using both Oasis MAX (6 mL, 400 mg) and Oasis MCX (6 mL, 150 mg) cartridges. Before the experiments, the extracts were pooled and evaporated just to dryness, then reconstituted in 5 ml of corn oil containing 8% DMSO as a solvent. Biological responses in rainbow trout were measured after intraperitoneal (i.p.) injections of extracts prepared from wastewater. Juvenile rainbow trout (n= 6-7 per treatment) were injected i.p. with doses of 5 µl of combined extract per g body weight. At 72 h post-injection, fish were were sacrificed and samples of blood plasma, liver and brain tissue were placed into cryogenic vials and snap frozen in liquid nitrogen. Tissue and plasma samples were stored at -80°C until analysis. Biomarkers included vitellogenin (VTG) in plasma, total glutathione (tGSH) and oxidized glutathione (GSSG) in liver, Thiobarbituric Acid Reactive Substances (TBARS) in liver, and serotonin in brain tissue. The results of the present study show that ozonation reduced the estrogenicity of wastewater, but catalytic ozonation further enhanced the reduction in estrogenic effects (i.e. VTG induction) in rainbow trout. Slightly elevated levels of tGSH in trout may indicate mild oxidative stress in trout treated with extracts from the ozonation treatment, but this response was not observed in the treatment with catalytic ozonation. No alterations in lipid peroxidation (i.e. TBARS) in liver or serotonin levels in brain relative to controls were observed in trout from any of the treatments. Overall, the data showed that ozonation in the presence of a Al_2O_3 based catalyst had a beneficial effect by reducing the estrogenic responses (i.e. VTG induction) in rainbow trout.

3.05.3

Novel insights into the toxicological and antibacterial perspectives of transformation products of antibiotics formed during UV-C/H2O2 oxidation in ultrapure water and wastewater effluent matrices

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The extent of oxidation during UV-C/H2O2 oxidation depends mainly on the chemical nature of the micropollutant itself and the reaction time; rarely leading to its mineralization, but rather to the formation of transformation products (TPs). The concern is whether these TPs retain the biological effects of the parent compounds or whether they develop new and undesired biological effects. This issue cannot be addressed merely by elucidating the structures of the TPs by chemical analysis. Instead, the assessment of treated wastewater toxicity is necessary for the optimization of UV-C/H2O2 treatment processes.The aim of this study was to investigate the transformation of selected antibiotics (i.e. azithromycin, ofloxacin, sulfamethoxazole and trimethoprim) during UV-C/H2O2 oxidation in ultrapure and secondary-treated wastewater effluents, as well as, the identification of their TPs by applying suspect and non-target strategies based on liquid chromatography quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS/MS). Moreover, 4 effect-based Chemically Activated Luciferase eXpression (CALUX®) bioassays were applied to assess multiple toxicological endpoints, i.e. genotoxicity (+/- S9), oxidative stress and cytotoxicity of the UV-C/H2O2 treated samples and of selected TPs. Finally, antibiotic susceptibility testing using the Minimum Inhibitory Concentration (MIC) technique for selected TPs and associated parent compounds was applied on selected strains of environmentally- and clinically-relevant bacteria (i.e. *Escherichia coli* (*E. coli*) ATCC 8739, *E. coli* ATCC 13597, *E. coli* DH5a, *Enterococcus faecalis* ATCC 51299, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Streptococcus G* ATCC 12394) using Clinical and Laboratory Standards Institute (CLSI) guidelines.In total, 23 TPs of the target antibiotics were formed and tentatively identified using suspect and non-target screening approaches. While the tested CALUX® bioassays presented quantifiable responses to the UV-C/H2O2 treated samples, testing of the individual TPs showed no activity indicating thus, the potential additive/synergistic effects of TPs in combination with their parent compounds and other mixtures of chemicals present in wastewater. The MIC of the TPs was approximately the same as the respective parent compounds indicating that TPs can retain their antimicrobial activity and thus, can act as an additional selective pressure to the spread of antibiotic resistance determinants.

3.05.4

Still Haven't Found What You're Looking For? Integrated Interdisciplinary Analyses May Be the Solution.

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Targeted chemical analyses, non-targeted chemical analyses, and bioanalytical tools each provide a unique but incomplete understanding of the chemicals in a sample. The U.S. Environmental Protection Agency and U.S. Geological Survey are collaborating to examine the sources, fates, and potential effects of contaminants of emerging concern (CECs) during de facto water reuse which occurs when treated wastewater is discharged to a source of drinking water. The project sampling design follows a surface-water flow path, with the collection of grab water samples at six sampling points: upstream of a wastewater treatment plant outfall, wastewater effluent entering the river, effluent mixing zone, downstream sampling point, drinking water intake, and treated drinking water. The study uses an integrated approach that includes a comprehensive analysis of over 200 targeted chemicals (e.g. pharmaceuticals, per- and polyfluoroalkyl substances); high resolution mass spectrometry to identify non-targeted chemicals (suspected or unknown); in vitro bioassays to assess biological activity (e.g. estrogenicity, androgenicity, metabolomics); rapid whole organism screens to assess cumulative bioactivity; and in vivo tests to address specific exposure and response endpoints. A rigorous quality assurance/quality control protocol was consistently applied from field to laboratory to ensure comparability of results between the various techniques. This integrated approach combines the strength of each technique and builds upon the standard CEC research approach by including environmental and toxicity endpoint assessments to more fully explore the potential effects to human health and the environment from chemical exposures.

3.05.5

Occurrence of human-excreted contaminants within a decantation tank: respective impacts of historical consumption and sediment properties

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In this work, we explored the potential of sediments accumulated in sewer systems to record the human activities through drug and illicit drug occurrences. The selected site is a decantation tank that collects wastewater and stormwater from the sewer network of the northern part of Orléans (France). This 20 m deep underground building constitutes a unique opportunity to study the historical evolution of the drugs and illicit drugs use. Six cores were acquired from 2014 to 2017. After sampling, 90 sediment samples were extracted with a mixture of water:methanol (1:1) prior to extract analyses by HPLC-MS². Sediments also underwent a large range of classical sedimentological analyses (granulometry, facies description, microscopic description, mineralogy and organic carbon analyses). The age model is based on 47 ^{14}C dates obtained on the 6 cores and

computed with OxCal v4.3.2 and Bomb13NH1 calibration curve. Oldest sediments are dated back to 1970. Sediments are stratigraphically organised into organic (TOC > 2%) and mineral (TOC < 2%) layers resulting from wastewater and stormwater inputs, respectively. Concentrations of 27 drugs and illicit drugs were measured in 90 samples on core CSA-02/2015-A that records the 1980-2010 time period. A strong control of speciation on the presence and concentration of drugs and illicit drugs is evidenced. Neutral and anionic compounds (e.g. carbamazepine, salicylic acid) present higher concentrations in the lower part of the core whereas cationic compounds (e.g. codeine, benzoylecgonine) display higher concentrations in the upper part. For the formers, their distributions are directly correlated with the distribution of organic carbon, whereas for the latters, a correlation with the relative abundance of inorganic surfaces is evidenced. As a result, the interaction mechanisms between targeted compounds and the sedimentary composition appears to be the most important control. The analysed sediments present important vertical variations of composition, due to distinct origin, especially emphasized by the organic carbon variations. Hence, drugs and illicit drugs distributions appear to be strongly controlled by their affinity with bearing phases. As a result, the distribution of these compounds with time cannot be directly correlated with the evolution of excretion amount in the catchment, as expected. Nevertheless, these patterns are very informative on the affinity between sediments and organic contaminants at a larger scale.

3.05.6
From soil to mouth: Assessment of the effect of organic fertilizers on the rate of incorporation of Antibiotic Resistant Genes in Lactuca sativa and Raphanus sativus. Real-scale studies.
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The reuse of organic amendments -organic waste- allows a better management of the finite resources we dispose for soil fertilization and food production. While they provide macro- and micronutrients to the soil, including carbon for the restoration of its physicochemical properties, they may also contain components that can be harmful for animal, plant, and human health. The presence of pathogens represents an obvious threat, but other pollutants, like antibiotics, pharmaceuticals and hormones may also represent a risk, as they may increase the number of bacteria resistant to multiple antimicrobial and antibiotic drugs. In this way, the potential transmission of antibiotic resistance (AR) from amended soils to crops and, ultimately, to consumers is a matter of major concern. The present study aims to assess the effect of three different organic fertilizers: sewage biosolid (Sludge), pig slurry (Slurry), and urban solid residue (USW), on the rate of incorporation of Antibiotic Resistant Genes (ARGs) in plants of agronomic interest (*L. sativa* and *R. sativus*), in comparison to the use of conventional chemical fertilizers. Thirty-two experimental plots between 3 and 10 m^2 were cultivated, depending on the crop (lettuce or radish), and randomly distributed by crop and fertilizer by blocks (n = 4). After the harvest, we extracted DNA from plants, from their respective soils and from the amendments used in each plot. We analyzed the levels of seven ARGs (*sul1, bla_{TEM}, $bla_{CTX\text{-}M\text{-}32}$, mecA, qnrS1, tetM, $bla_{OXA\text{-}58}$*), plus the integrase gene *intI1,* using quantitative real-time PCR methods. Regarding the amendments, we quantified *bla_{TEM}*, *intI1*, *sul1* and *$bla_{OXA\text{-}58}$*, in all of them, *qnrS1* and *tetM* in Slurry and Sludge, and *mecA* in Slurry. Moreover, we quantified *intI1*, *sul1* and *tetM* in soil and *sul1* and *tetM* in lettuce and soil. We observed a significative increase of *sul1* and *tetM* in lettuce and soil, and of *sul1* in radish for the samples amended with Slurry. This results correlated with the higher abundance of this two genes in the pig slurry itself in comparison with the other amendments. This evidence shows that the reuse of Slurry in agriculture can positively contribute to potential AR transference, and more processed organic amendments (Sludge, USW) may represent safer alternatives, at least in regard to ARG transmission. We also have data showing that composted pig manure is a far less hazardous soil amendment than the raw pig slurry.

3.05.7
Contaminants of emerging concern in irrigation water and fresh produce
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Treated wastewater (TWW) is frequently used in the agro-ecosystem for crop irrigation. On a global scale, irrigation with TWW is expected to increase due to prolonged droughts and depletion of freshwater sources. This Irrigation exposes the agroecosystem to pollutants of emerging concern including pharmaceuticals and personal care products (PPCPs). Once in the field, PPCPs may be taken up by plants which in turn may introduce them into the food chain. Currently, much of the data regarding plant uptake of PPCPs are based on studies done in controlled environments, while only a few reports have analyzed crops grown under field conditions. In this study, we aimed to evaluate the concentration of selected PPCPs in chosen soils, TWW, and fresh produce irrigated with TWW. A composite sample of fresh produce, soils, and TWW were collected from 450 fields irrigated with TWW across Israel. Analysis of 270 TWW samples, collected from the irrigation head at the field, showed that all TWW samples were contaminated with PPCPs. The most common therapeutic groups detected were antimicrobial, anticonvulsant, and antiparasitic agents: 100%, 91% and 99.6% (% detection), respectively. Anticonvulsant agents consisted of 3 target analytes: carbamazepine, gabapentin, and lamotrigine, and were quantified at the highest concentration in the TWW samples: 1039 ± 141, 1008 ± 125 and 630 ± 125 ng/L, respectively. Crotamiton, an antiparasitic compound, was detected and quantified (>LOQ) in 99.6% of the samples at an average concentration of 172 ± 18 ng/L. Similarly to TWW samples, PPCPs were detected in all 84 leafy green samples analyzed. Carbamazepine was detected in 96% of the leaves samples, at an average concentration of 322 ± 61 ng/g dry weight. In addition to the parent compound (i.e., carbamazepine), the metabolites: carbamazepine-10,11-epoxide and 10,11-dihydro-10,11-dihydroxy carbamazepine were detected in all of the leafy green samples at an average concentration of 189 ± 36 and 9.4 ± 1.6 ng/g, respectively. this finding supports lab and field experiments indicating that plants can metabolize carbamazepine and other PPCPs. Traces of PPCPs were detected and quantified in all analyzed samples (i.e., TWW and leafy green samples). Our data show that irrigation with TWW exposes the environment to PPCPs which may be taken up by different edible crops and enter the food chain.

Chemical Exposome of Human and Wildlife - Advancements in Non-targeted Analytical Methodology and Modelling Approaches

3.06.1
Non-targeted screening of markers of chemical exposure in human breast milk: development and challenges regarding sample preparation and data processing
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Our environment is vector of human exposure to hundred thousands of chemicals. This opened view of the chemical space contaminating the environment-food-human *continuum* is today supported by advanced chemical profiling instrumentation opening the door to holistic sample characterisation. One particular issue in this context is related to the early warning detection of emerging chemicals. Non-targeted screening (NTS) approaches are new valuable strategies aiming to detect unknown markers of exposure without any *a priori*. These screening approaches are associated to important expectations and promises, but also major challenges for scientists, particularly concerning sample preparation and data processing. In the frame of the workpackage WP16 "emerging chemicals" of the Human Biomonitoring for Europe project (HBM4EU, EU H2020), a NTS workflow was developed for human breast milk analysis. The sample preparation protocol included a recently released cartridge, Captiva EMR-Lipid, to remove lipids followed by a liquid-liquid partitioning. Extracts were analysed on both LC-HESI(+/-)-Q-Orbitrap and GC-EI(+)-Q-Orbitrap instruments in order to cover a wide range of molecules thanks to the complementarity of these techniques. A mix of 30 different halogenated contaminants (mainly pesticides and flame retardants) at four concentration levels (5-100 pg/µL fresh milk) was used for method assessment and to highlight method's limitations. Satisfying linearity (r^2< 0.975) and repeatability (RSD< 30%) were observed for 28 compounds at concentration level 10 pg/µL fresh milk. Compounds detected at higher concentration level represent method limitations in breast milk. The HaloSeeker [1] application was used to process data and to highlight halogenated signals of interest. In combination with the developed sample preparation, the hydroxy-chlorothalonil was identified in breast milk and structure was confirmed with analytical standard by MS/MS fragmentation. This work illustrates the real capability and complexity of NTS approaches in support of human biomonitoring and environmental-health researches. [1] Léon A, Cariou R, Hutinet S. 2019. HaloSeeker 1.0: A User-Friendly Software to Highlight Halogenated Chemicals in Nontargeted High-Resolution Mass Spectrometry Data Sets. Anal Chem 91:3500-3507.

3.06.2
Holistic approach for comprehensive xeno-metabolome coverage of Zebrafish embryos exposed to Benzotriazoles, combining orthogonal chromatographic modes, trapped ion mobility and HRMS
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There is clear evidence that benzotriazoles (BTs) persist in aquatic systems, as they are measured in almost every surface water sample. Thus, it is urgent to evaluate their potentially toxic effects to aquatic organisms. Zebrafish has emerged as a powerful model organism to study various aspects of developmental and cell biology, while it provides a promising alternative model for acute toxicological studies. The impact of the xenobiotics in the aquatic environment is evaluated in more depth when the whole xenometabolome of aquatic organisms is studied. Biotransformation is known to affect the internal concentration (C_{int}) and the uptake of the parent xenobiotics, while it constitutes a critical factor for toxic response. HRMS-based workflows, appear to be a powerful tool for the identification of bio-TPs. Despite HRMS high applicability and accuracy, separation of isomeric bio-TPs is not always possible, as they may pose identical chromatographic and spectral profile. Thus, additional "dimensions" of separation are required for reliable identification of isomers. The objectives of this study, were (1) to establish a high-end analytical platform that could combine multiple dimensions of separation with HRMS, to provide extensive experimental evidence for the identification of bio-TPs isomers. (2) A "biotransformation oriented" data treatment workflow, consisted of suspect and non-target screening approaches, was developed. Overall, the aim was to highlight a holistic approach for comprehensive xeno-metabolome and toxicity assessment of aquatic organisms exposed to xenobiotics. More specifically, the zebrafish embryo toxicity assay was used to calculate the LC_{50} of BTs, as well as for phenotypic evaluation of their toxicity. A holistic analytical platform was developed combining orthogonal chromatographic modes (HILIC and RPLC), trapped ion mobility and HRMS for the analysis of parent BTs and the identification and their bio-TPs. Retention time information in two orthogonal modes and collision cross section values provided additional evidence to the accurate mass measurements and facilitated the identification of bio-TPs isomers. Bio-TPs arising from both oxidative and conjugative metabolic reactions were identified. Overall, 26 bio-TPs were identified through suspect and non-target screening workflows. Finally, it was demonstrated that biotransformation data could be used complementary to the C_{int} of the parent BTs to interpret the induced toxicity.

3.06.3
Optimizing a suspect screening annotation workflow for large-scale application in human cohort

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The emergence of the exposome paradigm underlines the need for more holistic environmental exposure assessment to study causes of non-communicable chronic diseases as well as other physiological disorders. So far, data on human exposure to xenobiotics is limited to few chemical classes. Hence, new untargeted methods could be extremely useful to have a more global view on the human exposure to xenobiotic mixtures. The main challenge is that most of these xenobiotics are at trace levels in complex biological matrices. Hence, data pre-processing and annotation parameters have to be optimized to detect these low abundant signals. This work aimed at optimizing an annotation workflow for future large-scale application on a human cohort. Vendor softwares (MarkerView and Progenesis), open access (MzMine2), and open source software using R (XCMS) were evaluated for the detection of standards spiked at low level. Secondly, an automatized annotation workflow (2500 xenobiotics) based on confidence indexes on mass, retention time (experimental or predicted), and isotopologues, was developed in-house and tested on plasma samples from a mother child cohort. As a first result, this study showed that tuning of detection parameters is key to optimize the detection of low abundant signals for each software. Noise level was the key parameter to enhance detection of spiked standards in vendor software while peak width and wavelets scales were the most important parameters for XCMS and MzMine2. Once all the parameters were optimised, similar frequency of detection performances were observed for all softwares. However, vendor software such as MarkerView outperformed open access and open source softwares for computing time (< 3h for MarkerView compared to up to 29h for MzMine2). Annotation workflow based on three confidence indexes (m/z, Rt, and isotopologues) and combined into a global confidence index assessed on plasma samples from a mother child cohort allowed to detect xenobiotics such as pharmaceuticals, preservatives, fire retardants, and drug precursors. This study showed that in the specific context of application of suspect screening strategy in large-scale human cohorts, the use of vendor software can be considered as a fast and reliable solution for simple raw data processing. The in-house suspect screening annotation workflow was very efficient to remove false positives and allowed to detect xenobiotics to prioritize for targeted method development and quantification.

3.06.4
Combination of Non-targeted and Targeted Analyses to Identify Biomarkers of Pesticide Exposures

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Pesticides are largely used to control pests and represent a huge diversity of chemicals used in different contexts, leading to multiple exposure sources for human. Characterization of human exposure to pesticides still represents a challenge. The objective of this study was to associate non-targeted and targeted analytical approaches to monitor various pesticides and their metabolites in urine from pregnant women, and to assess the determinants of their exposure. Urinary samples from 300 women belonging to PELAGIE mother-child cohort (Brittany, France) were analyzed by UHPLC/OrbitrapMS with a non-targeted approach and by UHPLC/QTOFMS with a multiresidue targeted approach after a sample preparation step. According to data, frequentist and Bayesian statistical analyses were used to study the determinants of pesticide exposures. Non-targeted analysis resulted in the identification of 28 pesticide metabolites, corresponding to 3 fungicides (azoxystrobin, fenpropimorph and procymidone), 3 herbicides (quizalofop-p-ethyl, chlorpropham and phenmedipham), and one insecticide (carbofuran). Using the targeted approach, pyrethroids, organophosphorous (OP), phenoxypropionic acid derivatives, as well as chlorpyrifos and fluazifop-p-butyl were detected in > 60% of samples; prochloraz, bromoxynil, diazinon and procymidone were detected between 10 and 50%. Urinary concentration levels range was between < LOD until 600 µg/L for methyl-OP metabolites. The determinants of exposure were: (1) location in urban area (town > 20 000 inhab.) for phenmedipham and procymidone, (2) location in rural area for bromoxynil, (3) proximity with vegetable crops and presence of cereal crops in the town of residence (chlorpyrifos), (4) proximity with wheat crops (fluazifop); (5) diet (OP); (6) smoking (diazinon); and surprisingly, (7) absence of peas and potato crops in the town of residence (pyrethroids). The originality of this study was to combine non-targeted and targeted analyses on the same samples. Non-targeted approach allows the identification of pesticides (or corresponding metabolites) not frequently studied and not measured in biomonitoring studies due to the lack of analytical standards. Complementary, targeted approach allows the quantification of numerous compounds including low molecular weight polar metabolites. The generalization of this type of study would therefore be interesting to include new pesticide biomarkers useful for biomonitoring or epidemiological studies.

Chlorinated Paraffins - State of Science, Insights, Challenges and the Way Forward

3.07.1
Passive dosing vs. liposome delivery for bioaccumulation and toxicity assessment: method comparison using Chlorinated Paraffins as a model substance

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Standard aquatic testing, necessary during the environmental risk assessment of chemicals, is limited to readily water soluble chemicals. Passive dosing via silicone has been developed to accommodate hydrophobic organic chemicals, including CPs, allowing stable water exposures at environmentally relevant concentrations. Liposomes have been widely used in the pharmaceutical industry to effectively stabilize compounds with poor biodistribution and complex pharmacokinetics. In this communication, we aim to present and compare these two alternative aquatic testing methodologies developed for CPs. Experiments were conducted based on the 48 h Acute Immobilisation Test (OECD test 202). For the passive dosing approach, *Daphnia magna* were exposed to CPs via water and food equilibrated with CP-dosed silicone and via CP-loaded liposomes. Partitioning coefficients of CPs between silicone and water (log $K_{silicone\text{-}water}$) and *D. magna* and water (log $K_{oc\text{-}water}$, log BCF/BAF) were determined by quantifying the amount of CPs in each matrix (APCI-qTOF-MS). For the liposome mediated delivery of CPs, the amount of CPs in the liposomes, water and in *D. magna* were determined by radiometric analysis of the ^{14}C-labelled CPs. CPs were sucessfully incorporated into both silicone and liposomes (recoveries varied between 80 and 100%). Time to steady state in the animal (C_{SS}) was, on average, 33 hours when daphnids were exposed to CPs via passive dosing and 16 hours when daphnids were exposed to CPs via liposome dosing. Strickingly, when CPs were dosed to *D. magna* via liposomes, 50% immobilization was observed at 1 ng µg dw^{-1}, whereas through passive dosing, < 10% immobilization was observed up to 10 ± 1.5 ng µg dw-1. A full dose-response was obtained for the LMD (0.75-11 µg L^{-1} (total dosed µg CPs via liposomes, per litre) but not for PD (water concentration range 23 - 280 µg L^{-1}). We believe this is an effect of a faster uptake of CPs by the animals but also a result of direct uptake to specific, and possibly, critical tissues of the animal. The liposome mediated delivery of chemicals works by direct partitioning to the gut of the animals, after the liposomes are ingested as food particles, whereas passive dosing dosing occurs via water mainly via passive diffusion to the animal's body.

3.07.2
Environmental behaviour and bioaccumulation of chlorinated paraffins, dechloranes and PCBs in northern freshwater ecosystems
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Arctic ecosystems are viewed as net receptors of persistent organic pollutants (POPs). While concentrations of some legacy POPs have declined, there is increasing concern about emerging organic contaminants such as chlorinated paraffins (CPs). In 2017, short chain CPs (SCCPs) were included in the Stockholm convention and are now banned. However, medium chain CPs (MCCPs) are still not regulated and are produced in large quantities. The objective of this study is to investigate the environmental behaviour and bioaccumulation of S/MCCPs in Arctic freshwater ecosystems, currently representing a significant knowledge gap. Two lakes in Northern Norway were studied: Storvannet (ST) and Takvannet (TA). Both have similar food webs and the same top predator species but have different sources of organic contaminants. ST is located in an area with known local sources of legacy POPs and emerging contaminants while TA is a remote lake with no known local contaminant sources. Samples of sediments, benthic organisms, three-spined sticklebacks (Gasterosteus aculeatus), brown trout (Salmo trutta) and Arctic char (Salvelinus alpinus) were sampled and analysed for contaminants (polychlorinated biphenyls (PCBs), S/MCCPs, and Dechlorane Plus and analogues), stable isotopes (δ15N, δ13C) and lipid content. Laboratory procedures were performed in a clean room facility with strict quality control procedures. Preliminary results show that S/MCCPs were detected in char and trout from both lakes, suggesting these compounds can undergo long-range atmospheric transport, followed by deposition and bioaccumulation. Fish in both lakes had higher concentrations of MCCPs than SCCPs. There were no difference in SCCP concentrations between char and trout in either lake, but trout from TA had higher MCCP levels than char. Char from both lakes had lower S/MCCP concentrations than char from Bjørnøya (74oN 19oE), but higher than those found in Canada. Contaminants in sticklebacks, benthic organisms, and sediments are currently being quantified. Complete results for the whole ecosystem will be presented, also accounting for stable isotope analysis. Results will provide a comprehensive description of concentration, bioaccumulation and biomagnification factors of S/MCCPs, selected dechlorane compounds in comparison with PCBs. Solid mechanistic understanding of the link between emissions and Arctic ecosystem exposure is of key importance for scientifically sound chemical management strategies

3.07.3
Chlorinated paraffins and tris (1-chloro-2-propyl) phosphate in PUR foam - a source for indoor exposure?
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Improved insulation in houses reduce heat energy loss, and contribute to decreased use of fossil fuels. Therefore, the Dutch government financially subsidise house owners to improve the energy performance of their houses. Energy saving is not only financially attractive but also reduces the carbon footprint. Various insulation materials can be used to improve the heat energy performance of houses; one commonly used product is polyurethane (PUR) foam. PUR foams contains various chemical additives, such as plasticizers and flame retardants, to improve the physiochemical properties for insulation applications. These chemical additives may leach from the applied foam to the indoor and outdoor environment. In this study, we focused on the presence of short, medium, and long chain chlorinated paraffins (SCCPs, MCCPs and LCCPs) and the organophosphate flame retardant (OPFR) tris (1-chloro-2-propyl) phosphate (TCIPP) in PUR foam, and investigated the transmission of these chemicals through direct hand contact with PUR foam. CPs were detected in all ten new PUR foam products with levels ranging from 1.0% to 54% (w/w). The PUR foam products also contained TCiPP with levels ranging from 1% to 30% (w/w). The high levels of CPs and TCiPP used in PUR foam may pose a potential risk for human exposure. CPs and TCiPP are easily transferred from the PUR foam to the hands by direct contact. CP Levels up to 590 µg per hand were found. After hand washing substantial amounts of CPs remain on the hands. Further investigation is needed to assess potential exposure risks associated with general and occupational use of PUR foam. Further work will carried out on the emission to air, and landfill leaching potential of these chemicals when discarded.

3.07.4
Poster spotlight: What's in a name? Understanding the complexities and impact of chloro alkane nomenclature around the globe.
C. Howick, INOVYN / Product Stewardship
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3.07.5
Poster spotlight: New standards for chlorinated paraffins - Eurostars CHLOFFIN project
S. Valderhaug, Chiron AS
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Emerging Environmental Contaminants - Status, Trends, Challenges and Knowledge Gaps

3.08.2
Occurrence, risk assessment and prioritization of organic micropollutants: A multi-compartment analysis of freshwater systems in western Kenya
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The rising use and production of organic chemicals such as pharmaceuticals, pesticides and biocides has led to concerns over their increasing detection with potential adverse effects in the environment. However, as regular monitoring and legislation on organic micropollutants (OMPs) is done in Europe and other developed countries, there is still insufficient information from Africa. In order to close this knowledge gap, the main objective of this study was to determine the multi-compartment occurrence of chemical mixtures in freshwater systems. Specifically, we aim to: 1) identify OMPs present in the water, sediments and snails, 2) perform risk assessment using standard test organisms including fish, crustaceans, algae 3) derive candidate priority compounds for regulation in western Kenya and 4) determine the partitioning of these compounds between different compartments based on chemical activity. Sampling was carried out in 48 sites within western Kenya. Water, snails (if present) and sediment were sampled based on different land use systems for chemical analysis. Water samples were directly injected into a liquid Chromatography - high resolution mass Spectrometer (LC-HRMS). Pollutants in the snail tissues were extracted using the QuEChERS method while pressurized liquid extraction (PLE) was applied for sediment extraction prior to instrumental analysis. Out of the 423 target compounds, 78 compounds were detected in water, 45 compounds in snails and 60 compounds in sediments. Generally, pesticides and biocides were the chemical class with the highest detections in the three matrices. Concentrations ranged up to 24 µg L-1 (water), 480 ng g-1 wet weight (snails) and 7 µg (gTOC-1) in sediments with acetyl-sulfamethoxazole (A-SMX) and triethylcitrate predominantly in water and N-ethyl-o-toluenesulfonamide in all matrices. Snails and sediments acted as passive samplers to compounds like efavirenz, acetamiprid and thiacloprid not detected in water. Risk assessment through Toxic Units (TU) showed crustaceans having the greatest potential risk with diazinon, bendiocarb, carbendazim and pirimiphos-methyl driving this risk. Low but substantial risk was observed for fish and algae with TU up to 0.5. Based on frequency and extent of exceedance of acute and chronic thresholds, and by applying lowest Predicted No Effect Concentration (PNEC), compounds including diazinon, carbendazim, hexazinon and A-SMX by were prioritized for monitoring and abatement in western Kenya.

3.08.3
Comparison of Rare Earth Element levels in muscles of key species of Amazonian freshwater ecosystem close to an open landfill
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Improper disposal of urban solid wastes threaten the environment, particularly aquatic ecosystems. Among the concerns surrounding the release of untreated effluents into rivers adjacent to landfills is contamination by metallic elements. Metals like Cu, Zn, Pb, and Hg are usually studied in environmental risk assessment but others should be better considered: rare earth elements (REEs). REEs are 17 metals of similar physiochemical properties, scandium, yttrium and 15 lanthanides from lanthanum to lutetium. Because of their physical and chemical characteristics, they are often used in high tech everyday products However, despite their wide application, little is known about the ecotoxicology and bioaccumulation characteristics in organisms. The aim of this study was to determine the levels of REEs in key species of a freshwater ecosystem close to an open landfill. Three species were selected to represent different taxa: prawn (Macrobrachium amazonicum), crab (Uca mordax) and fish (Propimelodus eignmanni). These aquatic animals have a high socio-economic weight because they are captured, consumed daily by the population living on the banks of Aurá River and would also be resold in fairs. The study was conducted in Brazil, in the Belém Metropolitan Region (BMR). After the implementation of the Aurá landfill for almost three decades, this area has become the final destination of USWs from the BMR. The sampling campaign was carried out in May 2016. Biological parameters of organisms were measured, individuals were dissected to remove the muscles before to be stored at -80°C until the analysis by inductively coupled plasma mass spectrometer (ICP-MS) with rhenium (Re) as internal standard after muffle carbonization and acid digestion with microwaves. The REE levels in muscles were much higher than values found in the literature for China. They were significantly different comparing the three studied species, with up to 2 orders of magnitude. The highest levels were found for crab, followed by shrimp and fish even if they evolved in the same environment. These differences could be attributed to their specific habitat, their feeding behavior and their position in the trophic chain. No quantitative impact of the open landfill was highlighted on total REE levels but an enrichment of Eu and Gd could be attributed to it. This work allows to estimate the potential use of crabs as a very promising biomonitor of REEs and characterize the human exposure.

3.08.4
Combined analytical and effect-based monitoring of EU Watch List water samples

E. Simon, Centre Ecotox / Aquatic Ecotoxicology; A. Duffek, German Environment Agency / Water and Soil; M. Frey, Steinbeis Transfer Center for Applied Biological Chemistry; M. Scheurer, Water Technology Center TZW Karlsruhe; K. Swart, P. Behnisch, BioDetection Systems; J. Tuerk, Institute of Energy and Environmental Technology e.V. (IUTA); J. Bachmann, German Environment Agency (UBA) / Section IV2.2 Environmental Risk Assessment of Pharmaceuticals; R. Kase, State Secreteriat for Economic Affairs (SECO); E. Vermeirssen, Ecotox Centre CH / Aquatic Ecotoxicology; I. Werner, Swiss Centre for Applied Ecotoxicology, Eawag/EPFL

Three steroidal estrogens, 17α-ethinylestradiol (EE2), 17β-estradiol (E2) and estrone (E1), and the cyclooxygenase (COX) inhibitor pharmaceutical, diclofenac have been included in the first Watch List of the Water Framework Directive (WFD, Directive 2000/60/EC). This means that more Union-wide monitoring data are required on these potential water pollutants before they can be considered for inclusion in the list of priority substances. However, it is a challenge to detect these chemicals at bioactive concentrations (low pg/L) with existing routine analytical methods. Effect-based methods are promising bioanalytical tools that could circumvent such detection challenges by measuring total estrogenic activity or COX inhibition in the aquatic environment in a cost-efficient way at very low concentrations exerted by the above mentioned substances. To investigate the applicability of effect-based methods for monitoring specific groups of substances under the WFD a monitoring project was initiated by the Swiss Centre for Applied Ecotoxicology (CH) and the German Environment Agency (UBA, DE). Furthermore, the project aimed at providing high-quality monitoring data for these EU Watch List substances. Surface waters from Watch List sampling stations in 14 EU member states and 4 Swiss cantons were analyzed for steroidal estrogens and diclofenac along with other pharmaceuticals (COX inhibitors, e.g. ibuprofen) by LC-MS/MS and screened in vitro for estrogenicity by estrogen receptor transactivation assays (ERTAs, such as the human cell-based ERα-CALUX and the yeast estrogen screen, AYES) and COX inhibition. The presentation aims to summarize the major results and conclusions of this large monitoring project: All applied chemical analytical and effect-based methods fulfilled the required maximum acceptable method detection limit for the Watch List monitoring. All together high-quality monitoring data were produced and the applicability of effect-based methods was shown to complement targeted monitoring of specific groups of substances in the aquatic environment under the EU WFD. The applied effect-based method of steroidal estrogens is more sensitive than chemical analytics pointing out its good screening potential. At the same time, it also implies that analytical methods are faced with detection challenges of the target analytes at low concentrations. Chemical analytical and effect-based methods can be combined in order to get the benefits of both: o to receive indication on substances and their contribution to the measured effects, o to study variable chemical exposure, o to exclude false negative measurements, o to monitor specific groups of substances in the aquatic environment.

3.08.5
Water toxicity monitoring using CHO cell lines and genetically modified bioluminescent bacteria

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Examining the emerging cytotoxic, genotoxic, and mutagenic contaminants in source waters have gained much attention in order to supply pollutant-free water. The genetically modified (GM) bioluminescent bacteria sensitive to cyto-, genotoxic, and reactive oxygen mediated (ROS) toxic effects and Chinese hamster ovary (CHO) cell lines as a cytotoxicity bioindicators are utilized in this study to understand the water toxicity. Seven different sources of water samples of municipal wastewater treatment plant effluent (TWW effluent), Tabor stream, aquaponics farm, hydroponics farm, the effluent of wetland treating municipal wastewater, Kinneret Lake, and Amud stream was collected from northern Israel. Source water samples collected from TWW effluent, wetland, Tabor stream were comparably geno-, and cytotoxic on GM bioluminescent bacteria than other water types, but not inducing ROS mediated effects at all in any source waters. The XAD 2/8 extracted organics were several folds concentrated on measuring its cytotoxicity in CHO cell lines; in contrast, the bacterial toxicity assays do not require any such sample pre-concentration. The XAD 2/8 extracts from Tabor stream, TWW effluent, wetland, Amud stream, and aquaponics farm showed a significant cytotoxic (LC_{50}) effect at different concentrations on CHO cell lines. Comparing the sensitivity of two test systems, there was no correlation between the cytotoxicity of GM bioluminescent bacteria and CHO cell lines at equal LC_{50} eluate concentration tested, reveals that both the monitoring tools act distinctively. The acidification and XAD extraction procedure could underestimate the cyto-toxicants in source water samples by any chemical modifications. The difference in cyto- and genotoxicity observed on both biomonitoring systems highlights the composition of organic and inorganic contaminants are very diverse between the water sources utilized.

3.08.6
Development of a high throughput tool for improving hazard assessment using microalgae

A. Almeida, NIVA, Section 213 / Ecotoxicology and Risk Assessment; T. Gomes, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment; A. Lillicrap, NIVA Norwegian Institute for Water Research / Ecotoxicology and Risk Assessment

The algal growth inhibition test is a well-established toxicological endpoint required by regulatory authorities to analyse the toxicity of hazardous substance present in the aquatic environment. However, they do not provide any further information on the toxic mode of action by which contaminants affect the biological processes of microalgae. Flow cytometry can be used as a diagnostic tool to analyse cells' metabolic status. Other important effects of contaminants in microalgae are alterations in photosystem II (PSII). The present study intended to develop a high throughput methodology to discern how contaminants specifically affect microalgae as a complement to algal growth inhibition tests. This tool set included effects on cell viability, cell size and complexity, cell cycle and DNA content, formation of reactive oxygen species (ROS), natural pigments content, mitochondrial and cytoplasmatic membrane potential, lipid peroxidation (LPO) and PSII performance. A first experiment was made to analyse the sensitivity of this methodology to detect sub-lethal toxic effects of different bottled waters in *Raphidocelis subcapitata*. A second experiment was conducted to further evaluate the applicability of the tool set to analyse the mechanistic effects of two antibiotics, azithromycin and doxycycline in *R. subcapitata*. Exponentially growing microalgae were exposed along 72 hours, after which all the endpoints were analysed. The most sensitive endpoints reflecting the effects of the bottled waters were oxidative stress related endpoints (ROS and LPO), pigment content and the morphological endpoints size, complexity and cell cycle. On the other hand, the growth rate was one of the least sensitive endpoints. For azithromycin and doxycycline, only the general toxicity data from the algal growth inhibition test is available, indicating high toxicity of both antibiotics (EC_{50} of 0.051 mg/L and 0.060 mg/L for azithromycin and doxycycline, respectively). The data given by the tool set is currently being analysed and seems promising for indicating the specific mode of action of each antibiotic in *R. subcapitata*. The used methodology proved to be sensitive and high throughput, simultaneously gathering information on the morphological, biochemical and physiological status of microalgal cells. This is therefore a promising tool to be incorporated in the future in microalgal toxicity bioassays for testing contaminants, to improve current hazard assessment policies.

Mass Spectrometry Screening Strategies to Evaluate Environmental Exposures: What Have We Learned and Where Are We Going?

3.09.1
Development of an effect-directed method for the identification of toxic contaminants present in hospital effluents
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An effect-directed analysis protocol was developed and applied to a hospital effluent (Biscay, Basque Country), one of the bigger hospitals in the Basque Country holding more than 800 beds and offering more than 13 specialities, using the sea urchin embryo test (SET) as toxicological in vivo bioassay. The objective was to identify compounds responsible for the toxicity by combining state-of-the-art sampling, toxicology assays, fractionation and targeted and suspect screening techniques. A fractionation step was performed by means of a C18 (18 fractions) and an aminopropyl (15 fractions) semi-preparative column and toxic fractions resulting from the SET at 75% effective concentration (EC75) were analysed by liquid chromatography coupled to an Orbitrap high resolution mass spectrometer (LC-HR-Q-Orbitrap/MS). The number of candidates (suspect screening) was drastically reduced from 446 detected in the raw sample to 27, which were identified in the two significantly toxic fractions according to the SET. Among them, 4 (caffeine, sulfamethoxazole, fluconazole and bisoprolol) were confirmed with available chemical standards, 17 were structurally confirmed and 4 tentatively identified by the isotopic profile. According to values compiled in the literature the top-ranking drivers of toxicity were pharmaceuticals (eprosartan, irbesartan, verapamil) and fungicides (fenpropidin, spiroxamine) being also identified here in the toxic fractions by means of the target analysis. This study demonstrated to be a functional effect of directed analysis (EDA) strategy focused on the identification of toxicological effects in aquatic fauna, being the SET applicable in hospital effluents.

3.09.2
Ozonation transformation products in water and wastewater treatment with different post-treatments
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Ozonation is increasingly been used in water and wastewater treatment to abate micropollutants, however, formed ozonation transformation products (OTPs) are largely unknown. We used a new approach of a) smart mixtures and b) mimicked matrix with relevant ozone and OH radical exposures in laboratory experiments to identify OTP candidates of 70 environmentally relevant micropollutants with different functional groups, first as signals only using LC-HR-MS/MS. Thereafter, their abundance in real water matrices (wastewater and spiked drinking water) after ozonation with different post-treatments were evaluated. For these prioritized, environmentally relevant transformation products, chemical structures were assessed. More than 1000 OTP signals were found in the batch experiments, but only a fraction could be confirmed in the real water samples based on the exact masses and on similar retention times. We were able to elucidate OTPs of environmentally relevant micropollutants, increasing our knowledge on their abundance, but also for prioritization of ozonation reactions. OTPs were found to be better abated in post-treatments using activated carbon (granular activated carbon filters and powdered activated carbon treatment) compared to purely biological post-treatment in a sand filtration. We could confirm our hypothesis that biodegradability of OTPs depends on the functional groups of the respective parent compounds.

3.09.3
A chemometrics workflow for MS environmental data analysis: the ROIMCR procedure
R. Tauler, IDAEA CSIC / Department of Environmental Chemistry
In the last years, chemometrics has experienced a large expansion in the omics and environmental fields, because of its role in the analysis of the large volumes of data produced by the high performance analytical techniques used, such as mass spectrometry. There is an urgent need for an improvement, dissemination and automation of all the steps of chemometric data analysis procedures, to summarize and integrate the wealth of information present in MS data sets, and to extract from them the sought environmental knowledge. Most of the proposed strategies in MS chromatographic data analysis need the application of peak alignment and peak shape modelling approaches and they associate every "feature" (i.e., chromatographic peak) to a unique m/z measurement. Thus, the development of an alternative data analysis strategy applicable to most types of MS data sets, which properly addresses these issues, is still a challenge. In this work, the ROIMCR procedure is proposed to filter and compress the massive MS data sets produced in environmental and omics studies without the need of these data pre-treatment strategies nor of loss of the mass spectral accuracy and with the aim to resolve the spectral and concentration (elution) profiles of all the chemical constituents of the analysed samples. Two examples of application of the ROIMCR method will be illustrated in the investigation of two cases: a) the analysis of the effects of TBT on human cells, and b) the investigation of the polymer degradation products produced in different wastewater treatment plants. Overall, the hereby presented ROIMCR strategy demonstrates the usefulness of chemometrics in the analysis of the massive data from high resolution MS instruments and it is a valuable addition to untargeted analytical approaches. The applicability of the ROIMCR method is confirmed and presents some advantages compared to other strategies. On one hand, the principal benefit of performing the ROI filtering and compression steps is the capacity to minimize the primary dimensions of the data (gigabytes of storage) while preventing any loss of spectral accuracy. On the other hand, the main advantages attributed to the MCR-ALS analysis include: i) the possibility of immediate chemical identification of the components of the investiogated samples (metabolites, lipids, transformation products…) based on the MS information provided in the analysis, ii) the possibility to infer realtive quantitative information about them or even their absolute quantitation if analytical standards are available; iii) the high degree of interpretability of the results, iv) the flexibility in the structure and nature of the datasets that are potentially able to be analyzed and v) the added value as a preprocessing method that does not require peak modelling nor chromatographic alignment for the simultaneous analysis of multiple samples. *Supported by ERC-AdG-32073-Chemageb*

3.09.4
Poster spotlight: Community engagement and perceptions of targeted and non-targeted chemicals of concern for municipal wastewater reuse onto human food crops.
E.G. Nichols, NC State University / Forestry and Environmental Resources
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3.09.5
Poster spotlight: Non-target screening using two-dimensional GC coupled to high-resolution MS reveals complex pattern of contamination in Arctic marine zooplankton
I. Øverjordet, SINTEF Ocean / Environment and New Resources
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Measuring, Modelling and Monitoring of Pesticides Fate in a Regulatory Context

3.10.1
Initiative to Develop a Harmonised Framework for Spatially Distributed Leaching Modelling of Pesticides
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Spatially distributed leaching modelling (SDLM) of pesticides is a methodology to estimate the leaching potential over an extensive spatial scale. Percentiles of the leaching concentration can directly be derived from vulnerability maps of the area of interest. SDLM can also help setting groundwater monitoring into context. In FOCUS groundwater, 2014, SDLM is foreseen as higher tier leaching risk assessment on Tier 3b and for supporting monitoring studies (Tier 4).The report of the SETAC EMAG-Pest GW working group (Gimsing et al., 2019) highlights the importance of SDLM for selecting monitoring sites or setting existing results into context and shows examples of vulnerability maps. However, no harmonised framework is available. This results in uncertainty on how to conduct such modelling and which data or tool should be used. It also causes uncertainty by evaluators that need to assess the studies. In the 9th European Modelling Workshop 2018, it was agreed that a dialog on a spatial modelling framework and version control for high-resolution spatial databases for the EU is needed. Therefore, a group of scientists decided to bring together experts from academia, regulatory authorities and industry, to discuss options and requirements for a harmonized framework.A first workshop on SDLM was held on 24 May 2019 in Ghent, Belgium. The main discussion areas were "Input Data", "Modelling Requirements" and "Regulatory aspects" resulting in the main conclusions that a harmonized framework on SDLM would be valuable and should be developed, SETAC would be a good host, a stepwise process should be followed and it

should be started working on technical and methodological topics. At the FOCUS version control meeting in Piacenza in September 2019 it was agreed by most participants that taking geographical databases under FOCUS version control would reduce uncertainty by increasing transparency and reproducibility. However, capacity issues were seen by EFSA. In the joint meeting of the Ghent workshop participants and the SETAC EMAG-Pest GW working group also held in Piacenza it was confirmed that SETAC would be an appropriate host. It was concluded that clarification with the SETAC Europe council is needed whether SDLM should be a continuation of SETAC EMAG-Pest GW or a new SETAC working group. A steering group was formed to define the scope of the working group and to setup a functioning working group regarding subgroups, membership and type and frequency of Meetings.

3.10.2
Lipophilicity and translocation of (non-)ionised chemicals in intact plants - one curve fits all?
C.A. Schriever, BASF SE; M. Lamshoeft, Bayer CropScience AG / R&D
The Transpiration Stream Concentration Factor (TSCF) is implemented as surrogate value describing compound removal from soil pore water in environmental fate models for regulatory risk assessment of plant protection products. Latest EU guidance for regulatory environmental fate modelling recommends, as a higher tier option, calculating TSCF values based on the empirical relationship between logKow and TSCF published by Briggs et al. (1982) (uptake of 18 non-ionisable compounds by young barley plants, bell-shaped relationship between lipophilicity and uptake). The authors of this presentation evaluated TSCF data for non-ionisable and ionisable compounds reported in literature to elucidate whether the empirical relationship applies also for ionisable compounds and other crops. Papers reporting TSCF data from hydroponic experiments with different crops and compounds were included in the investigation, if they had been reviewed and assessed in the paper of Doucette et al. (2018), who also rated the reported values in terms of overall confidence in the data. Additionally, TSCF data published by Lamshoeft et al. (2018) were considered in the investigation, a paper that was not considered by Doucette et. al., since it was published at the same time. TSCF data that met the criteria for high and medium overall data confidence were analysed for the lipophilicity of a compound at the pH value used for testing. Model performance of the equation by Briggs et al. was quantified using the model efficiency proposed by Nash and Sutcliff (1970). Reported TSCF values were distributed over the log Dow range following a bell-shaped curve. The equation by Briggs et al. provided an acceptable description of the data (Nash-Sutcliffe efficiency (NSE) of 0.32). NSE increased to 0.52 after parameter optimization. The curve by Briggs et al. mostly overlapped with the 95% confidence interval of the optimized curve between log Dow of 0 and 3 and was similar or lower than the lower bound of the 95% confidence interval for negative log Dow and log Dow values greater than 3. In conclusion, a bell-shaped curve gives an appropriate description of the relationship between the lipophilicity of a non-ionisable or ionisable compound and its observed uptake by intact plants from an aqueous solution, if the pH of the solution and partitioning of all forms of the molecule present in the solution are considered.

3.10.3
Impact of 20-year FOCUS SW simulations on data storage capacity - practical solutions for data reduction
D. Weber, Exponent International Ltd. / Environmental and Ecological Modelling; M. Brauer, Exponent International Ltd.; D. Patterson, TSG Consulting; B. Erzgraeber, BASF SE / Agricultural Solutions - Global Environmental Fate Modelling; D. Schaefer, Bayer Crop Science / Environmental Safety; G. Spickermann, ADAMA Deutschland GmbH; T. Jarvis, Exponent International Ltd.
The FOCUS SW Repair Action working group will release the updated FOCUS SW model suite in 2020. An interim MultiYear tool was used to evaluate the impact of the planned switch from single year to 20-year simulations on the calculation times and hardware requirements. Beside the duration of FOCUS MACRO and FOCUS TOXSWA simulations at Step 3-4 for 20-year runs, the requirements for data storage and hard drive capacities were analyzed. It was identified that the data storage requirements increase significantly for each project, especially for the TOXSWA output files. Details of the outcome and practical solutions to reduce the amount of output data, including avoidance of redundant files will be presented.

3.10.4
Determination of plant uptake of TFA by inverse modelling of a long-term semi-field study - incorporating root imagery
K. Hammel, M. Kueppers, Bayer AG, Crop Science Division / Environmental Safety; F. Fois, Bayer AG, Technology & Sustainability / Screening Technology; M. Beckmann, Bayer AG, Crop Science Division / Environmental Safety; G. Reinken, Bayer Ag / Crop Science Division
The transpiration stream concentration factor (TSCF) of a chemical is a key parameter to quantify its uptake via plant roots. The TSCF describes the ratio between the concentration in the xylem and in the soil solution and typically varies between 0 and 1. Models used for pesticide registration usually require a TSCF value as input. Hydroponic studies (duration 1 - 2 weeks) are currently established via an OECD test guideline to experimentally determine TSCF values for this purpose. The test compound trifluoroacetic acid (TFA) is stable in soils and plants, and does not adsorb to soil. It is found in the environment from multiple sources, including the degradation of some pesticides To study plant uptake of ^{14}C-TFA over a complete vegetation period of winter wheat and in a natural soil environment under variable weather conditions a long-term semi-field container study was undertaken with two different soils. The weighted, freely draining containers (1.25 x 0.8 m, 0.5 m deep, filled soil column) were kept in a roofed vegetation hall and experienced approximate outdoor weather conditions; natural rainfall was simulated by corresponding irrigation. TFA was applied at BBCH 12-14 to the soil surface. Beyond the measurement of water and TFA mass flows leaching from the soil or directed to the plant we used high resolution in-situ root imagery to determine from which part of the soil at which time water and TFA were potentially taken up. Because the TSCF is difficult to measure directly it was determined by inverse modelling using SWAP and PEST. The roots grew relatively quickly down to the bottom of the containers and developed a system of similar density over depth with a slight increase towards the bottom. In contrast to the low TSCF values derived in the hydroponic studies with TFA the TSCF values derived here were substantially higher (cumulative uptake was more than 50 % of applied) and much higher than derived with the empirical Briggs equation. The two soils behaved very similar with respect to the water balance and the TFA mass flows including uptake. Due to the well controlled experimental conditions and the long study period the inverse modelling approach is considered robust to obtain reliable real-world TSCF values. The inverse modelling approach is considered robust to obtain reliable real-world TSCF values due to the well controlled experimental conditions and the long study period over one complete vegetation period.

Metal Speciation and Bioavailability: Mechanistic Links between Exposure and Effects in Multi Stressor Environments

3.11.1
Persistent Residues of Silver in Northern Pike (Esox lucius) from a Lake Dosed with Nanosilver
C.D. Metcalfe, Trent University / Water Quality Centre; L. Hayhurst, Lakehead University; D. Patch, I. Koch, Royal Military College of Canada / Chemistry and Chemical Engineering; K.P. Weber, Royal Military College / Chemistry and Chemical Engineering
We participated in a study in which suspensions of silver nanoparticles (AgNPs) were discharged over two ice-free field seasons (i.e. 2014 and 2015) into a boreal lake (i.e. Lake 222) at the International Institute for Sustainable Development - Experimental Lakes Area (IISD-ELA) in northwestern Ontario, Canada. As part of this study, we monitored the concentrations of total silver (Ag) in the tissues of Northern Pike (*Esox lucius*) collected from the lake. As reported previously, the pike rapidly accumulated Ag in all tissues during the addition phase, with the highest concentration of 5.1 µg/g wet weight detected in the liver tissue of one of the pike. Following cessation of lake dosing, the Ag residues in pike tissues declined within a few months and by October 2016, the concentrations in liver were in the range of 60-130 ng/g wet weight. However, monitoring over the 4-years post-dosing (i.e. 2016 to 2019) showed that Ag residues in liver tissue did not decline further in large pike (i.e. >4 years age) from the lake. X-ray absorption near edge structure (XANES) spectra generated for Ag species present in the liver of one of the pike sampled during the addition phase indicated that AgNP was present in the liver tissue, along with $AgNO_3$ and Ag-cysteine. However, it is possible that the spectrum identified as AgNP represents metallic silver (Ag^0). We hypothesize that the initial rapid decline in the concentrations of Ag from pike liver was due to the loss of labile species, while the more persistent residues are due the continued presence of non-labile AgNP or Ag^0 in the liver tissue. These data illustrate the importance of understanding how the speciation of metallic nanoparticles influences bioaccumulation.

3.11.2
Considerations on assessment of ecotoxicological risks of metals in mining affected boreal lake sediments
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One of the environmental impacts of metal mining is metal and sulphate releases to the aquatic environment. Sediments act many times as final storages for contamination and continue to do so also after original sources has ceased. Various approaches can be used in assessment of ecotoxicity and ecological risks. However, all approaches come with advantages and disadvantages. This presentation considers issues connected to ecological risk assessment of metal mining affected sediments starting from timing of sampling to conditions in laboratory studies vs. field. Seasonal differences due to changes in dissolved oxygen situation and depending on the assessment method used it is detectable or not. In addition, many models and approaches exist (BLM, SQGs, SSD etc.) for a

few metals, but for example validation ranges for water quality of BLM-models don't necessarily cover the variability seen in water quality in the boreal regions (e.g. pH, water hardness). In the field, late winter the oxygen concentration and pH were low and the risks of metals high as compared to autumn. In contrast, the use of aeration in the laboratory scale ecotoxicological test vessels led to low pH, which affected survival of the experimental organisms. Timing of the sampling and the methods used to assess ecological risks of metal contaminated sediments may lead to different conclusions in boreal lakes affected by metal mining. In addition, seasonality in dissolved oxygen concentrations and pH cause additional stress. In many cases, these are not considered in the methods used for assessments.

3.11.3

Integrated ecotoxicological assessment of Rare Earth Elements mixtures in freshwater bivalve

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Rare Earth Elements (REE) are essential in high-technology products including technology for the transition to sustainable energy and numerous industrial, medical, military applications. Consequently, REE are considered technological critical elements subject to geopolitical issues. For about twenty years, their increasing extraction, processing and use led to their release in the environment including aquatic systems. However, nowadays, little is known about the ecotoxicology of REE particularly in complex natural systems. Moreover, most of the available data concerning REE toxicity is focused on single elements, whereas they are found in mixture in nature. REEs are expected to have cumulative toxic effects on organisms, owing to their similar physicochemical properties, but studies on their mixture toxicity, more representative of realistic scenarios, are necessary to uphold this assumption. In this context, the ECOTREE international research program aims to understand better the fate, behaviour and the potential biological effects of REEs on freshwater ecosystems and, particularly in this presentation, on Asian clam, *Corbicula fluminea*. The bivalves were exposed during 4 days to 3 REEs representative of the lanthanide series: neodymium, gadolinium and ytterbium alone and in mixture in absence and presence of Dissolved Organic Matter (DOM: 8 mg/L of dissolved organic carbon including 6.8 mg/L of fulvic acid) to assess the influence of DOM on REE toxicity. Main physicochemical properties of the exposure media were characterized in order to study further REE speciation and predict their bioavailability. Both, total REE bioaccumulation and subcellular compartmentalization were measured by ICP-MS in gills and digestive glands. Additionally, toxicity responses of bivalves were evaluated using a multi-marker approach at different levels of the biological organization. For the physiological assessment, several biomarkers of general or metal-specific stress, involved in antioxidant (i.e. superoxide dismutase, catalase), antitoxic (acid phospatase) and neurological (acetylcholinesterase) functions, metal (metallothioneins, hemolymphatic calcium) and energetic metabolisms (i.e. triglycerides) and cell damages (i.e. lipid peroxidation, apoptosis) were measured by automated spectrophotometer and polarography and expressed with multivariate analysis. Some of these analyses are still in progress.

3.11.4

Empirical bioavailability corrections for nickel in freshwaters

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In the recent revision of the Australian and New Zealand water and sediment quality guidelines increased emphasis has been placed on incorporating bioavailability-based approaches into guideline value derivations for metals. Previously, only water hardness had been considered as a toxicity modifying factor for metals, although other water chemistry parameters, such as pH and dissolved organic carbon (DOC), are also important. Biotic Ligand Models (BLMs) are available for nickel that account for the influences of hardness, pH, and DOC on nickel toxicity, and can be used to normalize the ecotoxicity database to local conditions to derive site-specific. At present, no BLMs have been endorsed to derive GVs for Australia and New Zealand, an alternative approach that has gained considerable attention from regulatory agencies uses multiple linear regression (MLR) models. Predictive MLR models were developed for algae, plants, invertebrates, and vertebrates and validated against independent datasets in order to ensure their applicability to species other than those used for the model development. A generalised bioavailability model was also derived which is intended to be applicable to all aquatic organisms. The model developed for algae provided poor predictions for aquatic plants, suggesting the need for a separate model to predict the bioavailability responses of aquatic plants. The MLR development dataset covered pH values from 5.5 to 8.5, DOC from 0.25 to 26 mg/L, calcium from 0.1 to 128 mg/L and magnesium from 0.2 to 286 mg/L. The generalised model resulted in the lowest adjusted r^2 and predicted r^2 values, and provided the poorest fit to the validation dataset, with some of the data being poorly predicted. The use of a suite of MLR models developed for different taxonomic groups provides a potential alternative to the current BLM which is used for predicting site-specific nickel toxicity.

3.11.5

The impact of speciation and bioavailability on uptake and toxicity of metal mixtures in Tisbe battagliai

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Metal rich runoff as a result of deposited iron sulphide-containing bedrock after construction E18 highway in southern Norway enters coastal water in Kaldvellfjord. Thus, aquatic organisms were exposed to several trace metals. The focus of this study was to characterize metal speciation and link it to the uptake and observed adverse effects (mortality) in marine copepods *Tisbe battagliai*. Thus, complex study on selected copepod was performed in order to determine: 1) speciation of metal in seawater 2) toxicity ($LC50_{(24/48h)}$ values) for single metal and mixtures as well as 3) determine accumulated metal concentration in the whole-body tissues. Among many metals found at elevated levels in Kaldvellfjord, copper, nickel, zinc and aluminum were assumed to have a significant contribution to cumulative risk. Toxicity test (48h) were performed for both life stages: adults in age of 14 days and juveniles (6 days old) in fixed conditions (20 ± 2 °C, pH = 8,1±1, salinity 30‰). Determination of uptake was performed on both: single metal exposure and mixtures in concentrations of LC1, LC5 and LC10. Quantitative and qualitative analysis of metals in both, water samples and digested tissues were obtained using *inductively coupled plasma – mass spectrometry* (ICP-MS) method. For the determination of size distribution of selected elements in water samples, membrane filters and hollow fiber ultrafilter were used. Among examined metals copper was determined as the most toxic with the $LC50_{(48h)}$ value of 0.27 µmol/L for juveniles. Mixture toxicity test resulted with conclusion that the most toxic mixture is a combination of Cu and Zn with $LC50_{(48h\text{-}juvenile)}$ equal to 0,09 µmol/L. Results from uptake tests show that uptake of zinc is higher when present in each selected mixture. Although, percentage of LMM for copper is low in seawater (5% in 6 µmol/L solution) it doubles when present in mixture with zinc. Moreover, zinc accumulation is higher when mixed with copper, which can potentially lead to hypothesis that observed increased mortality is a result of joint effect of these two metals. These results lead to the conclusion, that speciation will affect the uptake and accumulation in organisms and thus potentially influence the toxicity of the metals to aquatic organisms as uptake is correlated to toxic effects.

3.11.6

Starvation causes changes to arsenolipid profile in Mytilus galloprovincialis: evidence for remobilization, consumption, and depuration

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The present study reports the changes in arsenolipids, naturally occurring lipid compounds that incorporate arsenic, in the Mediterranean mussel *Mytilus galloprovincialis* held under starvation conditions.Content of lipids and arsenolipids were tracked in individual mantles and digestive glands. Arsenolipid content and speciation was determined by using a HPLC-Mass Spectrometry coupling. Arsenolipids showed a behaviour similar to that ascribed to lipid classes under starvation conditions, with more polar arsenolipids (carbon chain, C < 20), decreasing, and less polar arsenolipids (C > 20) preserved.Less polar arsenolipids were stipulated to be to remobilize to the mantle. Arsenolipids in digestive gland, AsFA436, AsHC360 and AsHC404 had the most pronounced decreases; the long chain fatty acids (AsFA514, AsFA528, AsFA530, AsFA542 and AsFA558) were preserved or increased during starvation.During starvation seven arsenolipids (AsFA514, AsFA542, AsFA530, AsFA558, AsHC332, HC360 and AsPt546) present in the mussels were identified in the mussels' faeces suggesting that this was a depuration route. Three arsenolipids were exclusive to faeces; their origin was discussed.

3.11.7

Rigorous physicochemical framework for metal ion binding by nanoparticulate humic substances: implications for speciation codes and bioavailability models

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Humic substances (HS) are natural soft nanoparticulate complexants which play an important role in buffering free metal ion concentrations in aquatic ecosystems. It is widely assumed that the equilibrium concentration of the free metal ion is the relevant parameter for predictions of bioavailability. In this context, there is widespread use of equilibrium speciation codes, notably NICA-Donnan (incorporated in Visual MINTEQ) and WHAM, to compute free metal ion concentrations, which are subsequently used in ecotoxicological risk assessment models, e.g. BIO-MET and PNEC-PRO. The risk predicted by such bioavailability-based models is thus dependent on the parameters used by the speciation codes to compute the extent of metal ion binding by HS. In recent years a rigorous generic theoretical framework has been developed to describe the electrostatic and chemical contributions to metal ion binding by nanoparticulate complexants. The theory describes the way in which the reactivity of charged nanoparticles towards metal ions depends on particle size and particle type (i.e. reactive sites distributed within the particle body or confined to the surface), as well as the ionic strength of the aqueous medium, and the nature of the metal ion. HS dispersed in aquatic systems generally carry a net negative charge; the particle electric field has the potential to electrostatically impact on the chemical speciation dynamics. For the example case of soft environmental particles such as HS, we have delineated practical strategies for determining intraparticulate metal ion speciation, and for evaluating intrinsic chemical binding affinities. The heterogeneity of the chemical binding derived from our nanoparticulate treatment of HS is in good agreement with that independently determined by electrochemical measurements. In contrast, the outcomes of the NICA-Donnan and WHAM models lead to heterogeneity parameters that are not supported by independent experimental data. The presentation will discuss the findings in terms of a physicochemical analysis of the discrepancies generated by these equilibrium speciation codes, thereby revealing the a priori hypotheses adopted therein and the inappropriateness of some of their key parameters. It follows that attempts to correlate metal speciation computed by these codes to dynamic features such as lability, bioavailability, and/or toxicity are bound to suffer from physicochemically poor outcomes.

3.11.8
Assessing metal speciation with the technique AGNES

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Free metal ion concentration in a medium has been highlighted as a key factor in its (bio)availability to organisms, for instance, in the Free Ion Activity Model (FIAM) or the Biotic Ligand Model (BLM). Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) is an electroanalytical technique specially designed to provide free metal concentrations (such as Zn^{2+}, Cd^{2+}, Pb^{2+}, Sn^{2+}, etc.)[1,2]. One of AGNES' strong points is the simplicity of interpretation of the results, while one weak point is that its standard applications (with mercury based electrodes) is restricted to amalgamating elements. We will review the principles, limitations, developments and outstanding applications of AGNES including matrices such as river, estuarine and sea waters, soil extracts, humic acids dispersions, growth media, etc. As recent advances, we will explain how AGNES has been able to follow the kinetics of dissolution of ZnO nanoparticles by measuring the free zinc concentration (without any previous separation) in artificial saliva [3]. The solubility of $In(OH)_3$ at various pH values and other results on indium will be presented [4-6]. The application of AGNES to determine $[Sb(OH)_3]$ will be shown [7]. [1] Companys *et al.* 2017. A review on electrochemical methods for trace metal speciation in environmental media. Curr Opin Electrochem 3:144-162. [2] Galceran *et al.* 2004. AGNES: a new electroanalytical technique for measuring free metal ion concentration. J Electroanal Chem 566:95-109. [3] David *et al.* 2019. Dissolution and phosphate-induced transformation of ZnO nanoparticles in synthetic saliva probed by AGNES without previous solid-liquid separation. Comparison with UF-ICP-MS. Environ Sci Technol 53:3823-3831. [4] Rotureau *et al.* 2019. Towards improving the electroanalytical speciation analysis of indium. Anal Chim Acta 1052:57-64. [5] Tehrani *et al.* 2018. Free Indium concentration determined with AGNES. Sci Total Environ 612:269-275. [6] Tehrani *et al.* 2019. Determination of the lability degree of indium complexes and its application in measuring low free indium concentration. J Electroanal Chem 847:113185. [7] Pla-Vilanova *et al.* 2019. Antimony speciation in aqueous solution followed with AGNES. J Electroanal Chem 849:113334 *Acknowledgement* - The authors gratefully acknowledge support for this research from the Spanish Ministry MINECO (Project CTM2016-78798) and from European Union Seventh Framework Programme FP7-NMP.2012.1.3-3 [grant agreement no. 310584] (NANoREG).

3.11.9
A newly observed metal bioaccumulation route in intertidal mussels: cadmium uptake from mantle cavity liquid during low tide in Xenostrobus atratus

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Intertidal bivalves are the most frequently used biomonitors to indicate the status and trend of contamination in coastal waters. In intertidal zones, organisms are alternately immersed in water and exposed in air. It is tempting to speculate that the organisms will temporarily escape from the contaminants when they are out of water. However, this speculation has never been explicitly examined. In this study, we investigated cadmium (Cd) toxicokinetics in an intertidal mussel, *Xenostrobus atratus*, under the effects of tidal exposure using simulated tidal regimes. The uptake rate constant (k_u) of Cd ranged from 0.045 L g^{-1} d^{-1} to 0.109 L g^{-1} d^{-1}, whereas the elimination rate constant (k_e) of Cd ranged from 0.029 d^{-1} to 0.091 d^{-1}. Cd bioaccumulation was slightly higher in the continuously immersed mussels than the alternately immersed mussels, but much lower than what would be expected if assuming bioaccumulation being proportional to immersion duration. Cd uptake was observed even when mussels were exposed in air, due to uptake of Cd dissolved in mantle cavity fluid and internalization of Cd adsorbed on mussel tissues. Overall, tidal height showed limited effects on Cd bioaccumulation, consistent with the trend of Cd concentrations found in *X. atratus* collected from different tidal heights. The mantle cavity uptake mechanism is expected to be applicable to other contaminants and bivalves, and should have important implications in risk assessments for intertidal environment.

3.11.10
Exploring the link between cadmium stable isotope compositions and speciation in plants: a case study in plants from the Solanum species.

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The environmental pollution by ecotoxic heavy metals is a problem of increasing significance for ecological and human health reasons. Among these elements, cadmium (Cd) is of special concern due to its high mobility in the soil/plant system and its acute toxicity. To limit Cd contamination in plants, and to enhance food safety, it is critical to understand Cd biogeochemical cycle, in particular what controls the bioavailability of this element. Previous studies have demonstrated that Cd bioavailability in soils depends ultimately on its chemical speciation, whose investigation in plants that feed the world remains extremely challenging due to traditional X-ray spectroscopy technical limitations in low-concentrated samples (~100 ppm). In this study, we explore the link between Cd speciation and Cd stable isotope composition in plants from the solanum species (Solanum nigrum and S. Melongena) to assess to what extend Cd isotopes can trace changes of in Cd speciation in plants. S. nigrum and S. melongena were grown on clayey loam soil incubated with Cd nitrate (4 treatments: 100, 50, 25 ppm Cd and control experiment, 7 pots of two plants per treatment) for 14 weeks, then processed for X-ray absorption analyses, Cd stable isotope composition and trace and major element determination. Our XANES and EXAFS results demonstrate that Cd behaves differently in S. nigrum and S. melongena. In both roots and leaves of S. nigrum, results show the major role played by thiol ligands, while Cd transport form in the shoot involves binding with carboxilic acids. Our preliminary results suggest glutathion as a potential ligand for Cd. In S. melongena, results show a different fate of Cd between the roots and the leaves: while Cd speciation in the roots also involves thiol ligands, carboxylic acids likely play a major role in the binding of Cd in S. melongena leaves. XAS results demonstrate the role of Cd binding to different organic ligands (thiols and carboxylic acids) and changes in Cd speciation in S. nigrum and S. melongena plants when exposed to high Cd content. These different behaviours are very promising for the rest of our study, as theory predicts Cd isotope fractionation between –O and –S ligands. The upcoming results on Cd stable isotope compositions of these samples will help shed light upon the mechanisms that shape the fate of Cd in S. nigrum and melongena and assess the potential of Cd isotopes as tracers of cadmium speciation changes in solanum plant species.

3.11.11
Elucidation of chemical factors driving ecotoxicity of Flanders' freshwater and brackish sediments

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Sediment quality is crucial to aquatic ecosystem health: 'Legacy' pollutants reside in sediments and exert adverse effects on aquatic organisms.To assess these effects, bioassays evaluate toxic effects on organisms. Assays are only implementable in environmental risk assessment, if results are interpretable and can be (cor)related to test conditions/parameters. However, relations between sediment properties and bioassays are scarce and predictions based thereon remain elusive. We aimed to explain the outcomes of bioassays by investigating the relationships with concentrations of chemicals in sediment. For sediment concentrations from the Flemish Environment Agency, we applied recent and applicable information on speciation and bioavailability (as function of e.g. organic material, clay, redox and pH). We considered bioassays on *H. azteca* (detritivore), *R. subcapitata* (microalga), *T. platyurus* (crustacean) and *H. incongruens* (ostracod) and EC50/LC50 values from the RIVM/USEPA databases. Aquatic risk is dominated by heavy metals and ammoniacal nitrogen, whose effects can be accurately predicted by accounting for a suite of sediment characteristics. Pollution and biophysicochemical sediment characteristics vary geographically in Flanders. Highest concentrations were for nitrogen/phosphorous, followed by heavy metals. The Scheldt (esp. the upper Scheldt) contained much organics and metals; the Yser phosphorous/nitrogen. *H. azteca* was the most sensitive organism. 8 heavy metals and NH_4^+/NH_3 in the Scheldt, Yser, Albertcanal and moats explain 50-60% of the variance in the observed *H. azteca* mortality. Nitrogen and organotins appear to affect *H. incongruens* germination. 30-40% of toxicity is of undetermined cause; outliers/extreme values may relate to testing conditions/set-ups, species variabilities, incoherently documented exotic pollutants (e.g. Co, Sn, organochlorides) and additional biophysicochemical properties.

Micro(nano)plastics Occurrence, Fate and Effects: Mechanistic Approaches to Study Risks to Environmental and Human Health

3.12.1
Microplastic occurrence in urban sewage systems: Identification of sources for pathways into the environment

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All over the world, microplastic (MP) particles (particle size: 1 - 1.000 µm) are found in water, soil, air, biota and even food products. But plenty of these discussed findings are based on a very low number of real datasets, which are extrapolated to general projections. Furthermore, most data are not comparable because the strategies for sampling, sample preparation and detection methods are not harmonised/ or standardised. This would require extensive proficiency tests. Because of the ubiquity presence and the unclear risks, which might arise from those particles, various political and environmental organisations (i.e. OECD, UNEP, WHO) identify the reduction of plastic entry in the environment as a key challenge for now and the future. This challenge includes the identification of relevant entry pathways but also the demand of harmonised, meaningful and reliable analytical procedures. Regarding this task within the last few years, a fast practical solution for MP analysis has been developed, which includes the steps of representative sampling, adequate sample preparation and fast detection. Sampling is done by fractional filtration over sieves with mesh sizes of 500, 100, 50 and 5 µm [1]. The received samples are measured by ThermoExtraction/Desorption-Gas Chromatography-Mass Spectrometry (TED-GC-MS) [1,2]. for the most abundant polymers used in practice, which are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyamide (PA) and acrylate as well as styrene-butadiene-rubber (SBR), a main component of tires. The present presentation will give first-time insight in a comprehensive dataset of microplastic analysis for an exemplary urban sewage system. MP mass contents of different waters at several days, such as greywater, stormwater retention tank, influent and effluent of a wastewater treatment plant (WWTP) within an urban sewage system in Germany are determined. Furthermore, the mass of the polymers found in dry weather and rain weather flow are compared. The use of these large datasets allows first expressive conclusions regarding the contribution of urban sewage system to the MP entry sources in the environments. We found PP and PS in all different waters. Furthermore, there is SBR in influent and also in effluent of the WWTP. Surprisingly, we could also detect hugh amounts of PE in the effluent of the WWTP. [1] Bannick C.G., Szewzyk R., Ricking M., Schniegler S., Obermaier N., Barthel A.K., Altmann K., Eisentraut P, Braun U. 2019. Development and testing of a fractionated filtration for sampling of microplastics in water. Water research. 149:650-658 [2] Duemichen E., 2019. Automated thermal extraction-desorption gas chromatography mass spectrometry: A multifunctional tool for comprehensive characterization of polymers and their degradation products. J. Chrom. A. 1592:133-142 [3] Eisentraut P., Dümichen E., Ruhl A. S., Jekel M., Albrecht M., Gehde M., Braun U. 2018. Two Birds with One Stone-Fast and Simultaneous Analysis of Microplastics: Microplastic Derived from Thermoplastics and Tire Wear. Environ. Sci. Technol. Lett. 5:608-613

3.12.2
Occurrence and fate of microplastics in fat, grease, and sludge feeding to the anaerobic sludge digester

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Wastewater treatment plants (WWTPs) reduce the amount of pollutants discharged from urban wastewaters to the aquatic system and have repeatedly been studied with respect to microplastics (MP). However, the occurrence and fate of MPs in the internal process streams of WWTPs have not been studied to the same level. The present study investigates MPs in grease and sludge (before and after digestion) from Ryaverket WWTP, Gothenburg, Sweden. In addition, MP was analyzed in fatty slurries from restaurants, a bakery, a butter factory, and an ice-cream factory (termed external organic materials, EOM), which is fed into the anaerobic digester of the WWTP. The laboratory sample preparation varied as per the sample's nature. The excessive water from grease and sludge was removed for concentrating the particles and making a homogenous sample. About 2.5 g of grease, 400 mL of EOM, and 5 g of sludge (dry weight) were analyzed per sample. Different enzymatic treatment and oxidation steps were performed to remove cellulose, protein, and other organic materials. Floatation was applied for removing the inorganic particles. The extracted particles were analyzed by FTIR, namely µFTIR imaging (Agilent Cary 620/670 FTIR, FPA size 128X128, transmission mode) for MPs of 10-500 µm and ATR-FTIR (Agilent Cary 630 FTIR Spectrometer, diamond ATR) for MPs of 500-5000 µm. Furthermore, siMPle and OMNIC softwares were used to identify and quantify MPs. In total, 14 types of polymers were identified: Polyethylene (PE), Polypropylene (PP), Polyester (PEst), Polyamide (PA), Polystyrene (PS), Polyurethane (PU), Polyvinylchloride(PVC), Acrylic, Cellulose acetate, Acrylonitrile Butadiene Styrene(abs), Poly-vinyl acetate (PVAc), Polyamide acrylic fiber, Alkyd, and Aramid. The prevailing polymers were found to be Polyethylene, Polyester and Polypropylene, comprising 40%, 28%, and 9% of all identified MPs, respectively. The highest MP concentrations by particle number was found in digested sludge as 6.2×10^6 particle/kg and the lowest concentration was found in EOM as 2.47×10^5 particle/kg. Further, MPs in grease were larger than in the other matrices, which increased the mass concentration to 3.5 g/kg. A substantial amount of MP was also found in the other matrices, such as 0.308 g/kg, 0.183 g/kg, and 0.05 g/kg for digested sludge, pre-digested sludge and EOM, respectively. ***Keywords****:* Wastewater treatment plants, Microplastics, Grease, Anaerobic sludge digester

3.12.3
Spatial distribution of microplastic in surface water, sediment and biota along a river

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The interest in studying microplastics (MPs) in freshwater is continuously increasing. Still, one of the unknowns to date is the spacious variation of MP transport. Moreover, further field studies with freshwater organisms need to be performed. Here, we investigate the spatial distribution of MP in water surface (60 µm mesh size Neuston trawl), subtidal sediment, fish (GI of catfish, Silurus glanis), and macroinvertebrates (Hydropsychidae) along the length of the Ticino river in the North of Italy (six sites of an 80 km transect). In this study we aim to provide additional insight in (1) microplastic transport along a river and (2) sampling and extraction techniques for freshwater ecosystems. Environmental samples from different matrices require specific solutions and conditions to chemically digest organic material. Preliminary tests on MP extraction were already performed; the applied digestion protocol includes 30% hydrogen peroxide with iron catalyst (H2O2 + Fe) which is more appropriate for plant material and 10% potassium hydroxide (KOH) for animal tissues. Samples were digested for 1h at 50°C before sodium chloride solution (NaCl) was added for density separation (repeated once) and the solution filtered. For the planned analysis, particles on the filter will be visually identified with a stereo microscope and transferred onto silver membrane for the chemical analysis for polymer identification by Fourier transform infrared spectroscopy. Procedural blank will be included to assess sample contamination in the laboratory. Analysis will be completed in winter and results presented at the conference. Positive control samples of grinded, amorphous PS particles of different size categories were used to evaluate efficiency of the entire MP extraction techniques. Results from preliminary tests confirmed the presence of MP in surface water, sediment and fish GI content. The chosen solutions digested animal tissue, plants, plankton and other organic material in the samples sufficiently. The mass recovery of PS particles was 98.2% ± 0.96 (SD) for extraction using H2O2+Fe and 97.1% ± 2.4 for extraction using KOH. A first quantification of MP in water sample from the North of the Ticino revealed 5.6 MPs/m3. The concentrations are expected to increase with the length of the river towards the Po estuary. This study will provide insight into the extent of microplastic pollution in the Ticino river and give indications about major input sources based on the analysis of complex matrix samples from different sites. The use of positive control, which is highly recommended for an adequate assessment in high-quality research, verified a high mass recovery of MPs (PS). The extraction of further grinded, amorphous polymer types will be tested and the results presented at the conference. Moreover, the simultaneous analysis of water, sediment and biota samples along the sites of the river, could give an indication weather MP ingested by benthic invertebrates and fish reflect the environmental contamination.

3.12.4
Distribution of microplastics in abiotic and biotic compartments of Arcachon Bay during spring and summer.
C. Lefebvre, Environnements et Paléoenvironnements Océaniques et Continentaux; F. Le Bihanic, EPOC / Bat B eme etage; J. Latrille, M. Tanfin, L. Chassaigne--Viscaino, University Bordeaux UMR CNRS EPOC; C. Clérandeau, University of Bordeaux / EPOC UMR; S. Villette, UMR CNRS CBMN; B. Morin, University of Bordeaux / EPOC; S. Lecomte, UMR CNRS CBMN; J. Cachot, Université Bordeaux / EPOC
p margin-bottom: 0.21cm; direction: ltr; color: rgb(0, 0, 0); text-align: justify; p.western font-family: "Arial", sans-serif; font-size: 10pt; p.cjk font-family: "Times New Roman", serif; font-size: 10pt; p.ctl font-family: "Arial", sans-serif; font-size: 12pt; a:link color: rgb(0, 0, 255); Microplastics (MP) are ubiquitous in marine environments as they can be found from sea surface to hadal zone. It even enter trophic chain at different level due to there size inferior to 5mm. They are now considered as an emerging contaminant that erode water quality and damaged marine life. However, MP concentrations at meso-scale are not often reported for several compartment simultaneously. Considering distribution for different sea level could help understanding microplastic flux in a target area. Here, we display the distribution of microplastics in Arcachon Bay, a semi-enclosed lagoon located on the French Atlantic coast. Three abiotic compartments (surface, water column and sediment) and four species, the Pacific oysters (*Magallena gigas*), common seabass (*Dicentrachus labrax*), common sole (*Solea solea*) and spider-crab (*Maja brachydactyla*) were studied. Preliminary results were based on visual sorting under microscope of MP-like particles. At sea surface, concentrations range between 0.11 ± 0.03 and 0.01 ± 0.01 particle.m^{-3} while they reached 275.42 ± 43.41 particles.m^{-3} in the water column. Concentrations were 2 500 to 10 000 higher in the water column than from sea surface. The dominant particle shape at surface water was overall fragment while it was fibres in the water column. In sediment, the highest concentration reached 59.75 ± 73.87 particle.Kg^{-1} by dry weight and the lowest concentration was 22.75 ± 8.62 particle.Kg^{-1}. They were mainly fibres as they represent at least 91% of the recorded particle. At the two stations studied, wild oysters ingested 0.06 ± 0.09 and 0.37 ± 0.38 particle.gram^{-1} (fresh weight) that were characterised as fibre at respectively 70% and 92%. Our preliminary results show that every compartments were contaminated by MP-like particles but this will be examinate more accurately by Attenuated Total Reflectance Fourier Transformation Infrared Spectroscopy (ATR-FTIR) analysis. These data provide an overview of the contamination in Arcachon Bay and about its distribution among biotic and abiotic compartments. Combined with modelisation study, this will help in understanding MP sources and flux in a meso-tidal lagoon.

3.12.5
DETECTION AND QUANTIFICATION OF NANOPLASTIC IN NATURAL SAMPLES BY MULTI-SCALE CHARACTERIZATION
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Plastic pollution becomes increasingly documented and recent studies have revealed the large extent of this problem worldwide. A serious concern about plastic pollution is the occurrence of micro- and nano-metric plastic particles (MNP). But there are still scarce studies characterizing plastic pollution at this scale. This is explained by technical challenges we have to overcome like the concentration and isolation of MNP in order to provide their physico-chemical characterization and to develop quantification methods. Although degradation of polymers has been studied for a long time under laboratory conditions, the weathering conditions occurring in a complex environment (like in oceans or rivers) conduct to a combination of complex mechanisms. The mechanisms conducting to the formation of MNP are not clearly described2. The chemical nature of nanoplastics is not elucidate. For example we do not know how the macromolecule is organized in a nanoplastic. Finally nanoplastic have to be apprehended with their eco-corona that is to say with the natural organic matter and the ions it is interacting strongly with. In this presentation we will present the physico-chemical characterization of environmental samples (marine and river samples). We are developing a quantification method for nanoplastic by pyrolysis coupled to gas chromatography and tandem mass spectrometry (Py-GC-MS/MS)3. In addition, to better understand the structure of nanoplastic, we will present the microstructure characterization of marine plastic mesoplastic. Cross-sections of these materials were analyzed by several emergent mapping techniques. These characterizations highlight the deep modifications of the weathered materials within a layer a few hundred microns thick. The most intense modifications are macromolecule oxidation and a considerable decrease in the molecular weight. Fragmentation of the oxidized layer of the plastic debris is the most likely source of nanoplastics. The present work demonstrates that nanoplastics are composed of highly oxidized macromolecule chains that are significantly shortened in length. 1. Gigault, J.; Pedrono, B.; Maxit, B.; Ter Halle, A. Marine plastic litter: the unanalyzed nano-fraction. Environ. Sci-Nano 2016, 3 (2), 346-350. 2. ter Halle, A.; Ladirat, L.; Martignac, M.; Mingotaud, A. F.; Boyron, O.; Perez, E. To what extent are microplastics from the open ocean weathered? Environ Pollut 2017, 227, 167-174. 3. ter Halle, A.; Jeanneau, L.; Martignac, M.; Jarde, E.; Pedrono, B.; Brach, L.; Gigault, J. Nanoplastic in the North Atlantic Subtropical Gyre. Environ. Sci. Technol. 2017, 51 (23), 13689-13697.

3.12.7
Poster spotlight: Distribution of microplastics in Surface waters of ponds and channels in two South Ryadh and Al-Jubail (Saudi Arabia)
Y. Pico, University of Valencia / Environmental Quality and Soil
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3.12.9
Virgin and oxo-degradable plastic leachates effects in freshwater environment
s. schiavo, M. Oliviero, S. Chiavarini, ENEA CR; S. Manzo, ENEA CR / SSPT-PROTER-BES
Currently many discussions upon the environmental impact of oxo-degradable plastic leachates take place, however it has not been conclusively proven that there are no negative effects. The study on plastic polymers toxicity generally include the exposure of test organisms to virgin plastic pellets, supposed to be free from any additives and/or residual monomers. In this line, this study is aimed at studying the acute and chronic adverse effects of leachates, coming from virgin and oxo-degradable plastic polymers (PE, PP and PS), on test organisms belonging to several species and pertaining to different trophic levels: the bacteria *Vibrio fischeri* (acute toxicity), the plants *Sorghum saccharatum, Lepidium sativum*, and *Sinapis alba* (sub-chronic toxicity) the crustacea *Daphnia magna* (acute and chronic toxicity). To identify the possible ecotoxicological risk for freshwater biota the toxicity test battery integrated index (TBI) is used. Chemical analyses show the presence of metals in the range of µg/L (100% leachate) and of organic molecule for oxo-degradable polymers in the order of mg/L (for the major ones). It is possible to identify only 2,6-Diisopropylphenyl isocyanate and 2,6-Diisoproylaniline. Regarding toxicity assessment for virgin polymers, *Daphnia magna* shows the highest susceptibility in the chronic exposure tests (around 50 % of effect) while *V. fischeri* (around 25 % of effect) in the acute ones. No relevant toxic effects are observed on *S. saccharatum*, *L. sativum* and *S. alba* seeds. TBI allows to rank the toxicity risk associated to virgin polymers as follows: PP>PS>PE. In case of oxo-degradable polymer leachates *D. magna* survival is mainly affected by PS and PP leachates (72% and 61% effect, respectively) while PS notably reduce the reproduction rate. On plants, only PP exerts a negative effect (*S. saccharatum* IG% 32.4), while *V. fischeri* always shows values around 50%. The data integration, by TBI approach, allows to rank the oxo-degradable polymer leachates toxicity as follows: PE>PS>PP. This result could be mainly ascribable to the highest metals content in PE since no difference with organic compounds analysis is evidenced. The introduction of the oxo-degradable additive in the polymer seemed to increase the level of ecotoxicological risk of polymer leachates itself, suggesting that toxicity was ascribable to both additive occurrence and to enhancement of polymer (treated to be degradable) leachability.

3.12.11
Tackling the detection issues of effects caused by nanoparticles enable to build Adverse Outcome Pathways
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Engineered nanoparticles (ENPs) may end up in the environment. A growing body of evidence shows that some ENPs are taken up by organisms, distributed in their bodies, and consequently accumulated in various tissues and hence lead to effects. Currently there are quite limitations in characterizing and quantifying ENPs in biological media. Some techniques have recently been introduced and used to characterize and quantify ENPs. Most of these techniques are limited when applied to ENPs in biological media due to the complex and polydisperse matrices encountered there. I will present a generic multistep method for extraction of polymer-based ENPs from biological matrices. As well as present ecotoxicity tests results with zebrafish and how these studies thereby began to unravel a currently overlooked set of interlinked events in fish triggered by exposure. For the polymer-based ENPs, asymmetrical flow field-flow fractionation (AF4) was used where the retention and separation are caused by an external field (so-called crossflow). The theory behind AF4 and the mechanisms of functioning have been well described in the literature. AF4 can be used for the analysis of ENPs in liquid matrices, and we prove that this technique is a powerful tool to achieve information about the size distribution of NPs in complex matrices. Bioassays were performed according to stardardized FET tests, zebrafish embryo tests. The exposure concentrations used were derived from an initial dose-response analysis representing a no-effect concentration for mortality. Different ecophysiological endpoints were determined, such as glucose, cortisol levels as well as immuno-responses. Behavioral analysis was chosen as an apical more sensitive toxicity endpoint. The behavioral analysis was performed by subjecting the embryos to the light–dark challenge test. In conclusion, we illustrate that metal-based

nanoparticles induce immune-responses. And that polystyrene nanoparticle disrupt glucose homoeostasis with concurrent activation of the stress response system, eventually disrupting the swimming behavior.

3.12.12
Poster spotlight: Assessment of the Effects of Chronic Exposure of Polystyrene Micro- and Nanoplastics to Daphnia magna
O. Pikuda, McGill University / Chemical Engineering
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3.12.13
Poster spotlight: Impact of PMMA nanoplastics in the microalgae Rhodomonas baltica: effects of surface functionalization
T. Gomes, Norwegian Institute for Water Research (NIVA) / Section of Ecotoxicology and Risk Assessment
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3.12.14
No Trojan Horse In Sight: Wastewater Treatment Reduces Multigenerational Effects Of Polystyrene Microplastics On Daphnia magna
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The Trojan horse or vector hypothesis poses that microplastics can accumulate chemicals from the aquatic environment that are then released during the gut passage of organisms potentially increasing the toxic load. While wastewater treatment plants (WWTP) remove the majority of microplastics during the treatment process, the sheer volume of released effluent makes them a significant point source of microplastics in the aquatic environment. Currently, most studies address the Trojan horse hypothesis by combining of single substances and microplastics. In contrast, we simulated the passage of microplastics through a WWTP and compared the toxicity of those loaded particles in *Daphnia magna* to virgin particles. Irregular polystyrene microplastics were incubated in sterile filtrated raw wastewater and subsequently separated from the water through filtration and lyophilization. We exposed *Daphnia magna* in a multigenerational study in four consecutive reproduction experiments under food-limited conditions and investigated the effects of microplastics on mortality, growth, reproduction, and neonate size. While food limitation had no adverse effect itself, mortality generally increased over the generations in groups exposed to microplastics. Reproduction decrease as a function of particle concentration and over time. Effects were more distinct for the particles only incubated in ultrapure water as compared to those incubated in wastewater. Overall, our experiment does not support the hypothesis that the chemicals associated with wastewater increase the toxicity of polystyrene microplastics in a multigenerational setting.

3.12.16
The role of microplastics on the bioaccumulation, biotransformation and toxicity of the antibiotic clarithromycin on river biofilms
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Due to their ubiquity and potential to adsorb chemical contaminants, microplastics (MPs) have been recognized as a potential threat for non-target organisms and ecosystems, representing a growing environmental concern. Although MPs act as vector of other pollutants, there is still a gap of knowledge on the role that MPs play on this interaction and how this could be reflected in what concern to toxic effects, bioaccumulation and biotransformation of emerging contaminants as well as changes in the metabolism of non-target organisms.In this work, mesocosm experiments were used to better understand the interactive effects of polyethylene MPs on the toxicity, bioaccumulation and biotransformation of the antibiotic clarithromycin in river biofilms. River biofilms are a complex aquatic community, mainly formed by bacteria, algae and fungi, embedded in a mucopolysaccharide matrix. Due to their characteristics, river biofilms have been used as indicators of the water quality of aquatic ecosystems, appearing as a good model to evaluate the impact of organic contaminants. Short-term exposure experiments (72h) were performed with river biofilms that were exposed to 50 µg/L of clarithromycin and 1.0 mg/L of polyethylene MPs following single and combined scenarios. Although polyethylene MPs *per se* did not seem to impact river biofilms, results will be presented showing that the co-exposure with other emerging contaminants like the antibiotic clarithromycin could impact its toxic effects, bioaccumulation and biotransformation. Changes in river biofilms metabolism are also expected. **Acknowledgments** Work funded by the Spanish State Research Agency of the Spanish Ministry of Science, Innovation and Universities and European Fund for Regional Development (project PLAS-MED; FEDER-MCIU-AEI/CTM2017-89701-C3-2-R). Authors acknowledge Generalitat de Catalunya through Consolidated Research Group (ICRA-ENV 2017 SGR 1124 & 2017 SGR 1404). Lúcia H.M.L.M. Santos acknowledges the Juan de la Cierva program (IJCI-2017-32747) and Sara Rodríguez-Mozaz acknowledges the Ramon y Cajal program (RYC-2014-16707) from the Spanish State Research Agency of the Spanish Ministry of Science, Innovation and Universities (AEI- MCIU). José M. Castaño-Ortiz acknowledges the predoctoral grant from the Agency for Management of University and Research Grants (AGAUR) (2019 FI_B 00881REF).

3.12.17
Poster spotlight: Intracellular fate and toxicity of nano and microplastics and associated benzo(a)pyrene in mussel hemocytes in vitro
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3.12.18
Asymmetrical-flow field flow fractionation approach for the separation and characterization of nanoplastics in complex matrices
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Currently the global plastic production represents 359 million metric tons in 2018 [1] and the part entering the world's oceans yielded 4.8-12.7 million metric tons of plastic in 2010 [2]. These anthropic micro- and nano-particles are now considered as a new emerging risk with potential damaging effects on the trophic chain (human food chain as a result of their transfer to fish and other sea food) [3]. In contrast to microplastics, there are currently no robust analytical and standardized methods for detection, separation and characterization of nanoplastics in complex media such as environmental samples. In principle, some analytical techniques used for nanoparticles (NPs) should be applicable for the separation and detection of nanoplastics. Electron microscopy is widely used in polymer science and has been used over the years to characterize several types of nano-sized plastic structures and particles. In this work, asymmetrical-flow field flow fractionation (AF4) coupled with light scattering-based techniques will be described in a novel approach to clean, separate and characterize nanoplastics from environmental samples. As a proof of concept, a AF4 approach has been applied to the detection of nanoplastics in the digested tissues of an Ascidian animal, *Ciona Robusta* before and after exposition to "artificial" polystyrene beads. The sample preparation prior to AF4 measurement is based on the digestion of the complex matrix sample (biological sample) to obtain a consistency (viscosity and density) that enables the analysis. The tested biological matrix, *Ciona* Robusta, was exposed to polystyrene beads of 100 nm and 200 nm (Polysciences) in natural conditions, with environmentally relevant concentration of the particles and some *Ciona* Robusta, called control, were not exposed to the polystyrene beads to track the potential accumulation of nanoplastics from the sea water. The application of the AF4 method to the digested and cleaned biological matrix after exposure to polystyrene beads showed multiple populations (peaks) with sizes ranging from 1nm to about 400nm (Figure 1). The measurement by online MALS and DLS permitted to follow the exposed polystyrene beads and many other particles. The analysis of the control *Ciona* Robusta highlighted the presence of nanoplastics even before exposure, with a major population around (60-80) nm (Figure 1).The fractionation of each population has been conducted and the fractions were analysed by orthogonal techniques such as scanning electron microscopy (SEM) or confocal microraman. The comparison between the SEM images before and after AF4 showed cleaner particles with a lower impact of the corona and other free organic compounds in the biological matrix. The method developped here shows great potential for the detection, separation and characterization of nanoplastics in a complex matrix and it highligths the importance of the combination of different analytical techniques to track and fully characterize nanoplastic contaminations. One of the major results here is the detection of nanoplastics in an environmental matrix in the non-exposed *Ciona*Robusta samples.

3.12.19
Conventional drinking water treatment processes and nanoplastic particle removal
G. Pulido-Reyes, Eawag / Process Engineering; D.M. Mitrano, R. Kaegi, Eawag - Swiss federal Institute of Aquatic Science and Technology / Process Engineering; U. von Gunten, École Polytechnique Fédérale de Lausanne / School of Architecture, Civil, and Environmental Engineering
Microplastics have recently been detected in both potable water sources and bottled water, leading researchers to question the efficacy of current water treatment practices. While the magnitude of particulate plastic concentrations and compositions varied by water source and location, it is hypothesized that all waterways will continue to experience an influx of particulate plastic (including down to the nanometer scale) as degradation of larger plastic items continues. Ozonation and sand and activated carbon filtration are key treatment steps in drinking water treatment plants, yet studies addressing the impact of these

treatment steps on the retention of nanoplastic particles from finished drinking water are still lacking. Here, we evaluated the impact of ozone treatment on the physicochemical properties of two nanoplastic particles and the effectiveness of sand and activated carbon filtration for particle removal. For the first set of experiments, nanoplastic particles were assessed in terms of (change in) morphology, surface charge and particle aggregation state. The nanoplastic was reacted with ozone (0.5, 1 and 5 mg/L) in Lake Zurich water, which is used as the main source for drinking water production in the city of Zurich. Our results suggest that the hydrodynamic diameters of the nanoplastic particles remains unaffected by the ozone treatment. However, the surface charge of the particles significantly decreased with exposure to increasing ozone doses. Electron microscopy images recorded on pristine and ozone reacted nanoplastics revealed neither morphological changes or surface alterations. For filtration experiments, the movement of nanoplastic through packed columns was assessed by using nanoplastic particles developed in-house with an inorganic tracer (Pd) measured by ICP-MS. Factors which were suspected to influence removal, such as flow rate, grain size and particle roughness were also investigated. Experiments are still ongoing in column tests. Due to their small sizes, nanoplastic particles may pass through the filtration systems designed for drinking water treatments plants, therefore, the results of this work will help to assess the nanoplastic removal efficiency of conventional water treatment processes.

3.12.21
Plastic debris and PAHs: concerns in the southern retention basin of the Cheviré Bridge (Nantes, France)
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Retention and infiltration basins receive runoff waters from urban areas to protect from flooding, recharge of aquifers and improve downstream water quality. They are able to accumulate macro-debris and various pollutants. The waters and sediments of retention basin may have relatively high levels of microplastics and organic pollutants, including Polycyclic Aromatic Hydrocarbons (PAHs). The study site is the retention and infiltration basin of runoff waters from the southern part of the Cheviré Bridge, which is the main bridge in Nantes crossing the Loire River. This bridge constitutes a part of the ring road around Nantes and supports an average daily traffic of 90,000 vehicles. The retention basin represents an area of approximately 800 m2. A sampling campaign was carried out in February 2018. Since plastic debris are able to sorb organic compounds, PAH measures were carried out on plastic macro-debris collected around or inside the retention basin as well as in the sediments of the basin. PAHs in sediments were analysed by high performance liquid chromatography coupled to a fluorescence detector after lyophilisation, sieving and extraction with Accelerated Solvent Extraction (ASE). The same method was used to analyse PAHs extracted from plastic macro-debris. Moreover, the levels of microplastics were also determined. They were extracted from the sediments after drying, sieving, centrifugation, filtration and observation of filters using a microscope coupled to an infrared spectrophotometer. The total PAH concentration of the sediments varied significantly depending on the sampling point in the basin. Some concentrations were up to 1200 ng/g dry sediment, proof that the basin could be considered as polluted in PAHs. The results of the PAHs extracted from the plastic debris underlined a strong influence of the type of polymer. The highest PAH levels were observed for PE, with the same order of magnitude than in sediments. The sorption capability of PET was more limited compared to PE and much lower than in sediments. This study therefore showed that plastic debris could act as PAH wells, after sorption to their surface, depending on the polymer type. Even if the specific surfaces of microplastics are higher than those of macro-debris, taking into account their relatively small concentrations in the retention basin sediments, they did not represent an important exposure way for organisms compared to the sediments in this kind of ecosystems.

3.12.22
Fate of microplastics in the marine environment
C. AlChahir Bel Hajjar, CIRAIG - École Polytechnique de Montréal / Chemical Engineering; A. Boulay, CIRAIG - École Polytechnique de Montréal / Chemical engineering department; C. Bulle, Université du Québec à Montréal / CIRAIG, École des Sciences de la gestion; R. Samson, Ecole Polytechnique de Montreal / Department of Chemical Engineering
Fate of microplastics in the marine environment Carla AlChahir Bel Hajjar[1], Cécile Bulle[2], Rejean Samson[1], and Anne-Marie Boulay[1] [1] Chemical Engineering Department, Polytechnique Montréal, P.O. Box 6079, Montreal, QC H3C 3A7, Canada [2] ESG UQAM, Strategy, Corporate & Social Responsibility Department, CIRAIG, Montreal, QC H3C 3P8, Canada E-mail contact: carla.alchahir-bel-hajjar@polymtl.ca **Keywords:** microplastics, fate, marine environment, life cycle impact assessment Microplastics have been largely produced throughout the years because of their beneficial properties on the industrial and the usage scale. However, they are considered toxic affecting the human health, ecosystem, and the ocean. The mismanagement of these microplastics is leading to their accumulation in the marine environment. Life cycle assessment (LCA), used to assess the potential environmental impacts within the different life cycle stages of a product or service, does not consider microplastics as pollutants but rather as mismanaged wastes. Thus, it is important to integrate them. This project is part of a new working group named MARILCA (MARine impacts in LCA) under the UN Environment Life Cycle Assessment and FSLCI (Forum for Sustainability through Life Cycle Innovation). It aims to build a new framework to assess the fate of the microplastics in the marine environment. It is built on the multimedia modelling approach commonly used in toxic impact assessment in LCIA and in compliance with the USEtox framework. Within it, sub-compartments in the marine environment have been created to and from which the microplastics are emitted and transferred. This is important because of the difference in the main environmental mechanisms that affect the fate of these microplastics in each compartment. The mechanisms identified are the transport mechanisms (floatation, suspension, advection, and sedimentation), the mechanisms of formation (degradation of macroplastics), and the mechanisms of removal (degradation/fragmentation into nanoplastics). Different factors influencing the interaction between the compartments and the transport of the microplastics within them are also identified, which are the physical properties of microplastics, winds, waves, vertical mixing, and surface currents. The next step will be the development of algorithms for the quantification of each environmental mechanism and thus, the quantification of the environmental fate of the microplastics in the marine environment.

3.12.23
Poster spotlight: Biological fragmentation of microplastics by the freshwater amphipod Gammarus duebeni
A. Mateos Cardenas, University College Cork / School of Biological, Earth and Environmental Sciences
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New Approaches to Characterising the Global Chemical Exposome

3.13.1
A First Comprehensive Analysis of National/Regional Chemical Inventories
Z. Wang, Swiss Federal institute of Technology / Institute of Environmental Engineering; G. Walker, Australian Government / Department of the Environment and Energy; D.C. Muir, Environment and Climate Change Canada / Aquatic Contaminants Research Division; K. Nagatani Yoshida, UNEP / Asia and the Pacific Office
Chemicals, while bringing benefits to society, may be released during their lifecycles and possibly cause harm to humans and ecosystems. Chemical pollution has been recognized as one of the planetary boundaries within which humanity can safely operate, but has not been comprehensively understood yet. In this study, 22 chemical inventories from 19 countries and regions are analyzed to achieve a first comprehensive overview of chemicals on the market as an essential first step toward a global understanding of chemical pollution. It identifies that over 350,000 chemicals and mixtures of chemicals that have been registered for production and use, up to three times as many as previously estimated and with substantial differences across countries/regions. A noteworthy finding is that the identities of many chemicals remain publicly unknown because they are claimed as confidential (over 50,000) or ambiguously described (over 70,000). Coordinated efforts by all stakeholders including scientists from different disciplines are urgently needed, with (new) areas of interest and opportunities highlighted here.

3.13.2
Assessing Chemical Mixtures in Air at the Global Scale: Advances with PUF Disk Passive Air Samplers
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The Polyurethane Foam (PUF) disk sampler is being applied at various scales, ranging from global to personal, to assess the presence, transport, and exposure to a growing list of contaminant classes. This includes persistent organic pollutants (POPs) and POP-like emerging chemicals of concern, and more recently trace metals. The ability of the PUF disk sampler to effectively capture and retain particle-related species (in addition to gas-phase chemicals) supports further applications and target classes, inter alia, microfibres and microplastics and black carbon, and for characterising the toxicity potential of these chemicals mixtures for a variety of endpoints. Results are presented from the Global Atmospheric Passive Sampling (GAPS) Megacities pilot project, where PUF disk samplers were deployed across 20 major urban centres and analysed for organic contaminants (e.g. flame retardants (FR), trace metals, and toxicity endpoints). Targeted and non-targeted analyses of the samples are providing invaluable

information regarding chemical mixtures in air that contribute to a variety of toxicity endpoints. Levels of FRs in air were positively correlated with Gross Domestic Product and concentrations were dominated by organophosphate esters (OPEs). Highest concentrations in air were observed for New York City, USA and London, UK – 15100 and 14100 pg/m^3, respectively. Levels of trace metals were positively associated with total suspended particle concentrations and showed a different ranking, with highest levels in New Dehli, India and Cairo, Egypt. Results are also presented from controlled controlled oxidation flow reactor (OFR) experiments on parent OPE compounds, where potential degradation products are identified. This informs non-targeted analysis and suspect screening of air sample extracts, which is currently ongoing. Extracts of PUF samplers were also screened using an *in vitro* toxicogenomics approach to determine pathway-based impacts of the complex mixtures among the 20 cities. This new and complementary approach to contaminant research and monitoring provides 'real world' exposure information for air and contributes to more informed chemical risk assessment and risk management. The PUF disk sampler, which effectively collects and retains gas-phase and particle phase contaminants, in a time weighted sample, is a simple and cost effective tool for implementing a 'chemical mixtures' approach for studying the global chemical exposome.

3.13.3

Global chemical monitoring: Novel methodologies elucidating the extent and drivers of the riverine chemical exposome

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Active pharmaceutical ingredients (APIs) and other chemical contaminants are detected in the aquatic environment and their occurrence is suggested to elicit negative impacts. Knowledge gaps exist regarding the occurrence/effects of chemical contaminants, particularly in low to middle-income countries and those outside of N. America and W. Europe. Of the 196 countries recognised by the United Nations, data for the occurrence of pharmaceuticals is only available for 38% (75 countries). Here, we present the first global study of the chemical exposome using both novel sample collection, target and non-target analytical methods. The study comprised 133 sampling campaigns performed in 102 countries covering 1025 sampling locations across 254 rivers. A novel, hand-held, water sampling kit was developed and sent to all 75 partner intuitions who then collected 4 mL of grab sample water from 5-10 locations along their study river(s). Samples were sent frozen to the University of York for analysis using both in-house validated methods for target (n=61 APIs) and nontarget direct-inject high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). At least one of the 61 pharmaceuticals monitored via targeted HPLC-MS/MS was detected in 131 of the 133 sampling campaigns, indicating that contamination of aquatic environments is a global phenomenon. Detection frequencies ranged from 1% for the anti-malarial artemisinin (detected at 10 sampling locations, predominately in Africa) to 63% for the anti-epileptic carbamazepine (detected at 645 sampling locations). The highest levels of API pollution were observed in Africa followed by South America with mean total API concentrations of 25.9 mg/L and 20.3 mg/L across the respective continents. Specific pharmaceutical fingerprints varied from country-to-country with clear and statistically significant patterns emerging along spatial, therapeutic, potential toxicity and socioeconomic contexts. Non-target chemical analysis revealed regional patterns in wider chemical exposure. In addition to further medicinal contaminants not detected via the target HPLC-MS/MS method (e.g., antiretrovirals, antifungals, anthelmintics) other non-medicinal chemicals were identified. For example, industrial (e.g., octanedioic/ nonanedioic acid and tributyl phosphate used in plastic synthesis), agricultural/pesticides (e.g., DEET and carbendazim), illicit drugs (e.g., benzoylecgonine), among others were identified in the samples. The occurrence of chemical contaminants in rivers is a global issue and much work is needed to identify potential impacts in poorly-studied regions.

3.13.4

An exposome-based approach using Big Data to compare the health impacts of multi-chemicals and multi-stressor exposures

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High-throughput exposure strategies comparing exposure to various chemical, nutritional, and physical stressors are needed for exposome wide associations. This presentation shows how recent Big Data and models enable us to predict exposure and impacts for multiple stressors. a) Consumer exposures to chemical in multiple products (e.g. building materials, toys, cleaning products, personal care products) are assessed using stochastic usage and chemical contents databases for more than 15000 product-chemicals combinations. b) Occupational exposures to organic chemicals were assessed across the entire US industry, based on observed organic chemical concentrations at workplace for 49,000 measured sector-based concentration values for 235 organic chemicals. c) PM2.5 environmental exposures are assessed based on residence location and a multiscale model predicting local concentrations. In addition, exposures to chemical stressors are compared to d) Dietary exposures and nutritional impacts were assessed using NHANES survey and the decomposition of individual diet into 16 dietary riks factors, accounting for 400 risk-outcomes, stratified by 15 age groups and gender, coupled with food composition and consumption data for 20,000 individuals. e) Physical activity is charaterized using MET and outcome specific relative risk. All exposures are then coupled with corresponding dose-responses to obtain individual burden in µDALY/pers-day then translated minutes of healthy life lost or gained per person per day. Potential impacts are highly variables depending on individual behaviour and can typically range a) from 0 to 50 minutes of health life lost/pers-day for exposure to chemicals in consumer products, b) from 0 to 1400 minutes lost/pers-day depending on industry sector for impacts of organic pollutants on workers; c) from an impact of 0 to 50 minutes lost/pers-day associated with fine particulate 2.5µm, depending on residence location, d) from a typical impact of 40 minutes/serving-day lost from detrimental food, up to a benefit of 30 minutes/serving-day depending on food items, with main impacts associated with cardiovascular disease, diabetes and cancers; e) from a benefit of 0 to 40 minutes gained/pers-day as a function of physical activity. We enter an exciting era in which you can "Tell me when and where you lived and worked, what you consumed, exercised and ate… and I will tell you who you are (your exposome !).

New Methods in Environmental Analytical Chemistry: From Interrogation of Complex Matrices to Innovative Tools for Monitoring

3.14.1

Rapid, direct-injection LC-MS/MS micropollutant analysis and multivariate classification of international water samples

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The rapid quantification of 135 CECs in international surface and influent wastewaters in under 4 min using direct-injection liquid chromatography-tandem mass spectrometry (LC-MS/MS) followed by multi-site/sample classification using principal component analysis (PCA) is presented. Sample pre-treatment with solid-phase extraction (SPE) is often complex, inefficient or impractical for application at scale and is a bottleneck for wide analyte coverage. Direct analysis is often limited by MS data acquisition speeds for large numbers of compounds. Critical challenges for rapid, direct LC-MS/MS method development are presented. Method performance was excellent with low detection and quantification limits (e.g., median of 9 and 31 ng L^{-1}- in wastewater, respectively), inaccuracy and imprecision of < 12 %, linearity of R^2>0.9980 ±0.0037 (10-point calibration in matrix) and average matrix effects < 20 %. Quantitative analysis of >300 international surface and wastewater samples from the UK from USA, Germany and Mexico was performed and all were quantified within only a few weeks with 220 injections possible daily. For wastewater from the UK, USA and Mexico, >50 CECs were quantified including several European Commission 'Watch List' compounds, e.g., macrolide antibiotics. For illicit drugs, cocaine use in London was observed to plateau for the first time since 2016. Fenuron was quantified in wastewater from USA and Mexico, but not in London. However, it was detected in the River Thames and several rivers in Suffolk (UK), potentially indicating environmental persistence following restriction some years ago for pest control agriculturally. Following PCA, it was possible to show methamphetamine and opioid use was more prevalent for selected cities in the USA and Mexico than in London. For methamphetamine in particular, high concentrations of >10,000 ng L^{-1} were measured in one USA city and was the primary PCA loadings plot vector among all compounds detected. For river water, PCA was also applied to a 100-km portion of the tidal Thames as well as two smaller rivers in rural Germany to determine sustained sources of CECs from combined sewer overflows and wastewater effluent.

3.14.2

Analyzing unknown C2-C12 PFAA-precursors in environmental samples using optimized TOP assay

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As an excellent surfactant, per- and polyfluoroalkyl substances (PFASs) are widely used in products of daily life and industrial activities. Although legacy PFASs have been found widely occurring in the environment, unknown precursors may as well occur, which can be a potential source to the environment. Using top assay, we found a large contribution of unknown perfluoroalkyl acids (PFAAs) precursors in wet deposition samples collected across mainland China. The maximum predicted wet deposition fluxes of C6 and C8 unknown precursors can reach 3.1×103 ng/m2/d and 4.3×103 ng/m2/d, respectively. These unknown PFAA-precursors and ultra-short-chain perfluoroalkyl carboxylic acids (C2-C3) contributed significantly to the deposition fluxes, accounting for 6%-56% and 22%-91% of the total PFASs, respectively. This indicated that in addition to volatile PFAS precursors, there are soluble precursors or intermediates that can be removed by wet deposition in the atmosphere. Therefore, only focusing on the traditional known PFASs will significantly underestimate the contribution flux of PFASs in wet deposition to the surface environment. Furthermore, on the basis of the existing top assay method, we optimized the sample pretreatment using the BA/Ag/H IC solid-phase extraction cartridges, which effectively removed the high concentration of chloride and sulfate ions in the water samples. This made it possible to recover C2-C3 PFCAs in water samples of high salinity on WAX cartridges (62%-78%). With optimized TOP assay, we found the contribution of unknown C2-C3 PFCA-precursors was 12-93 mol% in the leachate samples from landfill, incineration plant, and waste transfer station in Tianjin. As a whole, the contribution of unknown C2-C12 PFAA-precursors remained 0.38-68 mol% in leachate samples after biochemical treatment. This indicates that a large number of PFAA-precursors in municipal solid wastes are released into leachate during the process of waste stacking and disposal treatment. The mass load of PFASs may be underestimated if not taking into account the contribution of unknown PFAA-precursors. Therefore, unknown PFAA-precursors have been found occurring in the environmental samples from both point and non-point sources of PFASs. Thus, it is necessary to further identify unknown PFAA-precursors in the environment, especially for C2-C3 PFCAs, using suspected and non-target screening techniques, so as to accurately assess the environmental risk of PFASs.

3.14.3
Method development for the monitoring of pesticides and transformation products in waterfowls' eggs
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France is characterized by the presence of very many small bodies of water (i.e. ponds), often neglected by studies, but whose cumulative surface area covers 0.92% of the territory (Banas et al., 2019). These small waterbodies play an important role in biodiversity preservation as some species like waterfowls come to eat and reproduce in these places. Often located in watershed heads, they may be surrounded by farming lands that are potential sources of pesticide contamination. These molecules can potentially accumulate in organisms and contaminate eggs which may compromise reproduction or development. Moreover, pesticides can be biodegraded or metabolized and transformation products, especially polar ones, are not well monitored concerning biotic compartments. However, only few studies are addressing this issue. The aim of this study was to develop an analytical method that allows the monitoring of pesticides in waterfowls' eggs, and apply it to real samples from the Sologne region (France). The high content of interferences in the matrix and the large range of physicochemical properties of the pesticides and transformation products targeted represent an analytical challenge especially for the quantification of traces. The developed protocol consists in a solid/liquid extraction based on QuEChERS approach, and an LC-MS/MS analysis. A total of 18 pesticides and 28 transformation products with wide range physicochemical properties were considered. Eggs from hens kept in battery were used as reference matrix for the protocol development when spiked with analyses, and as blanks. Matrix matched calibration was performed, and internal standards were used to characterize the representativeness of the reference matrix against the real samples. Most quantification limits obtained with the optimized analytical method were inferior to 10 ng.g-1 dry matter, which is compatible with traces analysis. A reliable and robust method for the characterization of pesticides in waterfowls' eggs, based on a matrix match and an internal standard approach was so validated. Moreover, one of its strength is to be relatively simple and can be easily transposed to other biotic matrices. Performances of the developed protocol were confirmed as terbutryn was quantified in a real sample. However, even with a sensibility compatible with traces analysis, only one pesticide was quantified, demonstrating that polar pesticides and transformation products may not be transferred to eggs.

3.14.4
Paper-origami device enabling low-cost and rapid microbial analysis
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Microbial detection is of significant importance for both biomedical diagnostics (e.g. infectious disease) and environmental analysis (e.g. microbial contamination in drinking water). This has traditionally been performed by culturing and typing the pathogens, however, these procedures often take several days. Molecular approaches (such as polymerase chain reaction (PCR) detecting genetic markers) will deliver faster turn-around times (< 1 hour) than culture-based methods, but currently require centralised facilities and skilled personnel to perform the assays and interpret results. There is, therefore, an urgent need to develop rapid and sensitive platforms that can provide rapid analysis of microbial both for biomedical diagnosis and environmental analysis. Here we report on a low-cost, deployable paper-based biosensor device for rapid analysis of microbes. Using a paper-microfluidic sensor with isothermal amplification technologies, we have demonstrated rapid, sensitive and easy-to-use sample-to-answer testing devices for rapid infectious disease of bovine, which was also tested in the field in India. Pathogen DNA was amplified with loop-mediated isothermal amplification (LAMP) and detected fluorescently, enabling a promising genetic DNA (from 10 to 100 copies per reaction) to be measured. Experimental results show that the data from our paper-origami device are able to be collected as a fluorescence signal either visually, using a low-cost hand-held torch, or digitally with a mobile-phone camera (Figure 1). We carried on double-blind tests for the semen samples collected from elite bulls at a germplasm centre, as a demonstrator for a low cost, user-friendly point-of-care sensing platform, for in-the-field testing in resource-limited regions, indicating for the first of time demonstration of the application of paper-origami devices for the diagnosis multiple infectious diseases from semen samples. We also show this device for the rapid test of microbial contaminations in drinking water, with a flexible sampling enrichment strategy with magnetic beads equipped on a syringe, enabling rapid analysis of microbial contamination in drinking water in low resource setting, and to address global water contamination issues.

Non-target Analysis in Environmental Sciences: The State of the Art and Future Perspectives

3.15.1
patRoon: Open-Source Software Platform for Environmental Non-Target Studies
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Non-target analysis (NTA) requires powerful software tools for effectively processing hundreds to thousands of detected chemicals. However, current software options often either lack functionality required for environmental analysis or solve only parts of the full workflow. patRoon [1], an R based open-source software platform, combines algorithms from commonly used software tools (e.g. enviPick, XCMS and MetFrag) with new functionality to provide a comprehensive mass spectrometry based workflow for automated. This software is designed to be flexible, user-friendly and both platform- and instrument-independent and has been applied to various environmental studies (e.g. [2-4]). The first study revolved around the development and validation of a new liquid chromatography coupled to quadrupole time of flight high resolution mass spectrometry method for the characterization of leaf litter derived organic material (OM) [2]. patRoon was then used to study aluminium-OM (co)precipitation by characterizing the molecular OM composition before and after aluminium treatment. Preliminary results showed that such precipitation favours larger/heavier and aromatic compounds, which was not observed for specific compound groups. The second study involved the elucidation of abiotic transformation products (TPs) for two commonly used biocides in cooling water towers: 2,2-dibromo-3-nitrilopropionamide (DBNPA) and glutaraldehyde [3]. The identities of two detected TPs from DBNPA were confirmed, whereas tentative identification efforts revealed several products resulting from the interaction between both biocides, which generally showed reduced toxicity towards natural biodegradation processes and daphnids. The final study involved NTA of chemicals suspected to contribute to bioactivity in a source for drinking water based on the results of a previous screening with effect-based methods [4]. patRoon revealed 49 suspects, for which the identity of 5 compounds was confirmed and for 16 others established with high confidence by library mass spectral data. The successful application of patRoon to these studies shows its suitability for a broad range of environmental NTA studies. [1] https://github.com/rickhelmus/patRoon [2] Brock O et al. (2019) European Journal of Soil Science. [3] Wagner TV et al. (2019) Journal of Hazardous Materials. [4] Albergamo V et al. (2019). Environmental Science: Water Research & Technology.

3.15.2
Combining in vitro bioanalyses with ion mobility-high resolution mass spectrometry as a refined screening workflow in environmental analyses
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High resolution mass spectrometry (HRMS), such as time-of-flight (TOF), is gaining popular in environmental research due to its powerful capability of wide-scope target, sususpect and non-target screening analyses which allow identifying all relevant chemicals present in a sample.The high mass accuracy and resolution, together with the extensive variety of available acquisition modes, make HRMS the technique of choice for monitoring of emerging contaminants. With the recent devolpment of ion mobility separation coupled to HRMS (IMS-HRMS) the capabilities of the instruments have been remarkably enhanced. However, dealing with such large data sets with many features is often time-consuming or even unaffordable, and therefore, feature prioritization strategies are pivotal for this task. In vitro bioanalyses enable the establishment of a certain toxicological fingerprint of a sample, and therefore measure the combined effect of all chemicals present in the sample. The comparison between least-active (ideally, non-active) and most-active sample in a certain bioassay enables the screening for most-active sample unique features, non-active sample unique features, or features present in both samples but at different concentration levels. Complementary, the application of target, suspect and non-target screening among the selected features would reveal the identity of the corresponding toxicity drivers. The application of this strategy will allow to focus in environmental harmful species that need to be monitored. In this study, the application of eight in vitro bioassays (aryl hydrocarbon receptor (AhR), estrogen receptor (ER) activation and antagonism , nuclear factor erythroid 2-related factor 2 (Nrf2), androgen receptor (AR) activation and antagonism and vitamin D receptor (VDR) activation and antagonism) werecombined with target, suspect and non-target screening by means of state of the art IMS-HRMS. In total, 11 surface waters from coastal lagoons from the Mediterranean coast of Spain were selected as a study case for this approach. The different toxicity observed between the samples permitted the application of the developed workflow revealing the presence of several organic contaminants.

3.15.3

Implementation of non-targeted analysis as a water characterization tool

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There has been growing concern about organic micropollutants due to their dispersion and their impact on the environment and human health. Current wastewater regulations focus only on a small number of micropollutants representing a limited portion of all contaminants dispersed in the environment. Furthermore, many relevant contaminants regarding their persistence on the environment or their toxicity may be still unidentified. The development of non-targeted methods appears to be a tool to develop knowledge on the changes at global scale during treatment processes and also of the impact of the treated wastewater discharge in the aquatic environments. The aim of this work was to study the evolution of the fingerprint (peaks inventory) of wastewaters and the corresponding treated waters and the impact of their discharge on the surface waters from the development and the application of a non-targeted method. Four sampling campaigns were conducted over a year. Raw and treated wastewater effluents and three different points on the french river Clain were sampled. Samples were concentrated by solid-phase extraction. Concentrated samples were analyzed by liquid chromatography coupled with High-Resolution Orbitrap mass spectrometry equipped with electrospray source. Compound Discoverer, Mzmine 2, R 3.5.1 and XLSTAT were used to process data and also to perform multivariate analyses. The results showed "fingerprints" with distinct profiles depending on the samples. Multivariate analyses were able to discriminate clearly wastewaters and treated waters. Thanks to Principal Components Analysis (PCA) correlations between some detected features and water origins were established. Finally, the comparison between surface water samples taken upstream of the treated wastewater discharge with those from the contact zone showed the emergence of new features into the river underlining a treated wastewater impact. The desappearance of numerous of these signals in the downstream samples tends to indicate a dilution effect in river. Accordingly, the representation of the fold change between samples and the Principal Components Analysis allow to better understand the impact of the treatment of the wastewater on the whole features detected by the non-target analysis. Using an approach that includes statistical treatments and data analysis, non-targeted analysis can be used to search for features correlated to the origin of the matrix and to extract features with distinct tendencies.

3.15.4

Poster spotlight: Suspect and non target analysis of emerging polar xenobiotics in water: workflows relevance

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3.15.5

Poster spotlight: Detection and prioritization of unknown compounds in water extracts from drinking water production

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Occurrence, Fate, Transport and Reactivity of Emerging Micropollutants in Aquatic Systems

3.16.1

Occurrence of micropollutants in fish collected from an aquatic ecosystem influenced by municipal effluent discharge and snowmelt

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In this study we examined seasonal and spatial occurrence of 218 organic micropollutants, including pesticides and their metabolites, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and other flame retardants in two fish species collected in East Canyon Creek, Utah, USA. The East Canyon Creek watershed is located east of Salt Lake City, Utah, USA. It is a unique semi-arid ecosystem influenced by effluent discharge from the East Canyon Water Reclamation Facility and seasonal snow melt, but often becomes effluent-dominated during summer months when instream dilution from snowmelt ends. Most of the annual precipitation (65-75%) is received as snow in the winter months. Two fish , brown trout *(Salmo trutta)* and mottled sculpin *(Cottus bairdii)* were collected from four sampling sites, with one reference site upstream, and three sites located at incremental distances downstream of the effluent discharge to evaluate spatial trends in contaminants levels. To examine seasonal trends, samples were collected during spring (May) reflecting highest flow from snow melt, summer (August) and fall (September, October) of 2014 representing lowest flow. We found some PAHs, pesticides and metabolites, PCBs, PBDEs, and flame retardants in mottled sculpin (11 analytes) and brown trout (18 analytes). Persistent organic contaminants: hexachlorobenzene (HCB), *p,p'*-DDE, PBDE-47 and common flame retardant triphenyl phosphate (TPP) were the most frequently detected contaminants in mottled sculpin and brown trout tissues, while PBDE-47 and *p,p'*-DDE were measured at the highest concentrations, reaching up to 73 and 19 ng/g wet weight, respectively. Measured PBDE-47 concentrations (22-73 ng/g ww) were the highest among other PBDE congeners, followed by PBDE congeners 99, 100 and 28. A spatial pattern was observed for PBDE congeners, where lowest concentrations were measured in fish from the reference sites, and highest concentrations were measured in fish collected downstream of the effluent discharge, indicating that municipal effluent discharge contributes to the elevated PBDE levels in fish residing in this effluent-dominated stream. Weekly consumption limits were calculated for target analytes detected in brown trout tissues following EPA guidelines.

3.16.2

Identification of Emerging Contaminants in Freshwater Invertebrates to Facilitate Toxic or Effect Pressure Estimation

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Contaminants of emerging concern (CECs), such as pharmaceuticals, illicit substances, persticides, herbicides, personal care products and their metabolites/transformation products have been found globally in aquatic environment, due to their widespread usage in healthcare, recreational/illicit use, and agriculture. Understanding these contaminants present in organism could further improve the risk assessment process. Acute and chronic effects of some specific contaminants on biota have been well studied.[1] However, these results were mainly based on exposure concentration levels measured external to the biota, (e.g. in water or sediment). This is due to the determination of contaminants in biota has been very challenging in terms of reliably separating different compounds and quantifying them at trace concentrations (e.g. pg-ng.g^{-1}).[2] Herein, determination of internal concentration of freshwater invertebrates and external concentration (*ie.* in

sediment and surface water) was achieved. *Gammarus pulex* and surface water were collected from 15 sites covering 5 river catchments across Suffolk. A total of 56 compounds were detected and ranged from < LOQ to 45.3 ng.g^{-1}, with cocaine and lidocaine frequently detected almost in all *G. pulex*. Pesticides that are no longer approved in the EU were also detected including fenuron reaching up to 16.1 ng.g^{-1}. While, 50 compounds were detected from surface water and ranged from < LOQ to 382.2 ng.L^{-1}. The successful identification of CECs at trace level was applied to another comprehensive study, covering different species of invertebrates (*G. pulex, Nereis diversicolor, Crangon crangon, Hydrobia sp)*, sediment and surface water samples, collected from 3 sites across the River Colne (Essex, UK). A total of 33 compounds, ranged from < LOQ to 90.8 ng.g^{-1} were detected across biota samples, while 40 compounds were detected from the sediments and ranged from < LOQ to 145.8 ng.g^{-1}. Out of these compounds, citalopram was found to be predominant in some biota (*Hydrobia sp.*), collected from one of the 3 sites and was also present in all sediment samples. A newly banned neonicotinoid pesticide, imidacloprid was also detected and only found in *N. diversicolor*. The presented data not only useful to show the concentration of CECs found at trace level in different fauna is affected by local land-use but also useful to estimate toxic and effect pressure of pesticides and non-pesticides pose to the environment.

3.16.3

Impact of Vanillic Acid and o-Methoxyphenol on Adsorption and Transformation of Lamotrigine by Manganese Oxide

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Lamotrigine, an anticonvulsant drug used for the treatment of epilepsy and bipolar disorder is considered a recalcitrant environmental pollutant. The parent compound, or its metabolites, are excreted from the human body, entering the environment. Lamotrigine and its transformation products are found in wastewater, drinking water, surface water and soils. Environmental behavior of lamotrigine can be influenced by its adsorption and transformation by redox-active minerals (e.g., birnessite, δ-MnO_2) and can be affected by the presence of reactive phenolic compounds, which are oxidized components of dissolved organic matter. Adsorption and transformation of lamotrigine by minerals has not been studied previously. Hence, we investigated the mechanism of lamotrigine adsorption and transformation by birnessite in the presence of phenolic compounds: vanillic acid and o-methoxyphenol. Batch experiments were performed at initial pH 5.5 for 72 hours. Both supernatant solutions and extracts from birnessite surface were analyzed by HPLC for lamotrigine and by LC-MS for transformation products. In the lamotrigine-birnessite system, 28% of added lamotrigine was removed after 72 hours and 17 transformation products were found. Based on the determined transformation products and their structures, transformation mechanism of lamotrigine by birnessite was constructed. The mechanism included three pathways of chemical reactions: oxidation, addition and dechlorination. The presence of vanillic acid significantly enhanced the removal of lamotrigine (92% of the added lamotrigine were removed after 72 hours) and increased the variety and number of transformation products up to 60. The presence of o-methoxyphenol resulted in removal of 48% of the added lamotrigine and formation of only 38 transformation products. Extensive transformation of lamotrigine in the presence of phenolic compounds can be explained by the oxidation of phenols by the birnessite surface, followed by the cross-coupling reactions of phenol transformation products (or their radicals) with lamotrigine. This study re-evaluates the environmental persistence of lamotrigine, elucidates the mechanisms of lamotrigine transformation, and demonstrates the role of redox-active minerals and dissolved organic matter in the environmental fate of lamotrigine.

3.16.4

Detection of the herbicide Glyphosate and its metabolite Aminomethylphosphonic acid in the Marine Environment

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Glyphosate is among the most applied herbicides worldwide. It has been frequently detected in freshwater ecosystems across the globe, together with its metabolite aminomethylphosphonic acid (AMPA). Nonetheless, to date there have been no reports on the occurrence of glyphosate and AMPA in the marine environment, presumably due to a lack of suitable analysis methods. The concentrations of pollutants like glyphosate in seawater are usually too low for direct measurement with analytical instruments, e.g., liquid chromatography coupled to mass spectrometry (LC-MS/MS). Therefore, analyte enrichment is required. For polar analytes like glyphosate, solid phase extraction (SPE) is often used. However, SPE enrichment from seawater can be hampered when salt and analyte compete for binding sites on the solid phase. A possibility to circumvent this issue is to desalt seawater with electrodialysis samples prior to SPE. In this study, we developed a method for the analysis of glyphosate and AMPA in seawater, specifically the Baltic Sea, since this semi-enclosed, brackish sea is heavily influenced by terrestrial input and anthropogenic activities. We evaluated three different materials for the SPE-enrichment of glyphosate and AMPA from Baltic Sea water: two ion exchange resins (IER) and one molecularly imprinted polymer (MIP). We further evaluated the possibility of using electrodialysis to desalt samples prior to SPE. We could show that glyphosate and AMPA can be successfully enriched with all three materials. However, both IERs were highly salt sensitive and could only be used in combination with electrodialysis. In contrast, the MIP did not exhibit salt sensitivity and could be used for direct SPE without previous sample desalination. The developed method was used to analyze seawater samples from the Baltic Sea. Glyphosate and AMPA could be detected in all samples. Concentrations ranged between 0.3 and 1.5 ng/L. AMPA showed a stronger concentration gradient between stations close to the coast and further offshore, indicating that AMPA is removed from marine surface water faster than glyphosate, e.g., through microbial degradation. To the best of the authors' knowledge, the presented data is the first report on the occurence of glyphosate and AMPA in any sea or ocean worldwide. The herein presented method enables monitoring programmes for glyphosate and AMPA in the marine environment. Moreover, it enables risk assessment studies that are based on empirical data.

3.16.5

Estrogenic compounds in the Pearl River Estuary

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The catchment area of the Pearl River comprises densely populated cities such as Guangzhou, Shenzhen and Hongkong, which potentially expose the river system and the adjacent marine environment to considerable anthropogenic pressure. In this study, surface water samples taken along the salinity gradient of the Pearl River were analysed for estradiol, estrone, estriol, ethinylestradiol, daidzein, genistein and zearalenon. The samples were investigated with a HPLC-MS/MS as well as with a biological yeast assay (A-YES) to estimate the estrogenic activity. Among the natural steroids, only estrone was detectable and present at all stations. The synthetic ethinylestradiol was present only at the sampling site closest to the South China Sea. Daidzein, genistein and zearalenon were detected at most of the stations. In general, estrogen concentrations decreased towards the sea. Estradiol equivalent concentrations were only detected at two stations close to the South China Sea. The investigated substances have gained concern as they interact with the hormone system of organisms and cause adverse health effects and are categorised as endocrine disrupting compounds (EDCs). As the removal rates of waste water treatment plants are often insufficient, EDCs have already been detected in effluents and river water which led to their transport into the marine environments. The actual effect on marine organisms is still not thoroughly investigated. However, it was shown that EDCs potentially affect organisms in low doses under laboratory conditions, and feminizing effects have already been observed, e.g., in eelpouts at the German coast of the Baltic Sea.

3.16.6

Sulfidized silver nanoparticles in abiotic and biotic compartments - A mesocosm study

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After release from consumer products, silver nanoparticles (Ag-NPs) will be sulfidized especially in waste water systems which are considered as major pathway components for the transfer of engineered nanoparticles to natural systems. Up to now no systematic studies were performed evaluating the environmental fate and effects of sulfidized silver nanoparticles (S-Ag-NPs). In this study we evaluated long term fate of S-Ag-NPs in the aquatic-terrestrial transition zone and their uptake by several organisms in an indoor floodplain mesocosm system. In order to evaluate the distribution of S-Ag-NPs between abiotic and biotic compartments, two replicate floodplain mesocosm systems were applied allowing to investigate aquatic and terrestrial aging of nanoparticles under consideration of flooding dynamics in meadow areas. The mesocosm systems were run with natural river water, quartz sand as sediment, and natural repacked floodplane soil. Leafs, benthic organism *Gammarus fossarum***,** and the filter feeding bivalve *Corbicula fluminea* were applied as biota. The total duration of the experiment was 16 weeks. The dispersions of S-Ag-NPs were injected as one single pulse (duration: 1 week) into the water phase. The results showed that the Ag concentrations in the water increased during the S-Ag-NP pulse. After the end of the S-Ag-NP injection the Ag concentrations decreased rapidly within 3 days followed by a slow decrease. The main fraction (ca. 90%) of Ag was found in the sediment top layer. This confirms our suggestion about aggregation of S-Ag-NPs and their rapid sedimentation. A high enrichment of Ag was observed for leafs and algae. The soft tissue of the mussels also showed an increasing Ag content during the injection of S-Ag-NPs followed by a slow decrease. The results clearly indicate that the Ag content of mussel soft tissue strongly depends on the Ag concentration in the water. Most probably, the mussels eliminate quickly the excessive amount of S-Ag-NPs. An enrichment factor of Ag remained constant for mussels during the whole experiment but increased in the course of the study for gammarides. This can be explained by the increasing sedimentation of nanoparticles and high enrichment of Ag on the surface of sediment which represents the natural habitat for these benthic organisms. High enrichment of Ag in leafs, algae, gammarides, and mussels can result in the magnification of S-Ag-NPs by trophic transfer and represents a risk for a long term biological impact.

3.16.7

INVESTIGATION OF THE SYNERGY OF NANOPARTICLES AND ANTIBIOTICS CONTAMINATION IN NATURAL WATERS - A FOCUS ON PYRENEES AQUATIC ENVIRONMENT

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Contamination of natural waters by emerging pollutants, has become a subject of global concern, because of the potential dangerous consequences to environmental and human health. One of the most important groups, within the category of emerging pollutants, is represented by antibiotics, which are of particular interest because of their high toxicity to algae and bacteria at low concentrations and diffusion of antibiotic resistance. Furthemore, upon release and emission, antibiotics may interact with natural occurring nanoparticles in the environment, possibly leading to a co-exposure of organisms and the occurrence of mixture effects. Nanoparticles (NPs) can facilitate the transport of other pollutants, the so called "Trojan horse effect", facilitating toxic actions on biota and humans [2]. NPs can also form reactive oxygen species that can have dangerous effects on biological compartments, therefore affecting the biota [1,2]. The presented study is designed to investigate three aspects: - antibiotic pollution in aquatic environment of the territory of interest - occurence of natural nanocolloids in this area - interactions between naturally occurring nanoparticles and organic pollutants with a focus on antibiotics. The study area covering the north of Spain and the south of France is characterized by intense animal farming resulting in potential release of veterinary antibiotics into the environment. A two-step methodology, based on Solid Phase Extraction followed by a liquid chromatographic separation coupled to tandem high resolution mass spectrometry was developed for detection and quantification of antibiotics in natural and waste water samples. Colloids were studied by field flow fractionation coupled with ICP-MS. At the same time the presence of antibiotics bound to nanocolloids is studied by SPE-LC-HRMS. A deeper insight into the interactions of emerging contaminants and natural (NOM) and anthropogenic sorbents was gained during model studies carried out using selected species spiked on the natural water matrices. **References** [1] Batley GE, Kirby JK, McLaughlin MJ., Acc Chem Res. 2013, 46, 854. [2] Naasz, S., Altenburger, R., Kühnel, D., Sci. Total Environ., 2018, 635, 1170. ACKNOWLEDGEMENTS: The work has been 65% cofinanced by the European Regional Development Fund (ERDF) through the Interreg V-A Spain-France-Andorra programme (POCTEFA 2014-2020). Project OUTBIOTICS EFA183/16

Persistent (P) and PMT/vPvM Substances in the Environment: Improving Experimental and Weight of Evidence Assessment Methods, Providing Solutions, and Informing Regulations

3.17.1

The P-Sufficient Approach: why high persistence is a source of very high concern

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The environmental persistence of synthetic organic chemicals has been an important element of chemical hazard assessment for 40 years. It is part of assessment schemes for the identification of persistent, bioaccumulative and toxic (PBT) chemicals and Persistent Organic Pollutants (POPs) at the national and international level. However, there are still many persistent chemicals on the market and the concern associated with the persistence of organic chemicals is only slowly fully acknowledged. Here we present the P-sufficient approach, which aims at highlighting highly persistent chemicals for management action on the basis of their persistence alone. In the same way as under existing assessment schemes, also under the P-sufficient approach the concern about a chemical in the environment is related to unwanted effects. However, as has been learned from many cases in chemical risk assessment, adverse effects are difficult to predict and the scientific knowledge about effects will always remain uncertain and incomplete. This is because the number of possible effects is infinite and it is epistemologically impossible to identify them all. If unexpected effects are caused by a short-lived chemical, it is possible to cease environmental contamination by restricting its use, which implies that no additional effects will be caused by that chemical. In contrast, for highly persistent chemicals, it is not possible to cease environmental contamination within a reasonable time frame by simply restricting their use. Environmental contamination by highly persistent chemicals (and the effects related to this contamination) will continue for years to decades. This problem of poor reversibility (or irreversibility) of exposure to persistent chemicals is a key reason why high persistence is a source of concern. We used a generic multimedia environmental fate model to compare the environmental concentrations of a short-lived and a persistent chemical in a scenario with equal emissions for both chemicals (increasing linearly for 10 year and then decreasing linearly). The concentrations of the short-lived chemical directly follow the emission curve. The concentration of the persistent chemical, in contrast, keeps increasing for another 4.5 years (overshoot) after the peak of the emissions in year 10, and it shows a tail of 25 years after the end of the emissions in year 20. Furthermore, it exceeds the concentrations of the short-lived chemical by several orders of magnitude.

3.17.2

Biodegradation of multi-ring aromatic hydrocarbons in soil - insights from OECD simulation testing

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Biodegradation is an important process for the breakdown and removal of chemicals from the environment. The OECD 307 test guideline describes the standard simulation test for biodegradation in the soil compartment. Aromatic hydrocarbons are a broad class of compounds which may enter the environment in a variety of forms through a range of natural and anthropogenic processes. They exist as constituents of petroleum and coal-derived substances, and have a wide range of physico-chemical and environmental fate properties. A selection of ^{14}C-labelled unsubstituted aromatic hydrocarbons – benzo[a]pyrene (BaP), phenanthrene (Phe) and biphenyl – with different physico-chemical properties and numbers of rings have been tested under OECD 307. Testing was conducted on four soils with varying physical properties, organic carbon content (C_{org}, %) and microbial biomass measurements in both non-sterile and sterile systems. At each time point extractable radioactivity, mineralisation and non-extractable radioactivity was determined. Parent compound was quantified in extracts, with the remainder assigned to a metabolite fraction. Degradation kinetics were determined using CAKE modelling. BaP demonstrated markedly different degradation in OECD 307 compared with Phe and biphenyl. There were notable differences in mineralisation, metabolite formation and the non-extractable fraction. In all cases formation of non-extractable radioactivity in sterile samples was extremely limited, suggesting a biological mechanism for this process. A correlation was observed between degradation rate and log K_{oc} of the test compounds, suggesting that differences in degradation kinetics are accounted for by differences in sorption behaviour. The differences in metabolite profiles, mineralisation and non-extractable radioactivity may in addition be attributable to differences in structure and molecular size. Comparison of the results of different soils show contrasting influences of soil properties, with soils with higher C_{org} demonstrating slower degradation of BaP despite having higher microbial biomass, and the opposite being the case for Phe and biphenyl. These studies demonstrated contrasting degradation behaviour of 5-ring versus 2- and 3-ring aromatic hydrocarbons. This is attributed to differences in sorption behaviour as well as structural and molecular size characteristics. These results indicate the delineation in environmental fate properties of this broad class of compounds.

3.17.3

The identification and assessment of PMT/vPvM substances under REACH

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There are substances with a specific combination of intrinsic substance properties that cause them to pose an inherent hazard to remote aquatic environments and the sources of our drinking water. These are 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). 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 15469 REACH registered substances as of May 2017. The list of PMT/vPvM substances is provided and discussed in terms of monitoring data, emission likelihood and current restrictions or regulations.

3.17.4

P chemicals and water resources TF: SETAC Dublin 2020 - Abstract submission for poster presentation

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Chemicals from various sources may be released into the environment and potentially reach ground- and drinking water resources. The PMT/vPvM-concept was developed by the German Environmental Protection Agency (UBA) with the aim to better protect these resources from such substances regulated under REACh. However, the exercise indicated that hazard-based data evaluation resulted in a serious number of false positive and a certain number of false negative candidates which indicates an urgent need for further adjustment of the concept. Furthermore, available monitoring data illustrated a broader range of substances, which are not REACh-related, that are present in ground- and drinking water and these substances will not be addressed by the REACH PMT/vPvM-concept. An ECETOC Task Force was established to elaborate relevant topics central to such an approach. These included a review of existing legislations, elaboration of the applicability of an alternative risk-based approach, review of existing monitoring data and the level of relevant metabolite concentrations, possibility of NER-formation, appropriateness of the chosen Persistence (P) Mobility (M)- and Toxicity (T) criteria. Results: A risk-based approach using available phys.-chem. and e-fate parameters is applicable. A survey of monitoring data revealed no correlation with logKoc. Employing a 0.1% as relevant metabolite threshold is not practically applicable due to expected low concentrations (≤1ng/L). In EU plant protection product (PPP) regulation, metabolite thresholds are 10% and 5%. NERs are not relevant for water-soluble substances. M cannot be a stand-alone criterion to identify substances with leaching potential to ground and drinking water resources. Including the additional T-criteria will not significantly contribute to an enhanced safety of drinking water. Discussion and conclusion: A risk-based approach is a suitable alternative, compared to the initial hazard based PMT/vPvM-concept, as it allows for the exclusion of both false positive and false negative candidates. For this purpose, additional higher tier data may be required on a case by case basis. The results from the monitoring survey suggests that PMT may not be predictive of actual exposures. A 10% and 5% threshold level for metabolites similar to REACH PPP is recommended. The criteria for 'T' as set out in Annex XIII of REACH already fulfils the protection goal of ensuring human and environmental safety in a conservative manner.

Potential Roles for Diffusive Gradient in Thin Film (DGT) Passive Samplers in Investigative and Regulatory Monitoring

3.18.1

Time-weighted average concentrations with Diffusive Gradients in Thin films (DGT)

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Passive sampling can be a cost-effective analytical approach to provide time-weighted average (TWA) concentrations of pollutants in natural waters. DGT differs from other passive samplers basically by incorporating a diffusion disc in between the accumulation element (binding phase) and the sample [1]. This allows measurements independent of the convective phenomena in the sample solution and a simple interpretacion of the accumulation based on Fick's first law. The effective concentration that results from this interpretation is called c_{DGT}, which stands for the metal concentration in a system with only metal that would lead to the same accumulation than the natural sample. In terms of the real species, c_{DGT} can be understood as the addition of the free metal concentration plus the labile fraction of the rest of metal species, the contribution of each one being dependent on the respective mobilities [2]. By deploying a DGT device in succesive solutions of different concentrations of Ni, Cd or NiNTA, the use of c_{DGT} as a surrogate of the average experimental total metal concentration for different deployment times and metal concentrations is discussed. When the accumulation in simple metal solutions approaches perfect sink conditions, it is shown that c_{DGT} provides a good estimation of the TWA metal concentration. Transient effects following concentration jumps are mainly dependent on the pulse time, but in a set of oscillating concentrations they tend to cancel out due to the opposite effect of the transient on the difference between c_{DGT} and the experimental average concentration. In presence of ligands, c_{DGT} tends to underestimate the average total metal concentration as seen in many cases. For a dominant strong complex and excess of ligand conditions, a simple factor applied to c_{DGT} can improve the estimation of the TWA concentration as seen in NiNTA solutions[3]. [1] Davison *et al.* Environ. Chem. 9 (2012) 1-13. [2] Altier *et al.* Environ. Chem. 15 (2018) 183-193 [3] Altier *et al.* Anal. Chim. Acta, 1060 (2019) 114-124 *Acknowledgement* - The authors gratefully acknowledge support for this research from the Spanish Ministry MCIU/AEI (Project CTM2016-78798) and FEDER UE

3.18.2

Can the existing Environmental Quality Standards (EQS) be adapted for Diffusive Gradients in Thin films (DGT), for the chemical assessment of transitional and littoral waters? Yes, we can!

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EQS (or related concepts) are being used worldwide based upon a plethora of legislations. As an example, the European Water Framework Directive (WFD; 2000/60/EC) establishes that the chemical status of water bodies must be determined by the comparison of the concentrations of priority substances with EQS. Within the WFD, although there are some EQS for biota, most of them are for water. And, specifically, as far as metals are concerned, they must be evaluated in the dissolved fraction. Within the plethora of systems or methodologies for integrated sampling, Diffusive Gradients in Thin films (DGT) are already widely used in investigative monitoring of metals and there is an increasing interest in their use for the environmental assessment of water bodies, within European policies requirements. However, at this time, it is not possible to use DGT to assess chemical status in water bodies within WFD, as it is not an officially established method and there are no regulatory EQS. Thus, in the context of MONITOOL European project, the overall objective is to adapt the already existing EQSs for water to those for DGTs, enabling their use for regulatory monitoring. In 2018, two campaigns were carried out in winter (rainy season) and in summer (dry season); simultaneous deployment of DGTs and high-frequency collection of spot water samples were carried out. The sampling campaigns were performed in four selected sites (transitional and coastal sites) in each of the eight Atlantic and Mediterranean European regions. Metals were analysed in three fractions: dissolved, chemical labile and DGT. From this data, EQS will be established through a transparent and scientific procedure, to ensure that they are at least as protective as EQS laid down for water, and therefore they will be proposed formally for regulatory purposes within the WFD.

3.18.3

Monitoring of pharmaceuticals, pesticides and perfluorinated compounds in wastewater using a hydrogel-based passive sampler (o-DGT)

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Several configurations of passive samplers based on diffusive gradients in thin films for polar organic compounds (o DTG) were introduced in the last years. The uptake of compounds to most samplers is limited by diffusion in water boundary layer (WBL), but in case of o-DGT, the layer of diffusive gel is thicker than typical thickness of WBL. Therefore, performance of o-DGT sampler is typically independent on hydrodynamic conditions, unlike other passive samplers used for polar compounds. In the presented work an o-DGT-based passive sampler variant was tested in the influent and effluent of municipal wastewater treatment plants for monitoring a broad range of pharmaceuticals, antibiotics and psychoactive substances, currently used pesticides, and PFAS substances. The study yielded information on uptake kinetics of contaminants by the samplers from wastewater, and also assessed how well the sampler survive under harsh exposure conditions. In-situ sampling rates were derived by comparing passive sampling results with the analysis of composite wastewater samples. The in situ sampling rates were compared with laboratory derived calibration data available from earlier work. Performance of the hydrogel passive sampler was evaluated in terms of time-integrative sampling period and substance specific sampling rates. The sampling rates are expressed as substance specific volume of water cleared of chemical per unit of time and calculated as ratio of the linear uptake slopes and the mean concentrations in water. Relationship of sampling rates with physicochemical compounds properties such as logK_{ow}, log$D$ and pKa will be discussed. For most substances that were quantifiable in the analysed wastewater samples, integrative passive sampler uptake was observed up to two weeks exposure. Longer exposures resulted in degradation of the diffusive hydrogel layer and related faster uptake to samplers, which may lead to biased aqueous concentration estimates.

3.18.4
Evolution of soil-solution transfers during one field season of viticulture: comparison of passive DGT sampling and active sampling

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Viticulture used copper sulfate (Bordeaux mixture) since 1885 to control mildew. These regular inputs of copper may lead to a copper long-term contamination of soils and thereby affect fauna and flora. The soil-solution transfer and copper availability are often studied under controlled conditions. But in the field, the temperature and humidity of soils fluctuate. To better understand the soil-solution transfers of copper in field conditions, we aimed to measure the in-situ availability during one year of culture. The application of DGT (Diffusive Gradient in Thin-film) on the soil is a good tool to evaluate the phytoavailability of trace elements. The objective of this study was to compare two sampling methods, active sampling and passive sampling with DGT, in order to evaluate if it exists most critical periods of potential phytotoxicity through one year of culture. Four vineyard soils were selected: 2 luvisols and 2 podzols with copper concentrations between 16 and 197 mg/kg and variable soil characteristics. Soil temperature sensors and microporous cups were installed. Soil solutions were sampled every 2 weeks if moisture conditions were sufficient. pH, DOC, copper concentrations were measured in each sampling solution. In addition, passive samplers DGT were deposited every 2 weeks in soils during 24 h at 10 centimeters depth, to estimate the availability of copper. An important increase of copper concentration was observed both for passive and active sampling at the beginning of spring when temperature increased. This is correlated with the increase of dissolved organic matter due to a recovery of mineralization by microorganisms. This phenomenon is observable for all the parcels but more pronounced for the highest concentrated soils. The concentration obtained with passive sampling was also the highest and the closest of those obtained by active sampling during these periods. However, the spreading period seems to not affect DGT copper concentration. The role of organic matter on copper soil-solution transfers is major. This study allowed to demonstrated an important variation of copper concentration in solution but also of DGT concentration, which is often estimated as constant, depending essentially of reactive constituents present in the soils.

Siloxanes and Related Chemicals: Environmental Monitoring and Behavior, Ecosystem Exposure and Energetic Efficiency

3.19.1
Analytical Bias in the Trace Analysis of Cyclic Volatile Methylsiloxanes

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Since the European Commission published a restriction on the use of octamethyl-cyclo-tetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) in wash-off personal care products (PCP) in 2018 there is increasing interest in the analytics of cyclic volatile siloxanes (cVMS) in the scientific and regulatory community. For that purpose accurate and robust analytical methods ranging from determination of residual cVMS in products to trace analysis in the environment are needed. However, long experience within the industry shows, that analysis of volatile siloxanes needs special caution due to specific properties of the analytes. The consideration of these peculiarities over the whole chain of the analytical process from sampling to measurement is key for accurate results. The unique ability of polydimethylsiloxanes to "equilibration", which they do not share with other classes of polymeric organic compounds, is a constant potential source for cVMS artifacts during the analytical procedure. This presentation provides background information on properties of the siloxanes, points out challenges in the cVMS analysis, especially when performed at trace levels, and how to overcome them. We demonstrate unexpected occurrences of direct contamination by unintended gas phase transport or changes in the product quality of laboratory commodities. We also show that inappropriate sample preparation procedures are prone to false positive findings. Especially analytical approaches aiming for "non-target" screening analysis, such as "Head Space Screening" or sample pre-cleaning utilising adsorbents (eg. "QuEChERS") have to be critically evaluated before employed. Acknowledgements: This work was partly funded by the Global Silicones Council.

3.19.2
Interim results of an EU-wide monitoring study assessing the effects of announcing use restrictions on methylsiloxanes

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In January 2018, the European Union issued a REACH restriction on the use of octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) in wash-off personal care products (PCPs), with a 2-year phase-out period. In 2019, ECHA proposed additional restrictions, which included dodecamethylcyclohexasiloxane (D6). The restriction goal is to reduce the amount of D4, D5 and D6 released into the aquatic environment via limits on mass loadings to waste water. The Silicone Industry in coordination with government agencies initiated a European monitoring program to assess the amount of D4 and D5 being released to residential wastewater treatment plants (WWTPs). In 2019, the program was expanded to include D6. A comprehensive field sampling protocol was developed to generate statistically representative data on analyte concentrations. The analytical method achieved sub-ppb detection levels. Method validation used both low density polyethylene (LDPE) to prevent losses of the highly volatile analytes, and 13C-labelled D4, D5 and D6 isomers as internal quantification standards for the GC-MS method. A quality control scheme ensured that sources of contamination or analyte losses were identified and minimized. Six WWTPs were selected across Europe, representing different use-patterns of PCPs. Three time periods are being monitored: pre-restriction for wash-off PCPs (2017/18), during the 2-year phase-out period (2019/20), and post-restriction for wash-off PCPs (2020/21). WWTPs are sampled 8 times during each period, using a stratified random sampling design that considers seasonal, weekly, and daily factors. The mass loading of D4, D5 and D6 of these WWTPs is calculated based on influent flow measured at the time of sampling and reported on a per capita basis based on population data from the Eurostat database. The data generated during the pre-restriction period and the first half of the phaseout period (2019) indicate that concentrations of D4 are in the range of the predicted post-restriction level at all 6 WWTPs. For D5, concentrations are approaching the predicted post-restriction level at all 6 WWTPs. All D6 concentrations measured in the program are well below levels calculated using the same approach that has been used to establish post-restriction concentrations for D4 and D5.

3.19.3
Occurrence of cyclic volatile methylsiloxanes D4, D5 and D6 in fish from German freshwaters

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Cyclic volatile methylsiloxanes (cVMS) are high-volume chemicals mainly applied as intermediates in silicon polymer production and as ingredients in personal care and household products. Due to their properties cVMS are suspected to undergo long-range atmospheric transport and to possess environmental persistence and bioaccumulation potential. Thus, octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), dodecamethylcyclohexasiloxane (D6) are listed as Substances of Very High Concern (SVHC) in the European Union since 2018. D4, D5 and D6 were determined earlier in freshwater fish in e.g., Canada, Norway and Sweden as well as in archived bream samples from the German Environmental Specimen Bank (ESB). Here, the levels of cVMS in different fish species from freshwaters are compared and the distribution of D4, D5 and D6 between fish fillet and carcass is determined. To this end, pairs of carcass and fillet composite samples of different freshwater fish from the German rivers Elbe, Havel, Moselle and Weser and Lake Starnberg and a Baltic Sea Lagoon were investigated. Biometric data (e.g., length, age, sex) were documented, age determined by examination of scales, trophic positions derived from $^{15}N/^{13}C$ stable isotope analyses, and fat contents measured. After solid/liquid extraction, speciation and quantification of the cVMS were carried out with a GC-ICP-MS/MS coupling method. Overall, in all riverine fish samples only D5 was detected. At the non-riverine sampling sites Lake Starnberg and the Baltic Sea lagoon no cVMS were detected at all. Highest D5 concentrations were found for perch and European chub from river Moselle with 480 and 350 ng/g wet weight, respectively. In general, cVMS concentrations in fillet composite samples were lower than in carcass samples. For example, in European chub fillet from river Moselle the D5 concentration was about 4 times lower than in the respective carcass sample. Compared to selected priority substances (PS), it is noticeable that D5 concentrations in carcass samples were clearly higher (up to 600 times) than those of the lipophilic PS HCB, HBCDD and PBDEs. Also, the levels of the non-lipophilic PS mercury and PFOS were lower in carcasses than D5 levels, but only up to 25 times. Other aspects that will be discussed are whether the cVMS levels correlate with biometric data, lipid content or trophic level. In addition, it will be examined whether it is possible to derive fillet-to-whole fish conversion factors.

3.19.4

Bioaccumulation of Linear and Cyclic siloxanes in Rainbow trout (Oncorhynchus mykiss)

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Volatile methyl siloxanes (VMS) are considered very hydrophobic and volatile substances that have the potential to bioaccumulate. The objective of the present study is to investigate six different VMSs and whether they meet the (B/vB) requirements using a dietary exposure test protocol to determine in-vivo biotransformation rates of various siloxanes in rainbow trout fish. Using depuration data from each of the studies we were able to calculate the dietary uptake efficiencies (ED), biomagnification factors, lipid normalized (BMFL), and bioconcentration factors (wet weight, total) (BCFww,t). Regulatory agencies across the globe use BCF data from aqueous exposures ubiquitously, while BAFs generated from dietary studies still do not have the same regulatory impact. Individual siloxane exposures to juvenile rainbow trout fish (D4, D5, D6, L3, L4, L5) were run at a concentration of 4.5µM siloxane/g food for and uptake duration lasting 35 days. Since the depuration rates for the cyclical siloxanes are very slow, and through previous work done in our lab, this high concentration should suffice to represent a depuration rate value, this will also save time and cost for the project. The depuration time points will differ between the linear and cyclic siloxane compounds due to the limit knowledge of depuration kinetics surrounding linear siloxanes. Our work indicates a few different points: (1) L3, L4, L5, D4, D5, and D6 bioconcentrate but do not biomagnify, (2) low dietary uptake efficiencies indicate considerable biotransformation in the intestinal tract, (3) D4, D5 and L3 exhibit slow rates of somatic biotransformation and (4) the bioaccumulation profile of L3, L4, L5, D4, D5, and D6 does not match that of PCBs and other highly bioaccumulative organochlorines.

Sources, Fate and Effects of Metals in the Environment: Advances in Risk Assessment, Regulatory Guidance, and Remaining Gaps

3.20.1

EcoRelevance: Population modelling for metal risk assessment

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Population modelling is increasingly considered as a useful tool for ecological risk assessment (ERA). Current case studies with population models are typically performed in the context of plant protection products (PPP). This use of population models is less applicable for other regulatory programs such as **REACH** or the**WFD** where ERA is often based on a **collection** of endpoints of **different species**. However, as REACH and the WFD are relevant for nearly all chemicals in Europe, the question on how population models can improve the ecological realism of ERA is highly relevant. In the **EcoRelevance** project, the potential of **population models to increase the ecological relevance** of the ERA of **two metals (Cu and Zn)** was investigated for nine species used in regulatory dossiers including fish, invertebrates and primary producers.**Individual based models (IBMs)** were used for most species to extrapolate Cu and Zn effects observed in toxicity tests to the population level. **GUTS and DEBtox** were used to model lethal effects and sublethal effects, respectively. For each species, toxicity models were calibrated on the available datasets. Population simulations were performed and, if possible, validated with experimental data. Finally, effect concentrations for population endpoints (growth rate, maximum and average density) were determined.Effect concentrations at the population level were typically higher than at the individual level, although differences were mostly limited (less than a factor 2). The differences also varied greatly between the **chosen population endpoint**: growth rate was typically more sensitive than population density endpoints. Another issue is related to the **bioavailability** of metal ions. Bioavailability modelling is a cornerstone for metal risk assessment but it is unclear how to include this in population modelling.Despite these issues, this study has demonstrated the potential of population models. In addition to predicting population effects, they also allow to derive **more information from** the available **toxicity tests**. Population models can help to identify the **mechanism behind toxicity** and determine whether lethal or sublethal effects are dominant at the population level. Additionally, they allow simulation of **untested scenarios**.Although issues remain to be addressed, population models thus allow for an unique insight into the ecological effects of metals using data already available in regulatory dossiers.

3.20.2

Integrating bioavailability of metals in fish population models

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Traditional ecological risk assessment of metals is based on effect concentrations derived from toxicity tests on individual-level endpoints. However, these effect concentrations are derived for a single time point at the end of the test while it is known that these effect concentrations are dependent on the exposure time. Furthermore, effects on individual level endpoints do not directly translate to a similar effect on the population level. The combination of population models and Toxicokinetic-Toxicodynamic models, such as the 'General Unified Threshold models of Survival' (GUTS), can help to overcome this problem. However, in the risk assessment of metals it is also important to consider the bioavailability of the metals, which is not currently the case for population models.Therefore, the main aim of this study was to investigate if we could incorporate bioavailability of metals into a population model of *Oncorhynchus mykiss* by means of introducing bioavailability models in the underlying GUTS model. For this, GUTS-IT (Individual Threshold) and GUTS-SD (Stochastic Death) models were calibrated for a zinc and a copper toxicity data set of juvenile rainbow trout (*O. mykiss*). The GUTS models were calibrated using the free ion activity of zinc and copper respectively. GUTS-IT and GUTS-SD models were separately parameterised for each test water and compared to identify the best model for each physico-chemical water characteristic. In addition, relations between GUTS parameters and the physico-chemical water characteristics were investigated. Then, we tried to develop a generalized bioavailability model (gBAM) for one of the GUTS parameters. As a final step, we used a combination of the GUTS model and a spatially explicit Individual Based Model to estimated effects of zinc and copper on an *O. mykiss* population.Overall, the GUTS parameter "median of distribution of thresholds" showed the strongest relationship with the physico-chemical characteristics. The developed gBAM for this parameter was able to predict the parameter within a two-fold error and was capable to make relatively good estimates of the LCx values after 30 days of exposure. At the population level, overall lower effects of copper and zinc were observed than at the individual level.

3.20.3

A Flexible Aquatic Metal Bioavailability Modelling Tool for Site-specific Risk Assessment

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Aquatic bioavailability models such as the Biotic Ligand Model (BLM) can be used to perform site-specific risk assessment for metals, but the complexity of the calculations involved has precluded widespread use of the available approaches. We have developed a tool to make the process of using bioavailability modelling as straightforward as possible, to facilitate the use of bioavailability approaches in aquatic metal risk assessment. The tool contains speciation models (WHAM with Model V, VI or VIII) and bioavailability models (the BLM and the generalised bioavailability model), parameter sets, and a database of laboratory toxicity test results. Computations require the use of toxicity data (endpoint metal concentrations and ionic composition of the test water), a speciation model, and a set of bioavailability models and their parameters. Users choose which combination of models and data to use from a pre-defined list of settings, making the computation process straightforward. The tool allows visual inspection of the toxicity test dataset, the resulting species sensitivity distribution, and computed measures of risk (e.g. the site-specific Predicted No Effect Concentration, PNEC, and the Potentially Affected Fraction, PAF). It can also be used to process data for multiple target waters, and the results may be saved for viewing in other applications. The tool has been applied to copper as an example metal, and has been used to perform calculations according to the approach previously used for the EU copper Voluntary Risk Assessment. The tool outputs closely match those obtained previously. The tool is extensible to allow updating of any component model or database, and so may be readily extended to other metals that have fully validated bioavailability models.

3.20.4

Validation of bio-met for the site specific bioavailability normalisation of EQS for copper, nickel, and zinc

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The importance of considering the bioavailability of metals in understanding and assessing their toxicity in freshwaters has been recognised for many years. Currently, Biotic Ligand Models (BLM) are being applied for the derivation of Environmental Quality Standards for metals under the Water Framework Directive in Europe. Bio-met is a user-friendly tool which was developed for implementing bioavailability-based EQS for metals in European freshwaters. This study validates the relationship between the full BLM predictions and the thresholds (HC5 values) predicted by bio-met in two stages. First, ecotoxicity data for specific species from laboratory tests in natural waters were compared against predictions by the full BLMs for the same species. Second, the site-specific HC5 values predicted by bio-met for the natural waters used for ecotoxicity testing were compared against those provided by the full BLMs. Both relationships were validated for the metals copper, nickel, and zinc. Calculations using a larger set of European natural waters additionally demonstrated the validity of bio-met over a broad range of water chemistry conditions. Bio-met is, therefore, an appropriate tool for performing compliance assessments against bioavailability-based EQS values in Europe, due to the demonstrated consistency with the toxicity test data.

3.20.5

Using national regulatory freshwater monitoring data to define ecologically relevant bioavailability-based EQS for metals: the example of France

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The starting point for the derivation of a bioavailability-based metal Environmental Quality Standard (EQS) hazardous concentration at the 5th percentile (HC5) that is protective of 95% of the waters for the region that is the most sensitive to exposures of that specific metal. This method has been applied to France for the assessment of nickel and zinc. The dataset utilised covered 4,565 sites in 96 departements covering 13 regions. Using the appropriate BLMs, the most sensitive regions for nickel and zinc exposures were determined to be Provence-Alpes-Côte d'Azur and Bretagne, respectively with 5th percentile local HC5 concentrations of 2.2 µg L-1 and 11.3 µg L-1. These derived values can then also be used as the compliance metric as part of a country-wide compliance assessment for sites where measured metal concentrations are available as part of a tiered approach. The large amount of data available for France indicates the utility of performing regional HC5 concentrations for derivation of compliance metrics; however, this approach is only scientifically robust where there is sufficient monitoring data available to assess the whole country. Where monitoring data is more sporadically available, there is significant potential for an under- or over-protective compliance metric to be derived.

3.20.6

Do counter-ions affect nickel toxicity?

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According to REACH guidelines, multiple substances may be classified into a single category if the determination is made that the substances display similar physicochemical and/or toxicological qualities as a result of their intrinsic properties. Under REACH framework and with respect to ecotoxicological studies, various nickel substances (e.g., nickel sulfate, nickel chloride, etc.) have historically been considered a single category. This classification has been primarily contributed to the release of the dissociated ion Ni2+ from nickel substances, which is the toxicologically relevant form in the environment. Consequently, the corresponding dissociated 'counter-ion' (e.g., chloride, sulfate, etc.) of a nickel substance is assumed to be toxicologically negligible. The intention of this research is to review the current available information related to various nickel substances under REACH and determine if there is validity in prioritizing the ecotoxicological effects of Ni2+ over the corresponding counter-ion. Although this study is focused on nickel substances, a similar evaluation should be applicable for other metals. Within this study, we aim to evaluate approximately five distinct lines of evidence including chemistry, natural water constituency, toxicology, risk assessment, and predictive modeling to determine the extent the counter-ion contributes to toxicity. Using data from the IUCLID database and available literature, we summarized LC50 values for three organisms (C. dubia, D. magna, and P. promelas) for their exposures to various metal (Ni, Na, K, Ca, Mg) and counter-ion (Cl, SO4, NO3, HCO3) combinations. The toxicity of Na, K, Mg, and Ca salts all have LC50s >510 mg/L (P. promelas, KHCO3), while the greatest LC50 (i.e., least toxic threshold) for any nickel salt was < 12.3 mg/L (P. promelas, NiCl2). The magnitude of the LC50 deviations provides evidence that compounds containing nickel are much more toxic than any toxicity thresholds than would be contributed by the counter ion, regardless of the which counter ion is present. Performing a thorough and logical progression of analyses to understand how counter ions may contribute to ecotoxicity is necessary for the justification of continuing to treat metal substances in a read-across framework under REACH. Ultimately, we expect to find that the toxicity thresholds are driven largely, if not entirely, by nickel concentrations in the environment rather than their counter-ion component.

3.20.7

Mixture toxicity of Ni, Cu and Zn on eight freshwater algal species: what determines a something-from-nothing effect?

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Metal mixture data is scarce for freshwater systems but is crucial to improve current risk assessment. This is particularly important for algae, the primary producers of ecosystems, as adverse effects on these species affect the functioning and the outcome of higher trophic levels (daphnids, fish). For example, nickel (Ni), copper (Cu) and zinc (Zn) often occur together in freshwaters at concentrations close to their individual predicted no effect concentrations (PNEC). Metals dosed at concentrations yielding < 10% adverse effect, may show a large effect when dosed in combination, commonly referred to as a something-from-nothing effect. However, it is still questionable how this mixture effect translates to a community level. A reduced effect would be predicted by the insurance hypothesis: less sensitive species take over the functions of the more sensitive ones when stressed by a toxicant. On the contrary, recent modelling of mixture toxicity on community level suggests a more likely occurrence of the something-from-nothing effect when the sensitivities to the individual metals across the species in a community are correlated. Therefore, metal mixture toxicity of Ni, Cu and Zn was tested on 8 freshwater algal species (*Ankistrodesmus falcatus, Chlamydomonas reinhardtii, Chlorella vulgaris, Desmodesmus subspicatus, Pseudokirchneriella subcapitata, Tetraëdron minimum, Synechococcus elongatus* and *Scenedesmus quadricauda*) in a fixed-ray design with a 1:1:3 ratio of Ni:Cu:Zn based on weight. Single metals were tested simultaneously and revealed a >10 fold observed variation in single metal sensitivities across the species. A significant, but weak correlation was found between all three binary combinations of Ni, Cu and Zn EC10 values, indicating that a something-from-nothing effect is indeed more likely on a community level. These correlations are probably caused by similar uptake and detoxification mechanisms of Ni, Cu and Zn, and species thus mainly differ in their overall metal detoxification strategy. This paves a way to testing mixtures on communities in which the next step is the assessment of interactions between species in presence or absence of stress.

3.20.8

Thirty-years of monitoring for metals in airborne particles (PM10) in the Canadian Great Lakes basin - trends and source apportionment

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Canada's Great Lakes Basin (GLB) Monitoring and Surveillance Program has been monitoring contaminants in air and precipitation since the 1990s, aiming at assessing the atmospheric deposition of contaminants to the lakes. Long-term monitoring for metals in airborne particles (PM_{10}) was conducted at three remote sites in the GLB; namely Burnt Island (1992-2013), Point Petre (1988-2017) and Egbert (1988-2008). Nineteen target metals were frequently detected, with concentrations ranging from not detectable to 850 ng/m^3. Dominant metals found were iron (Fe), zinc (Zn) and copper (Cu). The most populated site of Egbert shows the highest concentration of metals in PM_{10}, reflecting the anthropogenic origin of these substances. Changes in usage and emission patterns of metals in the Great Lakes region may be reflected in their temporal trends measured at these remote sites. Temporal trends were developed using the digital filtration method combined with the Seasonal Kendall test. Trend analysis suggested that the concentrations of most metals decreased significantly with time, with halving times ranging from 10 to 48 years, reflecting emission reduction during the sampling period. Sources of metals in the atmosphere were investigated by various techniques including enrichment factor analysis, positive matrix factorization analysis, and potential source contribution function analysis. The United States was found to be the major source area for metals in airborne particles measured at the three sites, mainly due to higher population and more industrial and transportation activities.

3.20.9

High-resolution spatial and temporal monitoring of trace metal speciation in a major European estuary

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Trace metal biogeochemical processes are very dynamic and their comprehensive study requires an accurate high-resolution monitoring at spatial and temporal timescales. We investigated the behavior of three EU (priority) hazardous contaminants i.e. cadmium (Cd), lead (Pb), and copper (Cu) along the Gironde Estuary salinity gradient and performed a diel cycle of measurements at the estuary mouth, in a period of intense primary production. Real-time measurements of the trace metal dynamic fraction, i.e. the potentially bioavailable fraction, were performed using an antifouling gel integrated microsensors arrays (GIME) incorporated in an innovative submersible miniaturized multichannel voltammetric probe. We also quantified metal levels in the (i) dissolved and (ii) "truly" dissolved phases (< 0.2µm and < 0.02µm, respectively), and the (iii) particulate phase by Triple Quad ICP-MS. Master physicochemical parameters and indicators of primary production were also measured. Similar 0.02–0.2µm dissolved concentrations observed for all metals studied, suggest that the 0.02–0.2µm colloidal fraction does not control Cd, Pb, and Cu transport in this system, while inter-comparison between dynamic and < 0.02µm dissolved fractions revealed the importance of smaller colloids (< 0.02µm) for the distribution of these trace metals. The first profiles for dynamic and dissolved fractions of Cd, Pb and Cu showed important variations in proportion of the dynamic fraction along the salinity gradient and through time (diel cycle). For Cd and Pb, the dynamic fraction represented between 20 to 100% of the dissolved phase while for Cu it ranged from 1 to 20%. Biogeochemical processes including organic (phytoplankton trapping/release, bacterial mineralization….) and inorganic (mineral-oxide adsorption…) particle-water interactions, may account for changes in trace metal speciation in space and time. These processes play a major role for the transport of trace metals under their supposedly most bioavailable fraction. The obtained results highlight the importance of performing high resolution monitoring of specific metal fractions coupled to master bio-physicochemical conditions to determine site-specific controls on metal reactivity, behavior and fate. In situ bioavailability-based high resolution monitoring methods may greatly contribute to the understanding of biogeochemical processes as a prerequisite for ecotoxicological risk assessment of metals and subsequent metal regulations.

3.20.10

Geochemical factors affecting metal solubility in saline waters: implications for ecotoxicity

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There have been few studies of metal solubility in seawater, kinetics of precipitation and adsorption, and changes in precipitate composition during aging, over timescales which are important for exposure pathways in field contaminated sites and laboratory ecotoxicological tests. The aim of the present study was to gain insight into the factors affecting aluminium, copper and lead solubility in seawater to improve exposure pathway predictions. Natural and artificial seawater test treatments were spiked with Al, Cu and Pb, and incubated for 28 days at 22°C, pH 8.15±0.05. Unfiltered and filtered (0.001, 0.025 and 0.45 µm) subsamples were taken over 28 days for analysis of dissolved and total metal concentrations and determination of the limit of solubility. A combination of elemental analysis and x-ray diffraction (XRD) was employed to investigate changes in Al, Cu and Pb precipitate composition over the duration of the tests. The limit of solubility of Al, Cu and Pb in natural seawater was 0.5, 0.6 and 2 mgL^{-1}, respectively. At concentrations up to the solubility limit, the dissolved metal concentrations stabilised and equalled the total concentration within a few hours. However, in the test solutions that contained total metal concentrations above the limit of solubility, the dissolved metal concentrations were dynamic, peaking at concentrations that exceeded the solubility limit over a timescale of hours to days before decreasing to values below the solubility limit. Higher total concentrations resulted in higher peak dissolved concentrations that decreased more rapidly. Ultrafiltered metal concentrations were always similar to the concentration in the 0.45 µm fraction, indicating negligible colloidal Al, Cu and Pb. The presence of DOC in natural seawater had no effect on the dissolved concentrations of aluminium or copper but resulted in a higher lead solubility than in artificial seawater (0.78 mgL^{-1}) without DOC, presumably due to lead complexation with dissolved organic ligands. The composition of the aluminium and copper precipitates changed as they aged over time, from amorphous $Al(OH)_3$ and $Cu_2(OH)_2CO_3$ to hydrotalcite ($Mg_6Al_2CO_3(OH)_{16}.4H_2O$) and clinoatacamite ($Cu_2,Cl(OH)_3$), respectively. The precipitate ageing coincided with decreased solubility, presumably due to a combination of lower limits of solubility of the aged precipitates and adsorption of dissolved metals onto precipitates. The composition of the lead precipitate did not vary over time and adsorption is likely to explain the lower solubility in the presence of precipitates. These findings have particular relevance for laboratory toxicity testing where there is a need to prepare test solutions with metal concentrations that span several orders of magnitude. The study highlights the need for experimental determination of solubility and characterisation of the mineral phases that precipitate from solution.

The Polar Regions: Pollutants & Environmental Change - Multiple Stressors, Ecosystem Response and Environmental Policy

3.21.1

How does Climate Change Affect Contaminants in the Arctic? First Results from a new AMAP Assessment

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The rapidly increasing temperature in the Arctic has effects on the physical environment and on ecosystems of the Arctic. These changes can also have implications for the levels and trends of persistent organic pollutants (POPs) observed in the Arctic, via changes in emission sources, transport processes and pathways, partitioning, degradation and exposure of wildlife and humans. The Arctic Monitoring and Assessment Programme (AMAP) has initiated a new assessment of effects of climate change on POPs and other contaminants in the Arctic, addressing the following main aspects: 1) Emissions and long-range transport of POPs (modelling approaches); 2) Influences on POP distribution and fate in abiotic media; 3) Food web changes that may impact POP exposure and accumulation; 4) Climate-related effects on temporal trends of POPs in biota. The asessment will also address implications of these findings and compile relevant data and knowledge gaps. Secondary sources can play an important role for the emissions and global transport of POPs as temperatures increase and the cryosphere is diminished. Primary emissions may also increase on the local scale, along with human activities in the Arctic. Emission inventories are key information in this assessment, but they are still largely missing, in particular for chemicals of emerging Arctic concern. Atmospheric POP measurements show recent increases of some volatile compounds, such as hexachlorocyclohexane (HCH) isomers and some polychlorinated biphenyls (PCBs), which could be related to volatilization from the sea. Correlations have been found between these compounds in air and certain climate parameters. As a consequence of changes in species distribution, food availability and accessibility, dietary composition has changed for several species, with implications for their POP exposure. To account for these changes, it will be relevant to monitor dietary biomarkers along with contaminant concentrations. Correlation studies have indicated associations between developments of POPs in biota and some climate parameters. While changes in emissions still seem to be the main driver of the long-term POP levels in Arctic biota, recent variations in POP levels might be better explained by climate-related phenomena, including changes in food web structures. Different driving forces are important factors in an effectiveness evaluation of international contaminant regulations, such as the Stockholm Convention on POPs.

3.21.2

Warmer climate and increasing productivity affect mercury biomagnification in subarctic lake food webs

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Subarctic areas are one of the most pristine regions on Earth, where indigenous people obtain a significant amount of diet from local fish and game. However, pollutants and toxic heavy metals like mercury (Hg), which biomagnify in aquatic food webs, are atmospherically transported across the planet and regularly deposited in circumpolar regions. In subarctic Fennoscandia, lakes are getting warmer and more productive by joint effects of rapid climate change and intensive land-use practices (e.g. forestry actions such as clear-cutting and ditching), with potentially increasing leaching of Hg into lake ecosystems. Thus, there is an immediate need to evaluate such environmental changes affect the level and magnification of toxic materials in food webs. In this study, we tested how the joint effects of climate and land-use influence mercury levels at the base of the lake food webs and identify patterns of biomagnification between total mercury ($log_{10}THg$) and organism trophic level determined from a stable isotope of nitrogen ($\delta^{15}N$) ratio. We sampled primary producers (algae) to top consumers, piscivorous fish, from a series of 19 subarctic lakes situated on a latitudinal (69.0 – 66.5° N), climatic (±1.5°C, ±15% precipitation) and catchment land-use gradients (from pristine to intensive forestry areas) in Norway and Finland. Our results reveal increasingly high THg levels in base of food webs towards warmer and more productive lakes. This lead to elevated concentrations of Hg in higher trophic level organisms. However, stronger THg biomagnification was observed as steeper regression slopes in the northern cold and oligotrophic lakes compared to warmer and eutrophic southern lakes. These results reveal the complexity of mercury dynamics, in which warmer climate and more intense land-use elevate mercury at the base of food webs. The highest mercury levels in locally consumed fish species, such as pike; *Esox lucius* and perch; *Perca fluviatilis*, were found in warm and productive lakes suggesting the importance to limit mercury inputs at the base of a food web. Further studies from lake food webs from other Arctic regions are needed to test, whether biomagnification slopes are consistently steeper towards the north.

3.21.3
Deposition of polychlorinated biphenyls to ice cores at opposite polar sites: Holtedahlfonna, Svalbard and Site M, Antarctica.
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Between 2001 and 2005 we collected ice cores from glaciers and ice sheets that are nearly polar opposites, Holtedahlfonna, on Svalbard (Lat 79.13 N, Long 13.27 E), and Site M, near the coast in East Antarctica (Lat 75.00 S, Long 15.00 E). Both sites are remote and at high elevations (Holtedahlfonna, 1150 m.a.s.l., Site M, 3470 m.a.s.l.) and likely receive contaminant inputs only from long-range atmospheric transport (LRAT) which may be different in these hemispheres. All samples were analyzed for 87 PCB congeners in 80 peaks. The core from the Holtedahlfonna site has a historic record from 1953 – 2005, and Site M, as one sample, from 1958 – 2000. The qualitative results of PCB congeners from the two sites show some similarities and differences. Most congeners at both sites are in TriCB, TetraCB and PentaCB homologues and at Holtedahlfonna these three comprise 82.5%, and at Site M, 89.2% of ∑PCB. Site M PCBs are dominated by PentaCB (47.6%), while Holtedahlfonna is dominated by TetraCB (44.9%). The dominant congeners in TriCB through HexaCB are the same at both sites (PCB-28+31, PCB-70, PCB-101, PCB-138). The total PCB flux at Site M was 51% of Holtedahlfonna which would only partly be explained by the additional 10-year record at Holtedahlfonna. We developed an LRAT frequency diagram showing that sites in South America are sources to Site M more than 2% of the time between 1958 and 2000, with coverage of all of New Zealand and parts of southern Australia at a frequency of greater than 0.1%. A similar diagram for Holtedahlfonna shows greater than 2% coverage of Northern Norway, Sweden, Finland and the western Kola Peninsula, as well as all of Novaya Zemlya and Franz Josef Land. The historical trend of PCBs at Holtedahlfonna shows the lowest flux from 1962-1971 during peak PCB production and use times in North America. The maximum flux occurred between 1980 – 1988 after PCBs were being globally restricted from further use. The earliest sample, 1953-1962 had only slightly lower ∑PCB flux than the surface sample from 1995 – 2005. There is no apparent trend to PCB deposition over time at Holtedahlfonna which has been observed elsewhere on Svalbard. These results suggest that PCB sources have continued to emit PCBs in the polar airsheds in both northern and southern hemispheres after the end of new uses in the 1980s.

3.21.4
Seasonal kinetics POPs in Antarctic pelagic marine waters and consequences for uptake in benthic organisms
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Persistent Organic Pollutants (POPs) may travel long distance through the atmospheric, which results in the transport of semi-volatile POPs to colder remote areas like Antarctica. In this presentation a recent study on the occurrence and seasonal dynamics of POPs in the coastal marine environment of Antarctica will be presented and drivers of their local accumulation in benthic organisms discussed. POPs (PCBs, OCPs and PBDEs) were analysed in seawater from Ryder Bay near the British Rothera research station at different time points throughout the season. Benthic organisms with different feeding traits (sea squirt (*Cnemidocarpa verrucose:* filter feeder),limpet *(Nacella concinna*: grazer),sea urchin(*Sterechinus neumayeri*: omnivore),sea cucumber *(Heterocucummis stein*: detritivore) and sea star *(Odontaster validus*: predator/omnivore)) were also collected. POP levels in the water were comparable to other studies, although they varied over the season. They were highest during melting of the sea ice, after which they dropped considerably likely due to increasing mixed layer depth of the water. An algal bloom developed and after its peak POP concentrations declined further, likely related to sedimentation with the organic particulate. Overall, POP concentrations in the water differed over 3 orders of magnitude over the season and results imply rapid depletion of POPs from the pelagic system and an effective transport to the benthic system. In the benthic organisms POP-levels differed between the species, PCBs and PBDEs were highest in the limpets and sea urchins. Of the OCPs, HCB and pp-DDE were found in the highest concentrations, followed by HCHs, heptachlor and oxychlordane. However, OCP concentrations generally did not differ among species. Limpets contained POPs with relatively higher logKow, likely due to their grazing behaviour and their potentially limited exchange with the water due to their shell. Sea squirts and cucumbers contained POPs with relatively high water solubility, which may indicate a water-borne exposure. Overall, the results show that POPs in the Antarctic marine system are effectively transported from the pelagic to the benthic system, which is related to seasonal sea ice dynamics and algal blooms. This is likely to be specific for sea ice dominated systems, and may change due to climate change. Once in the benthic system, uptake in organisms is related to species specific traits and chemical properties.

Tire Wear and Microrubber Particles - From Problems to Solutions

3.22.1
Towards a global quantification of TRWP Fate & Transport in the terrestrial and freshwater environment
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Tire and road wear particles (TRWP) consist of polymer-containing tire tread material with pavement mineral and binder encrustations. TRWP has been estimated to be approximately 50% tread, of which about half consists of a polymeric fraction. In 2018, the first integral model-based catchment scale TRWP mass balance was developed for the Seine and Scheldt basins (France/Belgium), describing the environmental fate and transport from road surfaces up to the river-estuary interface. For the Seine watershed, the TRWP mass generated at source was estimated as 27,600 t/y. The mass balance indicated that 19% of this mass was transported to freshwater and 2% was exported to the estuary, indicating significant trapping in freshwater sediments. This presentation discusses the first steps towards a continental or global scale TRWP mass balance model. The results from a sensitivity and uncertainty assessment for the Seine basin were further analysed to identify the most relevant knowledge gaps and model related uncertainties, not just for the Seine basin, but in a global modelling perspective. This resulted in focal points for model development. Recent literature was surveyed for opportunities to address knowledge gaps and alleviate model uncertainties. Also based on ongoing or anticipated, yet unpublished research efforts, a plan was developed for a geographically extended and methodologically refined TRWP mass balancing approach. A significant upgrade of the terrestrial fate and transport component of the existing Seine model was designed, aiming to provide better predictive capabilities for run-off induced transport, especially in arid regions. This is combined with a much higher spatial resolution of the model (1 km rather than 15 km), which allows for better predictive capabilities for "connectivity" between roads and waterways and better description of waterways morphology, and subsequent particle retention. The new model is being built using globally available datasets only, to avoid spatial inhomogeneities in model results. The presentation will include the overall model development plan and show first results from the above upgrades. The research presented shows how model refinement and extension is expected to lead to a global quantification of TRWP Fate & Transport in the terrestrial and freshwater environment in the years to come. This is an important basis for an enhanced global risk assessment of TRWP and cost-effective mitigation.

3.22.2
Quantification and characterization of tire and road wear particles in size-fractionated samples from road environments
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One of the main contributors to non-exhaust emissions are tire and road wear particles (TRWP). TRWP are heteroaggregates consisting of rubber particles abraded from tires and other particles that are deposited on the road surface. TRWP can be quantified by the use of marker substances, such as for example Zn. Derivatives of Benzothiazole, antiozonants and guanidine vulcanization accelerators are commonly present in tires and have been identified in tire particles as well as in environmental samples. In this study, samples from road environment were analyzed for TRWP contents and concentrations of selected organic analytes both in whole samples and in size fractions obtained by wet-sieving. TRWP was determined by Zn content in density enriched fractions using ICP-MS after microwave assisted acid digestion. Organic analytes were analysed with UPLC-TOF-MS after extraction with isopropanol, evaporation to dryness and reconstituion in 50% methanol. Road dust collected by road sweeping cars in the city of Leipzig (Germany), sediments from a highway runoff treatment facility in Berlin (Germany) and sediments from sedimentation ponds for runoff from highway A38 close to Leipzig were analyzed. The concentrations of TRWP in whole samples ranged from 3.7 to 480 mg/g. Samples from the treatment system in Berlin had by far the highest concentrations. The concentrations in road sweeping dust were similar between the different samples (6.7 – 9.4 mg/g). For the system from highway A38, concentrations were lower in the sampling spots close to the inlet of the pond than at the outflow of the ponds. Concentrations of organic analytes ranged from low ng/g concentrations to several hundreds of ng/g. Principal component analysis was conducted on the concentration of organic analytes and showed a clustering of samples depending on their origin. Three compounds with high influence on the first principal component were identified. Strong and statistically significant correlations between these substances and

TRWP concentrations were obtained. TRWP content and concentrations of organic analytes were also determined in size-fractions of wet-sieved samples. Size classes with highest TRWP concentrations varied between sample types. Findings suggest that TRWP size distribution differs depending on road type and may change with distance to the source. *Acknowledgement* - The authors thank Berliner Wasserbetriebe, Straßenreinigung Leipzig and Autobahnmeisterei Leipzig for provision of samples and the BMBF for funding the MiWa project (reference number 02WRS1378H).

3.22.3
Analysis of tire wear particles in soil samples from roadside using TED-GC-MS
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Tire wear particles (TW) are generated by the abrasions of tires on the road surface through traffic. These particles can be transported by air and surface runoff and might also infiltrate the soil and consequently affect terrestrial ecosystems. The estimated tire wear (TW) emissions are immense, with 1.33 106 t a-1 in Europe [1]. Despite this, only little is known about the environmental contents or the fate of TW. One reason for this knowledge gap is the challenging analysis of TW in environmental samples. Detection of TW with spectroscopic methods is problematic due to high fluorescence interferences caused by contained black carbon. One analytical approach is to use zinc (Zn), a typical additive in tires, as a specific marker for the quantification of tire wear. However, any Zn originating from the sample matrix must be separated beforehand and requires elaborate sample preparation [2]. Car tires consist partly of synthetic rubbers, such as styrene-butadiene-rubber (SBR). This SBR could be identified and quantified via Thermal-Extraction-Desorption-Gas Chromatography-Mass Spectrometry (TED-GC-MS) [3]. This newly developed and fast screening method allows the simultaneous detection of microplastics and TW mass contents and requires minimal to no sample preparation. Firstly the sample is thermally extracted in a thermobalance under a nitrogen atmosphere. The resulting specific decomposition products are sorbed on a solid phase adsorber, which is then transferred to a GC-MS via an autosampler, where the products are desorbed, separated and identified. Cyclohexenylbenzene is used as a specific marker for SBR. Here we investigated top layer soil samples, collected at the roadside of highly frequented German highways. Samples were analyzed without sample preparation, and SBR was detected in all investigated samples in mass contents ranging from 67.2 to 2230 mg kg-1. A correlation between SBR and Zn content in the soil was confirmed, while the correlation between SBR and Corg was hardly pronounced. We successfully demonstrated the application of TED-GC-MS as a screening method for tire wear in soil samples. The present study will discuss these analytical results in detail as well as sampling parameters like sampling depth and distance to the roadside, and the effect of the particle size on the particle transport by water runoff and air. References 1. Wagner, S.; Hüffer, T.; Klöckner, P.; Wehrhahn, M.; Hofmann, T.; Reemtsma, T. 2018, Tire wear particles in the aquatic environment - A review on generation, analysis, occurrence, fate and effects. Water Res. 139, 83-100. 2. Klöckner, P.; Reemtsma, T.; Eisentraut, P.; Braun, U.; Ruhl, A. S.; Wagner, S. 2019, Tire and road wear particles in road environment – Quantification and assessment of particle dynamics by Zn determination after density separation. Chemosphere 222, 714-721. 3. Eisentraut, P.; Dümichen, E.; Ruhl, A. S.; Jekel, M.; Albrecht, M.; Gehde, M.; Braun, U. 2018, Two Birds with One Stone—Fast and Simultaneous Analysis of Microplastics: Microparticles Derived from Thermoplastics and Tire Wear. Environ. Sci. Tech. Let 5 (10), 608-613.

3.22.4
The Effects of Tire Rubber Particles and Their Leachates on the Baltic Clam (Limecola balthica)
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Tire wear particles (TWP), generated from tires undergoing friction on the road, are proposed to be one of the largest sources of secondary microplastics. Tires consist of a complex mixture of natural and synthetic rubber polymers, carbon blacks, process oils, vulcanization chemicals, fillers and antidegradents. Some components of tire rubber (e.g. heavy metals and polycyclic aromatic hydrocarbons, PAHs) can leach out from TWP and cause harm to aquatic organisms. Due to the high volume of emissions and the chemical risks accosiated to TWP, there is a need to quantify the ecotoxicological effects of these micropollutants. The aim of this study was to follow the leaching of PAHs and heavy metals from tire rubber particles, and to investigate their accumulation to and effects on one of the key invertebrate species inhabiting the soft bottoms of the northern Baltic Sea, the Baltic clam (*Limecola balthica*). The study was performed using irregular tire rubber fragments (2–190 µm) cryo-grinded from used and recycled car tires, that were added to the mesocosms in concentrations representing their occurrence in the natural sediments (approx. 1.4 g/kg of dry sediment). The experiment consisted of acute (4 days) and chronic (1 month) exposures, and the clams were exposed either directly to the particles or indirectly to their leachates. The leaching of contaminants from tire rubber was verified by analysing water samples throughout the experiment, and their uptake by clams was analysed from tissues at the end of the experiment. The impacts of exposure were studied by combining physiological biomarkers to histopathological examination of clam tissues (gills, foot, and digestive gland). The results show leaching of heavy metals (especially zinc) and PAHs from tire rubber to seawater. For example, the concentrations of indeno[1,2,3-cd]pyrene and benzo[ghi]perylene measured from the water exceeded their environmental quality standards, indicating a potential risk for the clams. The exposure to tire rubber did not affect the survival of the clams, but accumulation of both heavy metals and PAHs to clam tissues were observed. The preliminary results of the integrated biomarker response also show an elevated stress in clams chronically exposed to tire rubber. The results demonstrate the potential of environmentally relevant concentration of tire rubber particles to affect the clams and emphasize the importance of tire rubber as a yet understudied environmental contaminant.

3.22.5
Comparison of chemical characteristics and biological effects of microrubber particle and leachate exposures from a pristine and road-worn tire to Hyalella azteca
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Acute and long-term biological effects of tire wear particles from a pristine tire (PT) were tested on *Hyalella azteca,* a freshwater amphipod, and compared to previous data from a worn tire tire (WT) of same make and model. Chemical characteristics (organics and metals) of both PT and WT particles and their respective leachates, extracted at 25°C, were investigated. The acute (48 h) mortality of *H. azteca* when exposed to the tire wear particles and leachate were studied. PT particles were more toxic (LC_{50} 0.19 ± 0.03 mg/mL) than WT (LC_{50} 0.91 ± 0.06 mg/mL), whereas leachate from WT and PT were not different. The leachate exposure data did not conform to a sigmoidal curve, and thus no LC_{50} value could be derived from these. Instead, comparing graphs it is evident that PT leachate is less toxic than PT particles throughout the concentration range, whereas WT leachate is more toxic in low concentrations until a plateau is reached after which WT particles become the more toxic component. Chemical characteristics of PT and WT particles and leachate were quantified by GC-MS and ICP-MS. Four compounds leached out to the water phase regardless of tire type: benzothiazole, indene, aluminium and zinc. Of these PT leachate had a slightly larger contribution of especially zinc, at the highest measured concentration in leachate at 5.53 µg g^{-1}, albeit this concentration is 29 times lower than that of PT particles, and did not seem to change the toxicity effect of the two types of leachate. In long-term tests PT particles had greater effects on growth and reproduction (0.12 mg/mL for both) than WT (0.28 mg/mL for both). Particles from PT contained 49 times more copper, 8 times more 1-octanethiol, 5 times more lead and 3 times more iron, phenanthrene and anthracene than WT particles, suggesting these might play a part in the increased toxicity observed with PT particles in both acute and longer-term tests. Considering the presence of different chemicals in particle and leachate fractions, the mechanism(s) behind toxicity continues to be of interest. Most of the tested PAHs and metals were not present in tire wear leachate at all, and were only present in equally low amounts in both WT and PT particles, hence they cannot explain the differing toxicity of these. Therefore, future efforts should be put into investigating other organic compounds such as antioxidants and ozonates that are added in the fabrication of tires.

Trace Metal Biogeochemistry and Fate in Ecosystems

3.23.1
Effects of metal mixture and temperature stress on metal accumulation and ionoregulation in Cyprinus carpio.
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Metal toxicity is often studied on an individual basis, but considering that we cannot predict the toxicity of a metal mixture on the basis of single compounds only, it is important to study mixture toxicity in order to understand the impact of pollution in the aquatic environment. Several studies already pointed out the adverse effects caused by waterborne metals as single and binary mixtures together with other stressors such as temperature. However, it is more difficult to find information on ternary mixtures using environmentally relevant

concentrations at different temperatures. Therefore, with the present study we will try to answer the following question: "Is the temperature a determining factor for metal toxicity and bioaccumulation in a long term exposure scenario?". In order to answer this questions we ran two parallel experiments for 27 days at 20°C and at 10°C, using a nominal metals mixture of Cu: 0.08 µM; Cd: 0.03 µM and Zn: 3.16 µM. The parameters investigated were metal bioaccumulation, disturbances in ionoregulation and induction of defensive mechanisms such as metallothionein gene induction. Our results in general show a rapid increase in Cu and Cd, with differences between the two temperatures that can only be observed from one week of exposure onwards. No ion loss was observed due to metal exposure for either temperature, however lower electrolyte levels were observed in fish exposed to 10°C. Regarding the defensive mechanisms an induction of metallothionein gene expression was recorded during the whole experiment.

3.23.2

Metal bioaccumulation in macroinvertebrates: linking body burdens to the chemical and biological quality of water bodies

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Many surface waters nowadays remain under threat by ongoing human activities or historic contamination. Even under the obligations of the EU Water Framework Directive (WFD), numerous rivers and streams have poor chemical status, i.e. pollutant concentrations exceed the current Environmental Quality Standards (EQS). However, poor chemical status does not necessarily correspond to poor(er) ecological status of the water body. The overall ecological status, as defined by the WFD, comprises biological, hydromorphological and physicochemical quality elements. In the Flanders region of Belgium, the biological quality metrics include the Multimetric Macroinvertebrate Index of Flanders (MMIF); where an MMIF larger or equal to 0.7 is considered to be good. In terms of the chemical and biological quality metrics, a location may fall into one of four categories: (1) good biological quality + good chemical quality; (2) poor biological quality + poor chemical quality; (3) good biological quality + poor chemical quality (4) poor biological quality + good chemical quality. Our initial field study focused on the third scenario for the case of metal ions, i.e. the EQS is exceeded, but the biological quality (MMIF) is considered to be good. The dataset has then been expanded to include all four scenarios, covering a total of 26 locations and more than 25 taxa. Others have already focused on the relationship between bioaccumulation in macroinvertebrates and the ambient total dissolved concentrations, or described changing community metrics with environmental concentrations. However, few have made a direct link between biological quality and macroinvertebrate metal bioaccumulation. As is often the case, rivers and streams are not only subjected to a single stressor; the ecological status derives from the overall habitat quality, which is affected by many interdependent physical and chemical factors, including e.g. hydrodynamic conditions in the water body, suspended particulate matter, dissolved oxygen, light intensity,... By characterizing the exposure patterns in terms of metal speciation in the water (dissolved, total, free M^{n+}, modeled) and the sediment (total, Simultaneously Extracted Metals - SEM, Acid Volatile Sulfides - AVS), we are investigating (i) whether biological quality can be partially explained by bioaccumulation, and (ii) if the chemical speciation in the exposure medium is related to the bioaccumulation in macroinvertebrates.

3.23.3

Evaluating the transport and fate of metal-bearing tailings dust into surface water

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In this study, an abandoned mine site in Canada with apparent aerial erosion of the tailings area and with nearby water bodies was investigated to assess the transport and fate of metal-bearing mine dust into surface water. A variety of dust sampling methods were used including passive air samplers, a high volume air sampler, rain catchers and sieved tailings samples (as a proxy for windblown dust). To provide a better understanding of seasonal trends and correlation to climate conditions, dust sampling was completed seasonally, occurring every three to four months from December 2017 to April 2019. Bulk geochemistry and mineralogy of the sieved tailings and filters was conducted to determine metal concentrations and the primary and secondary metal-bearing phases. Identification of these mineral phases is important because any potential metal leaching is partially controlled by the solubility of the minerals. Results indicate that metal-bearing phases present in the dust include a variety of sulfide, carbonate and (oxy)hydroxide minerals. For example, at least five different zinc-bearing minerals were found which would each uniquely interact with surface water. To investigate how the dust and identified mineral phases react in water, shaker flask tests were conducted. Metal concentrations in the shaker flask leachate were found to be elevated indicating that some metal-bearing minerals within the tailings dust are partially water soluble and capable of leaching metals into the environment. The shaker flask test leachate chemistry and water samples collected on-site were assessed using a geochemical modelling software (PHREEQC) to better understand dust-water interactions, by investigating potential mineral dissolution and precipitation reactions. This information, combined with dust deposition rates and distribution patterns will help evaluate the potential risk tailings mine dust poses to aquatic environments.

3.23.4

Plant and microbiota-dependent effects on arsenic behaviour in the soil-plant system

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Arsenic (As) is a trace metalloid known as "the king of poisons". As is also a class one carcinogen and its high toxicity correlates with a host of human diseases e.g. skin cancer. The speciation of As largely regulates its mobility, bioavailability and toxicity in the environment. As speciation is not only driven by inorganic parameters (pH, DOC), but also controlled by the interactions with plants and microbiota. Soil microbiota can enzymatically transform As from inorganic to less-toxic organic forms. We performed an experiment to investigate the effects of plant and soil microbiota on As behaviour in the soil-plant system as well as the physiological and biochemical responses of maize plants to As exposure. In the experiment, we set up nine experimental groups: three soil treatments (normal soil (NS), sterilized soil (SS) and first-sterilized soil reconditioned with microbiome (SM)) intersecting with three As concentration groups (0, 100 and 200 ppm). In each treatment group, 10 pots with maize plants and three pots without maize were cultivated. For the As treatments we spiked soils with different levels of As. After soil incubation for two months allowing for the equilibration of As with soils, we grew 99 maize plants and regularly determined As speciation in soil water with HPLC-ICPMS. The sum of organic As species showed positive correlations with DOC in soil water but negative relations with the pH values, which indicates that pH and DOC are two controlling factors regarding As methylation in the soil environment. Moreover, the maize phenotyping data revealed the harzadous physiological effects of As on plant health. With the increasing of As concentrations and the disturbtion of soil microbiome by initial sterilization, plants experienced smaller plant height, lower chlorophyll content and greater damage scale on leaves. Overall, microbiome do play a key role in As speciation and its effects on plant health, not only shown as the As concentration pattern, but also seen in the corresponding variation of As negative effects on plant growth (SS > SM > NS). Until SETAC Conference we will analyse the malondialdehyde concentrations to reveal the biochemical responses to As in plants. We will furthermore analyse various plant tissues (leave, stem, root and kernel) by means of ICP-MS and HPLC-ICPMS to determine the concentrations and speciation of As in the soil-plant system, which gains us a better understanding of As bioavailability to plants.

Water Quality under Pressure - Understanding Fate and Effects of Organic Pollutants in Rivers by Integrating Field, Laboratory and Modeling Approach

3.24.1

Measured and predicted natural estrogens from agriculture in tributaries of Lake Baldegg in Switzerland

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Estrogens in surface waters might have negative effects on reproduction and development of aquatic organisms [1]. Agriculture may be a significant source for natural estrogens in surface waters [2]. Therefore, we aimed to investigate systematically the prevalence of agriculturally derived estrogens in Swiss surface waters to understand better the exposition of aquatic organisms to estrogens. Our surface water monitoring campaign took place in the catchment of Lake Baldegg, which sustains the highest husbandry animal densities in Switzerland. At the beginning of the vegetation period 2019, we collected daily time-proportional composite water samples for 30 days of five Lake Baldegg tributaries. None of the sampling sites contained effluents of wastewater treatment plants. Thereby, we ensured that we measured only agriculturally derived estrogens. We conducted a liquid-liquid extraction, quantified estrone (E1), 17α-estradiol (E2α), 17β-estradiol (E2β) and estriol (E3) chemically with LC-MS/MS. Furthermore, daily discharge was measured for every sampling site to calculate the loads of natural estrogens in Lake Baldegg tributaries. In a second step, we predicted estrogen loads emitted to tributaries during our study period. First, we conducted a slurry monitoring to

determine average natural estrogen concentration in Swiss cattle and pig slurry. We calculated annual estrogen loads produced per catchment based on livestock numbers per catchment, estrogen concentrations in slurry and volume of slurry produced per species annually [3]. Slurry-derived estrogens were recovered in drainage water of a drainaged test field and likewise the emission rate to surface waters was calculated. The emission rate was multiplied by loads of slurry produced in a catchment to predict estrogen loads emitted to surface waters. These were substantially lower than measured estrogen loads in Lake Baldegg tributaries. We explain this underestimation by uncertainties in the volume of slurry applied by the farmers as well as in the emission rate. 1. Vethaak, A.D., et al., Chemosphere, 2005. 59(4): p. 511-524. 2. Johnson, A., R. Williams, and P. Matthiessen, Science of the Total Environment, 2006. 362(1): p. 166-178. 3. Walther, U., J. Rieser, and R. Fliesch, FAL Agroscope Reckenholz. Zürich, 2001.

3.24.2
Enrichment and attenuation of organic contaminants in an urban creek during a rainfall event

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Urbanization is a global phenomenon characterized by changes in land use. With increased urban landcover comes the creation of more impervious surfaces, which in turn results in increased runoff after a precipitation event. Watershed urbanization provides a mechanism for the transport of contaminants to surface waters. Mimico Creek watershed is a highly urbanized watershed in Toronto, Canada, which drains into Lake Ontario. In this work, we monitored the concentrations of four groups of organic contaminants - Polycyclic Aromatic Hydrocarbons (PAHs), Substituted Polycyclic Aromatic Hydrocarbons (SPAHs), Organo¬phosphate Flame Retardants (OPFRs) and Benzotriazole Ultraviolet Stabilizers (BT-UVs) in Mimico Creek. These concentrations were monitored before, during and after a major rainfall event in the summer of 2018. The aim of this was to explore the significance of stormwater as a source of contaminants to the stream and to observe the change in concentration of these contaminants in the stream during precipitation. Results indicate that the different contaminants behave differently as stormflow increases and decreases over time. Some contaminants show a sudden spike in concentrations, which coincides with the beginning of stormflow, while others show a dilution pattern. Our results suggest that in addition to land use, the nature of a contaminant and its phase partitioning properties influence its behavior in an urbanized watershed during precipitation. We also derived the sediment-water partitioning coefficient (Kd) for some chemicals from the measurements and observed that it changes during the course of the event, likely due to input of sediments with higher sorptive properties.

3.24.3
Assessing the mixture effects in in vitro bioassays of chemicals occurring in small agricultural streams during rain events

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Small streams can be impacted by micropollutants from both point and diffuse sources, including wastewater effluent and agricultural run-off. The current study aimed to assess the chemical burden in small agricultural streams after rainfall events using a combination of *in vitro* bioassays and chemical analysis. The water samples were extracted with solid-phase extraction and run in bioassays indicative of activation of the aryl hydrocarbon receptor (AhR), binding to the peroxisome proliferator-activated receptor (PPARγ), activation of the estrogen receptor (ER) and oxidative stress response. A wide dynamic range in effect and chemical concentration was observed. Iceberg modelling using the bioanalytical equivalent concentration (BEQ) approach was applied to understand which chemicals were contributing to the observed effect. The effect of chemicals detected at high concentrations or likely to contribute to the observed effect were fingerprinted in the assays. Iceberg modelling demonstrated that the detected chemicals only explained a small fraction of the effect (e.g. often < 1%), indicating that a large number of unknown chemicals present in the extracts were having an effect. The contribution of detected chemicals to the known fraction of effect (i.e., the tip of the iceberg) was calculated and mixtures of the top 17 chemicals explaining the known fraction of the effect were prepared for one hundred mixtures in the concentrations ratios they occurred in the tested water samples. The experimental mixture effects were compared with the "tip of the iceberg" mixture effects, which were experimentally determined based on the concentration ratios of the chemicals present in the water samples. These experimental mixtures generally agreed well with the predictions by the mixture model of concentration addition. Overall there was a high diversity of chemicals in the samples during rain events, though a few chemicals such as 2-benzothiazole sulfonic acid, diclofenac and diuron were important contributors to the mixture effects in different assays. This study shows that small streams can have a considerable micropollutant burden, emphasizing the need for re-evaluating regulatory monitoring measures that are based on a few selected chemicals.

3.24.4
Assessing benthic bioaccumulation of polychlorinated dioxins/furans (PCDD/Fs) and polychlorinated biphenyls (PCBs) in the lower Passaic River (NJ, USA) based on in situ passive sampling

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Passive sampling has emerged as a promising tool to assess the presence of hydrophobic organic contaminants (HOC) in water, sediment and biota, such as polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs) or polychlorinated biphenyls (PCBs). Previous work evaluated the ability of passive samplers to predict bioavailability of sedimentary HOCs mostly in the laboratory, often for marine organisms. The current study assessed the use of low density polyethylene (LDPE) to derive freely dissolved concentrations of PCDD/Fs and PCBs in porewater *in situ* versus *ex situ*, and in river water. The LDPE-based multisampler system was deployed at four locations along the lower Passaic River (NJ) in sediment and water column, where sediment and benthic species samples were also collected. Good agreement was generally observed for PCDD/F and PCB concentrations comparing *in situ* and the *ex situ* approaches (within 0.30 – 39%). Significant linear relationships were observed between log LDPE based – log lipid-based concentrations of PCDD/Fs and PCBs. Porewater passive samplers yielded better correlations than those obtained by using sediment OC,OC + BC or riverwater to predict lipid-based concentrations. This implies that the accumulated amounts of HOCs in LDPE sheets deployed in sediments are better predictors of lipid-based concentrations of HOCs and that the proposed porewater *in situ* LDPE based sampler could be effectively used to predict the bioaccumulation of HOCs in biota, or at least be used as a screening tool to estimate the potential for bioaccumulation.

3.24.5
Aquatic system pollution: A long way to reach water sustainability in developing countries Case study of Kinshasa (RD Congo)

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Anthropogenic activities are responsible of the introduction of an increasing number of contaminant in adjacent aquatic systems by the inflow of effluents from various origins such as municipal and industrial treated wastewater or urban/agricultural runoff. This contamination represent a major threat to human health and aquatic organisms as contaminants can return to the food chain. The situation is particularly alarming in developing countries, as freshwater is a major public resource. A large proportion of the population suffering of social and economic difficulties broadly use highly contaminated surface water, shallow wells and borehole for irrigation, domestic and drinking purposes. We propose to assess the impact of anthropogenic activities on the distribution of pollutant in aquatic systems of Kinshasa. 15 wells and 4 rivers (3 sampling point per river) were sampled for water and sediment in Kinshasa vicinity, Republic Democratic of the Congo. Samples were subjected to micro pollutant analysis (toxic metals, polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), poly brominated diphenyl ethers (PBDEs) and polycyclic aromatic hydrocarbons (PAHs)), bacterial contamination detection (*E. coli*, *Enterococcus* and *Pseudomonas spp.*) and antibiotic resistance gene detection and quantitation (ESBLs and carbapenems). The study reveals a high contamination of the studied aquatic systems by micropollutants including toxic metals (particularly Cr, Cu, Zn, and Hg), PCBs (Σ7PCB: 11.05 – 169.29 µg kg^{-1}), PAHs (Σ13PAHs: 128.71 - 2256 µg kg^{-1}), OCPs (Σ6DDTs: 1.23-270.4 µg kg^{-1}) and PBDE (Σ6PBDEs: 1.48 – 27.9 µg kg^{-1}); the contamination of wells (managed and unmanaged) and rivers by faecal indicator bacteria (FIB) and the dissemination of extending β-lactamases resistance and multidrug resistant bacteria in rivers. The results demonstrates the strong dissemination of various pollutants including toxic metals, PAHs, PCBs, PBDEs, OCPs, faecal indicator bacteria and antibiotic resistance genes by probable multiple diffuse pollution sources including the absence of solid waste regulation, open defecation and inadequate sewage collection near the site studied.

In most developing countries, rivers serves as a basic network for human and animal consumption as well as irrigation for fresh urban produces. The high level of pollutant indicate the human and environmental potential risk and the need to develop an efficient surveillance and protection of surface water.

3.24.6
Monitoring and modelling antibiotic resistance in a Southeast Asian river catchment
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The global increase of antimicrobial resistance (AMR) is among the greatest threats to human, animal and environmental health. The prevalence of AMR in rivers, including antibiotics, resistant bacteria (ARB) and genes (ARG), is greatest in low-and-middle-income countries (LMICs), where antibiotics are used less prudently and waste management is inconsistent. However, predicting AMR exposure is difficult because monitoring programmes rarely include antibiotic, ARB or ARG indicators. As such, an urgent need exists to characterise AMR in rivers in LMICs, both to define current conditions, but also to develop models for predicting conditions with limited data. This research combines new monitoring data with a hydrologic-AMR tracking model to predict AMR exposures in catchments in LMICs. This model can help local stakeholders to identify optimal interventions to reduce the health risk of AMR in rivers, including engineering and social options. Water samples were collected at eight locations during two seasonal campaigns across a rural-to-urban Malaysian river catchment. Analyses included standard water quality parameters, stream flow, antibiotic concentrations, culturable resistant bacteria, resistomes and microbiomes. A model using HSPF BASINS was developed to simulate AMR transport in the catchment. Monitoring showed culturable ESBL and carbapenem resistant coliform counts increase by two and one log10 respectively, as one moves from rural to urban parts of the catchment, although side tributaries caused spiked increases at intermediate locations. ARG and antibiotic concentration data suggest a similar trend with AMR pollution accumulating along the river. In the catchment, 13/22 monitored antibiotics were detected, with amoxicillin and ciprofloxacin exceeding predicted no effect concentrations for resistance selection. This was most notable during the dry season, where significantly higher antibiotic concentrations were observed compared to the wet season. Statistical comparisons of the collected data with national sampling data show river dissolved oxygen level is a good surrogate for predicting AMR exposure. As such, dissolved oxygen was used to model AMR transport in HSPF, which is being tested against new AMR data.

Assessment and Management of Wastewater Effluents

4.01.1
Assessing Risk to Sewage Treatment Plants from Upstream Chemical Spills: A Combined Screening/Modelling Management Approach
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The microorganisms utilised in the biological purification step of municipal sewage treatment plants (STPs) are exposed to a wide range of chemicals emitted into wastewater from industrial facilities upstream. There are several known cases where industrial down-the-drain spills of large amounts of chemicals have led to toxic shock of the microorganisms at the recipient STP. Toxic shock events can result in a prolonged (on the order of weeks to months) decrease in efficiency or complete inhibition of the pollutant removal typically affected by the microorganisms. This work outlines a methodology for identifying upstream facilities that use chemicals which if spilled—based on their amounts and toxicities—pose a risk to the purification capabilities of a recipient STP. We use the Käppala STP near Stockholm, Sweden as a case study. Data on the maximum amounts of industrial products held in stock were collected for 59 facilities upstream of the Käppala STP. To estimate chemical toxicity, predicted no-effect concentrations for STPs (STP PNECs) were web scraped from European Chemicals Agency 'Brief Profiles'. Valid use data and STP PNEC values were obtained for 1503 industrial products comprising 5121 constituent chemicals (1275 and 554 unique products and chemicals, respectively). Chemical concentrations were estimated for the activated sludge regions of the STP in a high-end spill scenario assuming all chemical in stock was spilled and arrived at the STP undiluted. Risk quotients were derived using the STP PNECs. In this scenario, 25 industrial products contain one or more chemical which, if spilled into the STP, pose a risk value >=1 for the smallest region of the STP, while 2 products show risk values >=1000. This high-end spill scenario provides a priority list of upstream customers and industrial products that can be used to mitigate the risk to the Käppala STP from spills of these substances. The steady-state, non-equilibrium STP chemical fate model SimpleTreat 4.0 is being modified for use in simulating the transient chemical influx scenarios under investigation here, and thus provide refined risk estimates for the high-end spill scenario. This risk assessment framework will be provided as an open access tool intended to help STP staff identify high-risk facilities and facilitate managing the potential for spills of chemicals of concern.

4.01.3
The use of bioassays to evaluate the efficiency of a reactor treating wastewater containing pharmaceutical compounds and LAS surfactant
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The presence of wastewater and emerging compounds have commonly been detected in Brazilian streams and they cause human and environmental concerns. Bioassessment is a useful tool to evaluate the efficiency of treatment, being among the requirements demanded by environmental agencies. This study analyses the impact of the hydraulic retention time (HRT of 36 hours - FI phase and 20 hours – FII phase) in the toxicity of the treated effluent (wastewater containing ibuprofen, triclosan, propranolol, diclofenac and LAS surfactant), that runs in São Carlos, Brazil. We applied bioassays to determine the toxicological effects on the survival of aquatic organisms. For this purpose, we exposed the organisms *Allonais inaequalis* and *Daphnia magna* into acute test using non-treated wastewater, treated effluent and some dilutions of them. The organisms are maintained in the Ecology of Aquatic Environments Laboratory (LEAA) at University of São Paulo and the effluents were obtained from the Wastewater Treatment Plant, being the raw effluent (municipal sewage after preliminary treatment) and the treated effluent coming from a pilot-scale fluidized reactor. Organisms were exposed to both effluents in three replicates per treatment and the exposure was performed at temperature of 25ºC and 12:12 darkcycle to *Allonais inaequalis* (tropical organism) and 19ºC and 16:8 darkcylcle to *Dapnhia magna* (temperate zone organism). The removal efficiency of the fluidized reactor was 90% (FI) and 88% (FII) to dissolved organic carbon and a minimum of 28% (FII) and a maximum of 85% (FII) to the drugs and surfactant. Regarding aquatic toxicity, we observed acute effective concentration on the survival of 50% of the organisms (EC50) decreasing from 70.77% dilution (FI) to 27.60% dilution (FII) for *Allonais inaequalis*. *Daphnia magna* was more sensitive to effluents, presenting EC50 of 23.56% on FII. However, despite the decreasing toxicity of the untreated effluent, these samples continue presenting a toxic capacity to contaminate water resources if discharged directly into the rivers, demanding improvements in reactor operating conditions or addition of a subsequent treatment to ensure aquatic life quality. Our results suggested that ecotoxicological tests are effective parameters to evaluate the impact of an effluent on aquatic biota and improve the treatment applied.

Assessment of Chemical Mixtures and Multiple Stressors: From Additivity and Synergy to Policy Options

4.02.1
Field species sensitivity distributions of complex mixtures can be derived, elucidate taxon-specific impact thresholds and provide latitude to improve polices
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Bioassessment is the art to diagnose environmental pressures from biological observations and is done to support protective or restorative management decisions. It has recently become feasible to also use bioassessments to diagnose effects of chemical mixtures based on monitoring data. We collected such data from Dutch waterboards and European data repositories, followed the bioassessment ideas, and derived field species sensitivity distributions (field-SSDs) for mixture exposures. That is, we derived taxon-specific thresholds of chemical pollution impacts for a suite of taxa, indicating the mixture exposure level above which taxon-abundance changes occurred. Because "all animals are unequal", the taxon-specific thresholds were unequal – which delivered us the field-SSD of mixture exposure. We discuss the findings in relation to chemical safety assessments (e.g., as under REACH), life cycle impact assessment (e.g., as in the EU-product environmental footprint) and especially environmental quality protection, assessment and management (e.g., as under the EU-Water Framework Directive). As yet, the evidence shows that the net degree of protection and restoration aimed at has not delivered us the ideal of the EU-policy goal non-toxic environment.

4.02.2
Ten years of mixing cocktails: a systematic review and quantitative re-appraisal of mixture interactions
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ECVAM; A. Kienzler, European Commission - Joint Research Centre / DG Joint Research Centre IHCP EURL ECVAM; N. Parissis, European Commission Joint Research Centre; A. Kortenkamp, Brunel University London / Institute of Environment, Health, and Societies

We present the outcome of a systematic review and quantitative reappraisal of 10 years' of experimental mixture studies. Unlike previous reviews, we did not limit our effort to certain groups of chemicals or specific toxicity outcomes. We covered ecotoxicological as well as human / mammalian mixture studies published since 2007. We created an inventory of 1,220 mixture experiments from which data were extracted and subjected to subgroup analyses. We developed an approach that allowed us to examine the internal validity of mixture studies by using a risk-of-bias tool tailored to mixture toxicology. For a subset of 388 entries that claimed interactions or provided indications for an interaction, it was possible to conduct a quantitative reappraisal of authors' evaluations of mixture effects. Strikingly, our reappraisal revealed that relatively few claims of synergistic, antagonistic or interactive effects stand up to scrutiny in terms of what we defined as regulatory relevance. In most cases, deviations from expected additive mixture effect doses (concentrations) were not larger than two-fold in either direction. A small proportion of experiments reported synergisms that we appraised as of regulatory relevance. Our efforts of pinpointing factors that might predispose to synergistic interactions did not reveal anything substantial. There were no clear trends when we analysed interactions in terms of the chemical composition of the mixtures. However, previous indications that synergisms are particularly frequent e.g. among pesticide combinations were confirmed. Our results confirm the utility of default application of the dose (concentration) addition concept for predictive assessments of simultaneous exposures to multiple chemicals.

4.02.4

Invertebrates at risk in agriculturally influenced Swiss streams

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Small streams are important ecosystems for macroinvertebrates and make up to 75% of the Swiss river network. Of those streams 14% of the flow length have >10% of agricultural land in their catchment area. It is necessary to assess the effects and risks of agricultural micropollutants pose to the macroinvertebrate community of these ecosystems. We conducted a comprehensive water quality assessment in 5 small streams over a period of 8 months involving highly resolved continuous chemical monitoring, measurement of acute and chronic effects of water samples on *Ceriodaphnia dubia*, calculations of the predicted mixture toxicity for daphnids using toxic units (TU), mixture risk assessment for the invertebrate community based on Environmental Quality Standards (EQS) and TU, as well as the evaluation of the macroinvertebrate community composition using the SPEAR-Index. Native stream water samples caused *C. dubia* mortality in 22% and reduced reproduction in 28% of samples tested. Acute and chronic sum of TU of the stressor mixtures (pesticides, metals and sulfate) for daphnids explained effects or no occuring effects observed in bioassays in 69% of cases. Observations in 31% of samples were not explained by TU, likely due to chemicals not measured or below detection limits. The TU approach further revealed that elevated sulfate concentrations were responsible for most of the observed toxicity measured at one experimental site. Metals were driving mixture toxicity to daphnids at the other 4 sites. Both mixture risk assessment approaches indicate a high acute and chronic risk for invertebrates. The determined SPEAR indices indicate also that Invertebrate communities were affected by pesticides at all sites investigated. The applied three different lines of evidence indicated an elevated risk to invertebrate communities in agriculturally influenced small streams. Bioassays proved to be a valuable tool that can integrate the combined effects of present chemical stressors and gives evidence about chemically not analyzed substances. Without consideration of metal concentrations, the toxicity observed in daphnid bioassays could not have been explained to the same extent. On the other hand, the influence of the naturally occurring sulfate at two sites has confounded the assessment of the pesticides. A mixture risk assessment in combination with bioassays is the way to obtain a realistic estimation of the duration and intensity of stressors for organisms in streams.

4.02.5

Impacts of pollution, global warming & an invasive predator on the survivorship and reproduction of a model gastropod, Lymnaea stagnalis

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Human activity is driving biodiversity loss at an alarming rate. Exposure of ecosystems to environmental stressors (factors that disrupt the dynamics of an ecosystem, population or organism) is becoming more prevalent as a result of human activity. It is highly likely that an ecosystem is exposed to different stressors. Therefor it is important to understand how different stressors impact different life-history traits of an organism. Here we assess effects of environmental stressors (global warming, pollutant mixture and invasive predator) using the great pond snail, *Lymnaea stagnalis,* which has been demonstrated as an effective model species for environmental and toxicity tests. Survival, growth and reproductive outputs were observed throughout the experimental period (122 days from hatching), across five treatment levels at environmentally relevant exposures for each individual stressor. Results from these single stressor studies have shown significant effects on the all observed endpoints. Firstly, hatching began earlier with increasing temperature however fewer eggs hatched at the highest temperatures (26°C and 28°C) compared to the control (20°C). Snails reared at intermediate to high temperatures (24°C, 26°C and 28°C) were consistently larger throughout the experimental period compared to the control. There was no difference in the number of egg masses produced between the control and temperatures up to 26°C, however fewer egg masses were produced at the highest temperature (28°C) despite egg production being correlated to body size. The pollutant mixture did not affect the time to hatch, however fewer eggs hatched at the highest concentration (50µg/l). Additionally, snails in the 50 µg/l treatment were smaller, produced fewer egg masses and had a higher mortality rate. Data collection for the invasive predator stressor is still ongoing and due to be completed by 15th December. Findings from this study will provide crucial information on how different stressors impact various life-history traits in aquatic organisms throughout the full life-cycle when applied at environmentally relevant levels.

Bioaccumulation and Biotransformation: Advances, Challenges, and State of the Science for Chemicals Regulation

4.03.1

A Novel Multi-Species Physiologically-Based Toxicokinetic Modelling Approach in Support of Chemicals Risk Assessment

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One of the greatest manmade threats to this planet is chemical pollution. Chemical risk assessment helps to understand the impacts of chemicals on the environment and supports the implementation of appropriate management strategies to minimize these impacts. A cornerstone of chemical risk assessment are standardized toxicity data which are generated in laboratory experiments with model species. However, model species are not necessarily representative of native species of concern in an ecosystem. A wealth of data from non-model species is available in the scientific literature but cannot be utilized in risk assessment because the data were not derived in compliance with test guidelines. Therefore, approaches are needed that enable inclusion of these data, such as models, that enable transposing these datasets into a format that is useful in risk assessment. One class of models that facilitate extrapolation between exposure conditions, chemicals and among species are physiologically based toxicokinetic (PBTK) models. Contemporary PBTK models for the prediction of bioaccumulation potential of chemicals in fish are based on single-species approaches. These approaches fail to include naturally occurring physiological variability between different species and among individuals of the same species. To overcome this limitation a novel multi-species approach is introduced in this study. By including inter- and intra-species variability of model input parameters through (i) the available physiological data of nonmodel species, and (ii) the incorporation of these data and their statistical distributions rather than as single values, this study developed a new and powerful multi-species PBTK modelling approach. In an extensive literature search, 2,777 single values were revealed, representing 71.9 % of families of freshwater fishes occurring in Canada. Model validation showed that bioaccumulation potential of 82 % of the modeled neutral organic chemicals were predicted within a 10-fold change respective to the corresponding measured data from the literature. This is in reasonable agreement with previously published single-species models while at the same time significantly improving the level of species diversity. As such, this model will potentially enable more environmentally relevant predictions using already existing data and could ultimately lead toward more sustainable use of existing data for risk assessment of chemicals.

4.03.2

Is additional complexity in models for BCF prediction worth the efforts?

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One approach to assess a chemical's bioaccumulation potential that requires less animal use is the prediction of bioconcentration factors (BCFs) using *in vitro* biotransformation data. For this purpose, differently complex BCF prediction models can be used: simpler models that need less input information and are easy-to-use or more complex models that represent the *in vivo* reality more accurately. However, these more complex models often require additional input data, have a more complicated structure and might thus be more difficult to apply. We evaluate whether this additional complexity is worth the efforts by comparing the results of a complex modeling approach with those of a much simpler modeling approach. We either used the simplest model possible, i.e. a pure one-compartment model that does not account for any limiting factors, or a multi-compartment model that represents the biotransforming organs (liver, GIT and gills) as separate compartments. By this, the multi-compartment model does not only represent blood flow limitation, but also considers the direct blood flow from GIT to liver and is able to represent the potential first-pass effect in the gills. For the calculations, we chose physiologically relevant parameter values for rainbow trout and hypothetical test chemicals with log K_{OW} ranging from 4 to 8. Comparison of the results of both models shows surprisingly similar results for most cases. For regulatory purposes, however, the use of the simple one-compartment might be critical because it is not a worst-case estimate due to the neglect of blood flow limitation.

4.03.3
QSAR prediction of in vitro biotransformation in human and rodents

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In the last 20 years, our research group has developed many validated Quantitative Structure Activity Relationships (QSARs) to estimate different physical-chemical properties and biological activities of organic chemicals and contaminants of emerging concern. Our most recent models focus on the prediction of the biotransformation potential of heterogeneous organic chemicals in multiple organisms. These models are particularly relevant in the context of chemical hazard and risk assessment, and in particular for the identification of potentially bioaccumulative chemicals. Recent studies have demonstrated the necessity to integrate biotransformation related information for the refinement of the bioaccumulation assessment. Reliable toxicokinetics data are also required to apply *in vitro* bioactivity (hazard) data in risk-based contexts. To this end, it has been suggested that data from *in vitro* assays may provide biotransformation rate data that can be scaled using in vitro-inviivo extrapolation (IVIVE) models to refine bioaccumulation estimates.Therefore we have developed new Multiple Linear Regression QSAR models for the prediction of intrinsic *in vitro* clearance measured in human and rodents liver medium. The chemicals were grouped according the most likely reaction pattern to developed specific models for each possible reaction. These models are now available in the QSARINS-Chem software, and easily applicable to generate predictions for new and existing chemicals, in the respective structural applicability domain. The software is freely downloadable from the internet (http://dunant.dista.uninsubria.it/qsar/). This work demonstrates how QSARs can be easily applied to profile the potential *in vitro* biotransformation of chemicals with the support of the QSARINS-Chem software, which allows for further analysis of the reliability of pedictions in the domain of the original models. These QSARs and the QSARINS-Chem software may be helpful to improve bioaccumulation assessment. In addition they can also be used to estimate *in vitro* biotransformation rates that further extrapolated to *in vivo* activities by *in vitro-in vivo* etrapolation (IVIVE) models.

4.03.4
Food web on ice: A pragmatic approach to investigate the trophic magnification of chemicals of concern

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The trophic magnification factor (TMF) may be considered the 'gold standard' of bioaccumulation assessment for a chemical since it integrates bioaccumulation processes over an entire food web and under realistic environmental conditions. Thus, TMFs could be useful for the regulatory risk assessment of substances of concern. Currently, it is also discussed to use TMFs in the context of the European Water Framework Directive (WFD) to apply them for the derivation of Environmental Quality Standards (EQSs) for priority substances, or to normalize biota concentrations of chemicals from different species to one common trophic level for EQS compliance testing. Although TMFs for numerous chemicals have been derived in the past twenty years, a standardized approach for TMF studies is still lacking. To allow the evaluation of the advantages, benefits and pitfalls of the TMF concept, field samples were retrieved from Lake Templin near Potsdam, Germany, in 2018. All samples were frozen after sampling, cryomilled and kept stored under cryogenic conditions, similar to the protocols applied for the German Environmental Specimen Bank (ESB), to generate samples forming a food web on ice. This set comprising plankton, mussel and fish samples gives the opportunity to analyze the trophic magnification of multiple chemicals, but also to investigate general aspects of biomagnification within the same material without performing additional samplings. For the food web samples bulk stable isotope signatures (for trophic level calculations), lipid content of samples (for normalizing of biota levels of lipophilic compounds) and other parameters were determined. Analyses of benchmark chemicals (e.g. Hg/MeHg, PCBs) serve as proof of concept for the biomagnification in the sampled food web. It is currently under investigation whether the TMFs derived from the sampled food web are reliable and comparable to data from similar ecosystems.

4.03.5
A tiered testing strategy for rapid estimation of bioaccumulation via modelling and in vitro data

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The overall objective of this research is to reduce uncertainty related to the estimation of toxicokinetics and bioaccumulation of organic chemicals in fish in ecological risk assessment. The propensity of chemicals to accumulate in biota is of high concern because it may result in significant internal exposure in organisms, potentially leading to toxicity, and be a source of chemical exposure to organisms higher in the food chain. The traditional in vivo test with fish to derive accumulation data, expressed as bioconcentration factor (BCF), is not only ethically questionable but also excessively resource intensive relative to the number of chemicals in need of testing for BCF. Thus, alternative strategies are being sought, with the most developed strategy being to use computational models in which fish liver S9 or hepatocyte-derived biotransformation rates are incorporated to predict an in vivo BCF. In a CEFIC-LRI project (Eco34), we asked whether biotransformation rates from extrahepatic tissues derived by additional in vitro models, along with a critical evaluation of model complexity, would further reduce in the uncertainty of BCF predictions. We first derived at a list of nine candidate chemicals for in vitro testing based on model discrepancies, availability of reliable in vivo BCF and BMF data and in vitro biotransformation rates. The resulting chemicals were divided into three Kow categories: group I: log Kow < 4 (aqueous exposure dominates – gill and liver models); group II: log Kow 4 - 5.5 (mixed exposure routes – gill, liver and intestine models); and group III: log Kow>5.5 (predominantly dietary exposure dominates – liver and intestinal models). For in vitro testing, biotransformation rates, determined as parent compound loss over time, were determined in the following rainbow trout (Oncorhynchus mykiss) – derived models: (a) primary gill cell cultures grown on permeable support; (b) S9 fractions from liver and intestine; (c) three permanent cell lines respresenting gill (RTgill-W1), liver (RTL-W1) and intestine (RTgutGC). All in vitro systems proved capable of biotransformation though not all test chemicals were biotransformed. The primary gill cell system and the RTgill-W1 cell line were comparable with regard to the resulting clearance rates, with gills being overall less efficient in clearance compared to liver and intestinal cells. Biotransformation of acenaphthylene was below the limit of detection in the gill cell models but was cleared from the liver cell line. Deltamethrin and 2,4,6-tribromophenol were biotransformed in the intestinal S9 but not in liver S9 or any of the cell based models. Our data demonstrate an overall agreement for tissue-specific biotransformation rates derived from different in vitro models but also provides indication for tissue-specific clearance. Extrahepatic clearance was important. Incorporation of these experimental data into different computational models is ongoing and will be used to evaluate needed model complexity and improvement of BCF predictions.

4.03.6
Lipid Normalizing the Biomagnification Factor

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The biomagnification factor (BMF) of a chemical in fish is a bioaccumulation metric that currently is receiving much attention in regulatory circles (Hashizume et al. 2018). One of the challenges in using the BMF for bioaccumulation assessment is the apparent variability in BMF measurements. Hashizume et al. (2018) illustrated substantial variation in the biomagnification factors of

Hexachlorobenzene (HCB) in common carp and that lipid normalization of the concentration of the chemical in the diet to a diet with a 5% lipid content ($BMF_{L,5\%}$) reduced this variability. This approach, which is different from that included in the OECD 305 guidelines for bioaccumulation testing has received much interest. In this study, we investigate this alternative method of lipid normalization on (i) bioaccumulation theory; (ii) bioaccumulation testing in the laboratory using dietary bioaccumulation test results from OECD-305 style tests for 238 test chemicals; and (iii) bioaccumulation in the field using biomagnification and trophic magnification factors determined in field studies. In 19 dietary bioaccumulation tests for HCB (excluding Hashizume et al. (2018)), the BMF_L ranged between 0.61 kg lipid/kg lipid to 2.8 kg lipid/kg lipid with 11 BMF_L values exceeding 1. In contrast, the $BMF_{L,5\%}$ ranged between 0.16 to 0.72 kg lipid/kg lipid and between 0.15 and 0.27 kg lipid/kg lipid in the study by Hashizume et al (2018) using non-growth corrected $BMF_{L,5\%}$ values. Also, in the dietary tests investigated, the BMF_L identifies 35 chemicals with biomagnification potential, while the $BMF_{L,5\%}$ only identifies 12 chemicals to have a biomagnification potential. Chemicals for which the BMF_L was greater than 1 and the $BMF_{L,5\%}$ was less than 1 include PCBs 118, 187, 195, 209, mirex and HCB. We conclude that biomagnification models and data clearly illustrate that biomagnification factors are a function of the lipid content of the diet. Differences in the biomagnification factor of chemicals due to differences in the lipid content of the diet are therefore not experimental artefacts or error but reflect the fact that biomagnification factors are greater if the chemical is present in food with a higher lipid content. Normalizing the biomagnification factor to a dietary lipid content of 5% tends to underestimate the biomagnification potential of lipophilic substances in aquatic food-webs by a considerable margin. The use of the lipid normalized biomagnification factor (BMF_L) appears to provide more realistic estimates of biomagnification in the field than the $BMF_{L,5\%}$.

Bioremediation and Phytoremediation of Contaminated Ecosystems

4.04.1
Green roofs in Milan metropolitan area: runoff water quality and quantity
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Green roofs provide many ecosystem services in urban areas, such as thermal insulation of buildings, reduction of stormwater runoff, mitigation of heat island effect and noise pollution, aesthetic improvement. A less investigated aspect is the contribution to the reduction of urban runoff pollution by absorbing pollutants deriving from stormwater. An experimental research in two areas, North and South Milan (Brugherio and San Giuliano Milanese), was carried out to test the potential of this technology in a heavily impervious metropolitan area. A total of 24 different green roof arrangements (each with a 0.38 m^2 surface area and 2 replicates, for a total of 25.5 m^2) were equipped by combining the following characteristics: substrate type (3 types, i.e. 2 different soil types with about 5% organic matter, and type 2 enriched with organic matter up to 10%), substrate depth (8 cm vs. 12 cm vs. 15 cm), vegetation type (*Sedum* spp. vs. grassfield vs. no vegetation), slope (2% vs. 10%), fertilization (no fertilization vs. fertilization). The performance in retaining stormwater, analyzed between May 2018 and September 2019, resulted in 73% runoff reduction. Macro- and micro-element, nutrient and PAHs concentrations and loads in green roof runoff were compared with those of stormwater in three precipitation events. In November 2019 and April 2019 events, experimental green roofs retained 88% of $N\text{-}NH_4$ deriving from stormwater, as well as 10%-35% of Zn, Ni, Pb, Se, Co e Cd. Vegetation and substrate type resulted as key factors determining the capability to retain part of precipitation and pollutants. In the winter event (February 2019), retention capability was strongly reduced, probably due to inactive vegetation and frozen substrate, except for PAHs (60% retention) probably due to the absorption on the organic fraction of the substrate. However, green roof runoff was characterized by a strong enrichment in suspended solids (30±40 mg/L), DOC (13.3-55.3 mg/L), nutrients such as TP and TN, cations as K e Mg, macro- e micro-element such as Al, Fe, Mn, Ba, Sr e Cr, leached from substrates, fertilizers or drainage systems. If correctly designed and managed, these systems may be an effective adapting strategy to climate change, achieving a strong capability to reduce stormwater annual runoff in impervious areas. However, materials and arrangements need to be correctly chosen to reduce significantly pollutant loads.

4.04.2
Phytostabilization of heavy metal contaminated soils in urban and rural communities in Wisconsin, USA
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Reducing human exposure to lead (Pb) through ingestion and/or inhalation of soil-Pb is important in urban and rural communities. Given widespread soil Pb contamination from paint, gasoline, and mining operations, typical remediation practices, like excavation and soil capping, are not cost-effective and remediation needs outstrip community resources. Alternative methods of reducing Pb-exposure are needed. Phytostabilization, the immobilization of soil contaminants by rhizosphere processes, is suitable for reducing bioavailable lead and lowering overall Pb exposure risk. As differences in contaminant source and land use drastically impact exposure scenarios, site-specific investigations are needed to ascertain phytostabilization viability. Southwestern Wisconsin is home to a historic lead and zinc mining district (1820-1950) where local populations concentrated around mining operations and are still threatened by mine tailings that persist in local soils today. Milwaukee, U.S.A., like most urban areas, has widespread soil-Pb from Pb-based paint and leaded gasoline. This research explores 1) the establishment of native prairies in mine-scarred agricultural land to restore ecosystem services and 2) the effectiveness of a Pb-pyromorphite forming turfgrass in urban soil to reduce Pb human bioavailability. Prairie forbs and grasses native to Wisconsin were direct-seeded in mine impacted soil for a greenhouse study, as well as direct-seeded and transplanted into a mine-scarred field site amended to investigate improved plant growth. Lime (2.94 kg/m2), manure (3% wt/wt), and biofertilizer (Trichoderma sp.) were applied in a split block design with native plant species grown in each soil amendment type. Milwaukee Pb-contaminated soil and Pb-spiked Plano soil (600 mg kg-1) were direct-seeded with 3 turfgrasses, one known to induce Pb-pyromorphite formation (Agrostis sp.). For 7 weeks, weekly treatments of Ca(NO3)2 and (NH4)SO4 (4.88 g N/m2) were added to induce alkalization and acidification, respectively, as these processes are hypothesized to induce Pb-pyromorphite formation. Plant shoot and root metal concentrations from both studies will be determined. Mehlich 3, Relative Bioaccessible Lead Protocol, DTPA, and X-ray florescence will determine soil metal bioaccessibility to plants and humans. Results will reveal methods suitable for phytostabilization in mine soil and urban contaminated areas.

4.04.3
Managing water evaporation and trace element accumulation using tree species at a red gypsum landfill
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Nowadays, the mining companies are increasingly confronted with problems related to manage contaminated sites and soils. The red gypsum landfill of Thann presents environmental and ecological context representative of the typology by-products storage of Ti extraction activity (high content of some Trace Element (TE): Fe, Mn … and low biodiversity). Moreover, this red gypsum is stored on a waterproof structure that requires appropriate management of the incoming and outgoing water masses. The aims of this study was to evaluate the impact of revegetation on the TE and the water status for red gypsum landfill. Our hypothesis is that the selection of tree species for TE accumulation capacity would not be antagonist of the selection of species with high transpiration rate. The opportunity of the 5 year-old demonstration plantation (18 tree species) was used to screen transpiration and TE accumulation in leaves (n=9 by species). For estimating the leaf area, we used remote-sensing airborne drone. A pot experiment with the anthroposol recovered from the study site, was initiated in the growth chamber at the laboratory for confirming the transpiration rate of the whole plant (by weighing lost method), and to measure Mn. In the field, there was a significant difference in maximum stomatal conductance between tree species, *O. carpinifolia, M. pomifera,* and *R. copallina* had the highest stomatal conductance. Regarding the Mn concentration in the leaves, *O. carpinifolia* and *B. pendula* had significantly higher levels than other tree species. The relationship between stomatal conductance and Mn content showed that tree especies that had a high Mn content, had high stomatal conductance (*O. carpinifolia*, *B. pendula*, *S. aquatica grandis*, *S. alba*) excepted *R. copallina*. The tree species with the lowest stomatal conductance have also low concentration of Mn. The results of the pot experiment reinforced the field results. *O. carpinifolia* transpiration rate was significantly higher than for other tree species tested. Although while *B. pendula* had intermediate transpiration, it accumulated the highest leave Mn content (1000 mg/kg DW). *O. carpinifolia*, *S. aquatica grandis*, *M. pomifera*, and *U. pumula* are the species that have the highest canopy volume. They showed higher growth performance than other tree species. The selection of tree species with hight transpiration capacity, would be therefore combining a high leaf surface area and a high maximum stomatal conductance.

4.04.4
Leverage Effect Of The Relationship «Belowground-Aboveground» For The Rehabilitation Of Contaminated Marginal Lands
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It is well known the existence of ~1,350,000 hectares of land in Europe deemed less favourable for conventional agriculture. Nowadays, these soils are identified as "marginal lands" which include contaminated and degraded soils, such as trace element (TE) contaminated sites. To overcome the problem of poor fertility of

these soils biofertilization seems a promising way. It consists in improving the soil properties by inoculation of biological species (earthworms (EW), fungus, etc.) in soils in order to promote the recycling of nutrients. It is however necessary to quantify both the positive and negative externalities induced by this practice on the development of non-food biomass crops. The aim of this study was to highlight the soil-plant-EW interactions in the case of a poorly in situ contaminated soil, with the dual objective of 1) determining and prioritizing the biotic and abiotic parameters that influence these interactions, and 2) assessing the importance of the belowground-aboveground relationships on the sustainability of certain ecological functions provided by soils. A pot experiment in controlled conditions was carried out with a Cd, Zn, and Cu contaminated sandy soil during 2 months. Pot experiments included treatments with or without EW (*Aporrectodea caliginosa*) and with or without plant (*Lolium perenne*). For each condition, 6 replicates were established and when needed, 30 seeds and/or 10 EW per pot containing around 2kg of soil were inoculated. Environmental TE content was quantified by measuring TE concentrations in soil solution and environmental bioavailability by measuring TE concentrations in depurated whole EW bodies and plant aerial biomass. Finally, toxicological bioavailability was investigated by measuring EW survival rate, body weight changes, plant biomass and leaf chlorophyll concentration at the end of the experiment. In general, although EW presence increased the availability of all the studied TE, Cd and Zn plant uptake was reduced and plant biomass increased when compared to the condition without EW. Moreover, the TE bioaccumulation in EW did not produce a direct toxicity, according to the EW survival rate and body weight results. Finally, our pot experiment confirmed that even in contaminated soils, the presence of *A. caliginosa* promotes plant adaptation, reduces TE uptake and improves biomass production. However, further studies are required to establish potential toxicity due to the accumulation of TE in EW and plants at long-term.

4.04.5

Assessment of compost and biochar in improving plant-assisted bioremediation of a multi-contaminated area

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Nature-based solutions for recovering contaminated areas, such as plant-assisted bioremediation strategies, are cost-effective, long-lasting and aesthetic. The effectiveness of a plant-assisted bioremediation strategy for remediating soils from pollution is affected by physical/chemical soil characteristics, plant species, bioavailability and biodegradability of contaminants. Many works proved that the application of soil fertilizers (e.g. compost and biochar) is an effective agronomic practice to promote rehabilitation of soils contaminated by heavy metals (HMs) or xenobiotics, such as polychlorinated biphenyl (PCBs). The main goal of this research was to assess the capability of two different fertilizers (a municipal solid waste compost and a biochar obtained from the pyrolysis of plant biomass) in enhancing remediation processes of a multi-contaminated site in Southern Italy, in which a plant-assisted bioremediation strategy has been applying since four years. On 2015, about 750 *Monviso* poplar cuttings were planted to remediate a heavy metals (HM) and polychlorinated biphenyls (PCB) contaminated area, located close to Taranto city. In March 2019, a compost or biochar treatment was performed on six different contaminated plots, selected in the poplar-treated area. The results of the chemical and microbiological analyses carried out on soil samples collected in fertilizer treated and in un-treated plots (controls), before and 6 months after the fertilizer treatment are here reported. In particular, PCB and HM determinations and microbiological analyses (microbial abundance, viability and qPCR assays) were performed.

4.04.6

Application of in situ biorremediation strategies in soils amended with sewage sludges

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Actual soil loss and the scarcity of natural or agricultural lands require new strategies for reuse. In this sense, the recovery of polluted soils (for other uses) offers a chance for social and economic regeneration. With this purpose, several decontamination technologies/treatments have been developed. Most of them, employ physical and/or chemical technologies with high cost and big impact for the ecosystems. By contrast, biological technologies are more cost effective, but also more time-consuming. Among biological techniques microbial bioremediation, vermiremediation and phytoremediation are widely known technologies. However, although their individual remediation yields are known, little is known about the impact/influence of each technology upon the rest. In fact, remediation performances could be enhanced when different biological strategies are combined. In the present work combinations between microbial remediation, vermiremediation and phytoremediation were applied in a landfill (Landfill 17; employed as sewage sludge disposal point for, at least, two decades) located in Gernika-Lumo (Basque Country) in search of the treatment (single) or combination of treatments with best remediation yields. Thus, 7 experimental groups (with 3 replicates each) were obtained by introducing earthworms (E), bacteria (B), plants (P), bacteria +earthworms (B+E), bacteria+plants (B+P), plants+earthworms (P+E) and plants+bacteria+earthworms (P+B+E) in the experimental plot (in the Landfill 17). As well as a control group without treatment. To assess the efficiency of each treatment, a study of soil status was carried out before and after remediation, integrating both chemical and ecotoxicological approaches. The results showed dieldrin as the pollutant with the highest removal rates with degradations between 50% and 78% in all experimental groups. For heavy metals and BaP, removal rates of around 20-25% were achieved (Cd 15%-35%; Cr 7%-39%; Pb 15%-33%; Ni 24%-37%; BaP 19.5%-28%). Highest reduction in contaminant concentrations were obtained in P+E, B+E and P+B+E treatments. Ecotoxicological tests (especially in those carried out with worms and plants) pointed to P+B+E treatment as the one that provides best soil health among those treatments with better elimination yields . Best results were obtained with the combination of phytoremediation (P), bioaugmentation (B) and vermiremediation (E). Acknowledgements: Basque Gov (IT810-13; ITO18-16), CTM2017-87766-R, AGL 2015-64481-C2-1-R and AGL2016-76592-R from MINECO, PhytoSUDOE-SOE1/P5/E0189.

4.04.7

Poster spotlight: Hexachlorocyclohexane bioaccumulation in Alnus glutinosa - height, age, season and isomer specificity

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Complex Mixtures in User Products and the Environment: Chemical and Toxicity Profiling, and Modelling to Identify Risk Drivers and Estimate Footprints

4.05.1

Chemical fingerprints of WWTP effluents - performing a large-scale target analysis for LVSPE samples of 52 European WWTPs

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Most waterbodies in Europe are exposed to a large variety of chemicals, released by industry, traffic, during agricultural processes or simply in daily routine. These include categories such as pesticides, pharmaceuticals, personal care products, etc. The combination of different chemicals and the overlay of multiple tributaries lead to complex mixtures of substances, impeding statements on particular toxicities and biological effects in general. Simplifying and understanding the composition of waterbodies, like rivers and lakes, are therefore of special interest in the field of ecological and human health risk assessment. One approach is to subdivide these mixtures depending on their origin in order to obtain source related so-called “chemical fingerprints”. This study is focusing on wastewater treatment plants (WWTPs) representing important point sources for the anthropogenic impact on surface waters. A large-scale target screening was performed by investigating effluents of 52 European WWTPs from in total 15 different countries. Large volume solid phase extraction samples (LVSPE) were taken and analysed by liquid chromatography-high resolution mass spectrometry (LC-HRMS). An extensive target list of approx. 600 substances in combination with retrospective analysis of additional compounds resulted in a total of approx. 1200 target substances. The final results were evaluated using multivariate statistics. Several hundred individual chemicals have been identified and quantified in European WWTPs in concentrations up to the several hundred µg/L range. By clustering chemicals and sites, chemical fingerprints specific for different groups of WWTPs have been identified and evaluated in the context of the European region, size class and treatment technology of the WWTPs. To this end, it was possible to show the influence of additional treatment stages like ozonation. Component-based risk assessment using the toxic unit (TU) approach and multiple substance potentially affected fractions of species (msPAF) identified major risk drivers in the effluents and helped to group WWTPs according to environmental risks taking into account the dilution factor in the receiving waters. Finally, the present study reveals a detailed overview on the impact of WWTP effluents to contamination patterns in surface waters, allowing to define the most severe drivers of toxicity.

4.05.2

High levels of brominated dioxin-like activity discovered in plastic toys using a combination of in vitro Aryl hydrocarbon receptor-based reporter gene assays and GC-MS analysis

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While health outcomes related to the exposure to dioxins may vary (altered behavioral and cognitive functions, altered reproductive functions, cancer, etc.), the ligand-binding interaction of dioxins with the aryl hydrocarbon receptor (AHR) is a known and consistent molecular initiating event of dioxin-like toxicity. This relatively straight forward mode of action led to the development of sensitive and quantitative in vitro cell-based reporter gene methods like DR CALUX. The DR CALUX is a well established and validated method for sensitive quantification and reproducible hazard assessment of dioxins, dioxin-like compounds, and other AhR activators. It is known that the DR CALUX does not only respond to chlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) but also responds to their brominated counterparts (PBDD/Fs). There is now evidence that significant levels of PBDD/Fs can be found in a variety of matrixes such as plastics. Reported levels of PBDD/Fs indicate that they could potentially contribute to the daily human background exposure to dioxins. Although both PCDD/Fs and PBDD/Fs show similar molecular and health effects, the latter chemical group is significantly less regulated and PBDD/Fs are not listed under the Stockholm POP convention. Using the well-known rat-cell based AhR reporter assay, DR CALUX, and a human variant thereof in HepG2 cells (DR_{human} CALUX), we first determined relative potencies of various PBDDs and PBDFs congeners. All chemicals tested were active in both DR CALUX and DR_{human} CALUX assays. Then we evaluated dioxin-like activity in plastic toys which were also analyzed by GC-MS for PBDD/Fs content. In plastic toys, we found very high DR CALUX and DR_{human} CALUX bioanalytical toxicity equivalents (up to 6,880 pgBEQ/g), likely due to the presence of PBDD/Fs. Such levels suggest that the toys were potentially manufactured with plastic containing high-levels of PBDD/Fs, which could represent a potential major source of exposure of children to dioxins at a sensitive age.

4.05.3

Advancements in effect-based surface water quality assessment

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Traditionally, chemical water quality is determined by monitoring surface waters for the presence of priority substances. However, concentrations of priority substances in surface waters are decreasing, and industries have switched to a plethora of alternative compounds which enter the aquatic environment and seriously impact water quality. Consequently, a large portion of toxic effects observed in surface waters cannot be attributed to compounds measured by water authorities, and toxic risks to aquatic ecosystems are thus caused by mixtures of a myriad of (un)known, unregulated and unmonitored compounds. Understanding of these risks requires a paradigm shift, that allows for new monitoring methods that do not depend solely on chemical analysis of priority substances, but contrastingly consider biological effects first. Therefore, there is a need for effect-based monitoring strategies that employ bioanalytical tools to identify environmental risk. To answer this need, the Smart Monitoring project aimed to further develop and integrate time-integrative sampling, bioanalytical tools, and in-depth chemical analyses for application in surface water quality assessment. This platform presentation will summarize the findings and insights generated during four years of research, starting with an initial monitoring campaign that explored the potential of effect-based tools in water quality assessment. Subsequently, advancements in passive sampling, bioanalytical tools and chemical analysis transcending priority substances lists were integrated to innovate effect-based surface water quality assessment methods, incorporating sediment quality as well. The project overview ends with a second nationwide measuring campaign where the innovated toolbox was used to explore the link between ecotoxicological effects of water and sediment and the ecological structure and functioning of surface waters. It is concluded that effect-based water quality assessment allows prioritization of sites based on ecotoxicological risks, can identify the presence of hazardous compounds regardless of being listed as priority substances, and meanwhile can prevent costly chemical analysis at sites with low ecotoxicological risks.

Dealing with and Communicating Uncertainties in Environmental Risk Assessment While Ensuring Trust Among Stakeholders: Mission Impossible?

4.06.1

Identifying and Characterising Uncertainties to Improve In Silico Models for Toxicity Prediction

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In silico approaches to predict the environmental and human health effects of chemicals include quantitative structure-activity relationships (QSARs), grouping and read-across. QSARs have been used for several decades to predict the toxicity, fate and properties of chemicals and, along with grouping and read-across, have found great utility for regulatory applications such as REACH in the EU. The key to the practical application of predictions is their acceptability for regulatory purposes, this has historically been guided by the OECD Principles for the Validation of QSARs as well as copious guidance on grouping from OECD and ECHA. Evaluation of in silico methods and acceptance of predictions for toxicity and fate could be improved, and in some circumstances made more formulaic, in particular including better consideration of uncertainties, variabilities and areas of bias. To this end uncertainties, variability and areas of bias have been defined for QSARs and read-across. The aim of this study was to utilise uncertainty schemes for QSARs for environmental toxicity to demonstrate their applicability (both of the schemes and QSARs), review their use and attempt initial quantification. The schemes to assess the uncertainty, variability and areas of bias of QSARs were applied to some recently published QSARs. Each QSAR was evaluated according to questions defined by the scheme, with responses undergoing semi-quantification on a integer scale of 1-3 (low to high uncertainty). In addition, areas of poor definition, ambiguity or repetition in the characterisation of uncertainties were noted and recorded to assist in the further improvement of the scheme. The evaluation of the QSAR models found that they were generally well described and presented although for some models provenance of the data was uncertain e.g. the original source data were not cited or checked. Areas where QSARs could be improved included the mechanistic evaluation and justification of the models which was either absent or, in the opinion of the reviewer, incorrect. In addition, the compound / data set collection was often not well defined. The criteria were found to be easy to apply and gave confidence to predictions from the QSAR models. The purpose of these criteria is not to necessarily to imply that a particular model is “validated” or fit for purpose, although this could be part of their use, but to identify areas where more information may strengthen confidence in the model.

4.06.2

Modelling can help understand and apprehend uncertainties

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The world in which we live and try to protect is filled with uncertainties. These uncertainties influence our everyday life and our decisions. When these decisions concern critical domains like human, animal, or more broadly, environmental health, proper quantification of these uncertainties become crucial. Uncertainty and variability arise in several forms. They can concern the organisms or environment themselves with inter- and intra-species as well as the environmental variability. They can also concern the mechanisms used to measure and analyse the organisms or environment of interest with noise, errors, design and assumptions made in the measurements or the modelling. Understanding and assessing these uncertainties is therefore of upmost importance in Environmental Risk Assessment, as this allows calculating an acceptable level of risk. Mechanistic modelling and its associated tools can be used to better understand and apprehend these uncertainties. The utilisation of specific inference techniques as well as tools like the sensitivity and uncertainty analysis renders possible gaining insight on the natural and intrinsic variabilities, on the model properties, or even on unknown mechanisms. I present case studies where uncertainty and sensitivity analyses were used to improve the understanding of biological mechanisms, improve modelling, and ultimately, the risk assessment.

4.06.3

Bayesian Evidence Synthesis in scientific assessments of risk

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A successful communication of uncertainty in scientific assessments of risk is important to ensure that decision makers do not to put too much, or too low, confidence in the conclusions made. A complete communication of uncertainty follows when assessors have characterised uncertainty, in terms of both uncertainty in the range, distribution or likelihood of decision relevant variables (outcome uncertainty), and the strength of the knowledge behind the assessment. A quantitative characterisation of uncertainty in parameters and variables is advantageous as it allows for a separation between epistemic and aleatory uncertainty, and a transparent evaluation of the impact of multiple sources to uncertainty on the outcome uncertainty. Assessments of the strength of knowledge in an assessment are currently mostly limited to expert-based quality appraisal and qualitative measures like “confidence” scores and sensitivity analyses. Scientific experts or assessors face the challenge to find and motivate how they choose to deal with uncertainty. This is not always a trivial task, especially since a scientific assessment of risk often becomes a daunting challenge to integrate multiple sources of, sometimes limited, evidence and judgements from experts. Bayesian Evidence Synthesis (BES) is a highly flexible method of inference suitable for scientific assessments of risk. BES has its roots in evidence-based health care, as a marriage of meta-analysis and decision analysis. A BES is a fully Bayesian model which enable integration of expert judgement (as priors) and data from empirical observations, and where epistemic uncertainty measured by subjective probability. A risk assessment can be framed as a BES, by replacing the decision analysis with an assessment model and the multiple sources of evidence are the data informing parameters of the assessment model. The benefit of using BES compared to "quantifying uncertainty in one parameter at a time" is that there is an unbroken link from data to the quantification of uncertainty and that it allows for multiple sources of data informing the same parameters. A BES opens up for more efficient integration of several sources of evidence and learning as new data or expert judgements becomes available. I demonstrate a BES for a hazard assessment of a chemical substance relying on species sensitivity distribution and multiple sources of toxicity estimates with errors and biases.

4.06.4
Identifying uncertainty trade-offs of ecological risk assessment objectives to guide model complexity
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Risk managers are charged with interpretation of Ecological Risk Assessments (ERA) and need to ensure decisions reflect a strong scientific basis of the models used and understand uncertainties of both the models and assessments. Model uncertainty is directly connected to model complexity, which is a critical element of good modelling practice; however, there is little guidance on selecting the level of model complexity appropriate for specific ERA objectives. We present a framework that provides guidance for developing and applying models of varying complexity in regulatory decision-making using population models. The framework is centered on the trade-offs of generality, realism, precision, and complexity for both ERAs and models with consideration of resource investment. The framework considers what uncertainties are acceptable for an assessment based on its objectives and uses those uncertainties to guide model development. In doing so, our framework links the trade-offs of an ERA objective with those of model building. Also central to our framework is the concept that complexity is not independent of these trade-offs, increasing as models move both from general to realistic, and from lower to higher precision. Complexity can increase such that knowledge-based, or qualitative, uncertainties are reduced by adding mechanisms or model functions that represent real-world scenarios. Complexity may also increase such that the level of precision, or confidence in model output, is increased through the addition or improvement of mathematical functions that reduce quantitative uncertainty. Application of the framework first identifies ERAs by their level of generality, realism, precision, and associated complexity which is then used to guide commensurate trade-offs in model development. For both ERAs and models, realism increases with species, spatial, and temporal specificity and inclusion of real-world complexity, whereas generality is based on surrogate species, contains less defined spatial and temporal scales, and has broad applicability across locations. To demonstrate these concepts, we use case studies that represent ERAs and associated models that represent various trade-offs within the framework. While the framework presents an approach that will guide the development of population models of varying levels of complexity based on the objective of the ERA, the principles that define the framework can be applied to other types of models used in ERA.

Effect Modelling for Regulatory Environmental Risk Assessment: Current Applications and Future Directions

4.07.1
On the model choice for the prediction of chronic lethality
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The effect assessment of pesticides is based on acute (short-term) and chronic (longer-term) toxicity tests with various species. In aquatic assessments, acute testing focusses on lethality, while for chronic toxicity, sublethal endpoints such as feeding, growth and reproduction are of additional importance. Usually, chronic effect concentrations are lower compared to those derived from acute toxicity tests. Toxicokinetic-toxicodynamic models allow for the extrapolation of chronic toxicity based on acute test results as the explicitly account for mechanisms behind and processes leading to an effect. In this study, we analyzed acute and chronic effects for the case of imidachloprid, thiacloprid and flupyradifurone in the non-biting midge *Chironomus riparius*. The three insecticides share a similar mode of action but have different ecotoxicological profiles. We disentangled the mechanisms behind those profiles by a combination of Dynamic Energy Budget (DEB) modeling and applications of the General Unified Threshold Model of Survival (GUTS) to account for both sublethal and lethal effects. Using this example, we will illustrate how lethality and sublethal effects may work in concert on evoking chronic toxicity and that choosing the relevant model type is key to successful extrapolations.

4.07.2
Modelling sublethal effects of chemicals: DEB model options and challenges
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Dynamic energy budget (DEB) theory is the leading candidate to model the growth and development of animals, which is then perturbed by toxic exposure. However, many different variants have been developed and used as the basis for toxicokinetic-toxicodynamic (TKTD) modelling, each with their own data requirements and limitations. We present a unified naming convention for the predominant model variants within DEB theory and specify the assumptions each variant relies upon. The result is a set of recommendations for the best model variant to use based on knowledge of the species, stressor and the problem which the model must solve. The considerations discussed and their implications naturally imply a hierarchy of model complexity which allow the use of DEB-TKTD to be maximised while minimising model complexity and amount of data required.

4.07.3
Revisiting simplified DEBtox models for regulatory risk assessment of pesticides
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Mechanistic effect models are gaining increasing interest in environmental risk assessment (ERA). At the individual level, such models are termed toxicokinetic-toxicodynamic (TKTD) models, aiming to replace descriptive hypothesis testing and dose-response fitting as data-analysis tools. Since TKTD models explicitly deal with the factor of 'time', they can be used to analyse and predict toxic effects due to time-varying exposure, something that is essential for ERA of pesticides. In 2018, EFSA published a scientific opinion on TKTD modelling for regulatory risk assessment of pesticides, including DEBtox for sub-lethal effects. The term 'DEBtox' was first used in 1996 for a software package that included a specific set of simplified dynamic energy budget (DEB) models to analyse standard aquatic toxicity data. However, after more than 20 years, the classical set of simplified DEBtox equations needs an update. There are three specific reasons for this update: 1) the classical DEBtox is not consistent with the broadly-accepted GUTS framework for survival (which argues for a central position of 'damage'), 2) the success of reserve-less DEB models for invertebrates (DEBkiss), and 3) the specific needs for risk assessment of pesticides (time-varying exposure conditions). In this contribution, an update to the classical DEBtox equations is presented. The model is based on DEBkiss, and formulated in terms of easy-to-interpret compound parameters such as maximum body size and maximum reproduction rate. This update also makes DEBtox consistent with the GUTS framework, and allows for dealing with toxicant-induced (or environmentally-induced) starvation. Furthermore, a case study will be provided to illustrate how DEBtox can be used in practice to analyse toxicity data.

4.07.4
Testing DEBtox model predictions of effects of time variable exposure to a fungicide on reproduction and survival of Daphnia magna
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In 2018, the Panel on Plant Protection Products and their Residues (PPR) of the European Food Safety Authority (EFSA) published a scientific opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) models in prospective environmental risk assessment (ERA) for pesticides and aquatic organisms. In this scientific opinion, an overview of existing TKTD models is given for lethal and sublethal effects for animals and primary producers, and an evaluation of the state of science of these models is done. DEBtox models (Dynamic Energy Budget toxicity models) account for sublethal effects of pesticides on growth and reproduction. The EFSA scientific opinion identified some suggestions for improvement of DEBtox models which would make them better suited for use in regulatory risk assessment. The importance of having more examples for applications of DEBtox models for the analysis of the effects of time-variable concentrations of chemicals on growth or reproduction of aquatic organisms was underlined. This study provides such an example, here studying effects of the fungicide azoxystrobin on the water flea *Daphnia magna* following the recommendations for model calibration and validation given in the EFSA scientific opinion. Effects of azoxystrobin on growth, reproduction and survival of *D.magna* were analysed for semi-static and pulsed exposure conditions. Under semi-static exposure of up to 250 ug.L, *D.magna* showed no impacts on survival and growth over a period of 21 days, but an inhibition of reproductive output. A DEBtox model was calibrated to these data using the BYOM package in Matlab,

and the calibrated model was used to define 2 time variable exposure profiles, differing in the no-exposure-interval between two pulse exposures of azoxystrobin. The exposure profiles were tested in validation experiments at low, medium and high concentration levels, and results compared with the effect predictions. The model predictions for the effects under pulsed exposure were matching the observations for reproduction, but in the pulsed exposure effects on survival were observed where the model did not predict any. This mismatch can be traced back to uncertainty about the elimination rate constant in the DEBtox model. The results indicate that for DEBtox models knowledge about the TK parameters is key, and uncertainties in the TK can considerably influence the quality of model predictions.

4.07.5
Assessing effects of salmon lice treatment (H2O2) on shrimp populations: combined exposure, effect and population modelling
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Hydrogen peroxide (H2O2) is used as anti-parasitic biocide in salmon farms worldwide. Since the treatment water is discharged to the sea, there are concerns about effects of H2O2 on the coastal ecosystems. Our study focuses on the effects of this biocide on populations of the Northern shrimp (Pandalus borealis), which is an economically and ecologically important species in Norwegian fjords. Shrimp may be exposed to multiple stressors including repeated exposure to salmon lice biocides from surrounding aquaculture farms, commercial harvest, and climate change. Laboratory experiments have demonstrated that even short pulses (3 x 2 h) of exposure to H2O2 in concentrations well below the prescribed treatment concentrations result in mortality of shrimp. The aim of this study is to make this information more applicable for risk assessment and management of Northern shrimp populations. We apply a chain of three models. (1) An oceanographic model for the distribution of H2O2 in shrimp fields following salmon lice treatment in different locations and seasons. (2) A mechanistic effect model for individual survival probability (General Unified Threshold model of Survival). The GUTS model is calibrated to the experimental data, and used to predict survival probabilities under different scenarios of H2O2 exposure profiles in the field. (3) An age-structured population model to project the effects of H2O2 exposure on shrimp population decline, under different scenarios of H2O2 treatment in different locations and seasons. The purpose of this model chain is to predict effects of H2O2 exposure in combination with other stressors on population-level endpoints such as long-term abundance, and to assess the risk of shrimp population decline below threshold abundances. With this combination of lab experiments and complementary modelling methods, we demonstrate how the risk from H2O2 exposure to shrimp populations can be affected by factors such as local oceanographic conditions, the season of the H2O2 treatment, and the degradation time of H2O2.

4.07.6
Unexpected recovery and non-effects predicted with a mixture toxicity implementation in a population model
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Current regulations in risk assessment are very substance-based and only regard effects observed in lab-standardized tests on individual organisms. However, in the environment organisms are exposed to mixtures of chemicals that vary in concentration and composition. In addition, effects on individuals will have an effect on populations and higher levels of organisation in the ecosystem. Mechanistic, individual-based models have been proposed to tackle this issue as they can integrate effects observed at the individual level to make an extrapolation of effects at the population level. In addition, mechanistic models can be used to describe the effects of mixtures. Mixture toxicity implementations of the General Unified Threshold theory for Survival (GUTS) and the Dynamic Energy Budget theory (DEBtox) are applied here: damage addition and independent action. Integrating these models in an individual-based implementation, a model is obtained that describes effects of mixtures on *Daphnia magna* populations. Two population experiment were conducted exposing *Daphnia magna* mixtures of different compounds (Cu, Zn, alfa-HCH, dicofol and pyrene). These compounds have been selected for their suspected toxicological mode of action. The populations were exposed for 2 months to these compounds, their binary mixtures, and a ternary mixture. The population density over time was recorded bi-weekly in all of the treatments. We calibrated an individual-based model for *D. magna* based on the individual-level effects of the different substances. We validated the implementation at the population level with the data from the population experiment. We evaluated the different mixture toxicity implementations (i.e. damage addition vs independent action). For Cu, the model was able to predict (unexpectedly) recovery of effects over time. The independent action approach was able to predict the effects for the Cu and Zn mixtures. Overall, we highlight the applicability of mechanistic population models for predicting mixture toxicity effects at the population, based on effects observed on the individual level with individual substances.

4.07.7
Calibrating and Validating the BEEHAVE model to field data
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The honeybee (Apis mellifera) is a key species worldwide for pollination, both in terms of an ecosystem service and economically for professional beekeepers. In recent years there have been reports of significant over-winter colony losses, which has been a cause for concern for many.There are multiple potential causes of honeybee colony loss, most notably the parasitic mite Varroa destructor and its accompanying viral diseases. However, it is also important to consider the impact of pesticides on the honeybee colony, especially for the risk assessment of these chemicals.The BEEHAVE model is a large-scale integrated model of the honeybee colony. It includes a cohort-based colony model simulating the in-hive processes and regulations controlling the growth of the colony; a landscape module allowing a landscape to be defined, comprising of many individually parameterised food patches to represent the real landscape in question; a foraging module providing realistic use of the landscape patches by the foraging bees and; a Varroa and virus module, implementing the impacts of the Varroa mite and its related diseases.In this work, we explore a calibration method for the BEEHAVE model, and attempt to validate the model against a large, field-level dataset. The dataset consists of 2 French apiaries, one in Alsace and one in Picardie, each containing 6 colonies. The colonies were assessed over 4 years and the weather and beekeeping practices applied were recorded and have been applied in the model.Firstly, we performed a sensitivity analysis of key parameters in the model, both one-at-a-time as well as including interactions.To calibrate the model, we used a Latin hypercube method to provide 2000 parameter combinations spanning our parameter space. We then ran the model using each of the parameter combinations with 10 replicates. This method then gives options for the “best-fit” parameter values, which was then used to provide a calibrated model for use in the validation.When calibrating the model, we found that there are often many parameter combinations giving similarly good fits, however it was possible to attain a good fit to the empirical bee population data from the field studies. Initial attempts at validation to independent field data set showed good matching of the different patterns of colony development in the 4 different years.

4.07.8
On the use of population models in ecological risk assessment
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In contrast to the individual protection foreseen for humans in toxicological risk assessments, under the auspices of the regulatory risk assessments for ecotoxicology, protection of populations is assumed to be of greater relevance than protection of individuals. This adds an extra layer of complexity to the risk assessment, as determination of what effects, and what level of those effects, would result in a population level effect remains a large unknown. As a conservative measure, it is generally assumed that any effects which would result in massive acute toxicity or effects on reproduction would result in population level effects. Furthermore, conservative assumptions are made for the exposure of the entire population (it is assumed that the entire population will be exposed at the same, high, level). However, while it is assumed that this is a conservative path, it is unlikely to be realistic for most uses being assessed. It is also not known to what degree these levels of uncertainty cover the uncertainties inherent to the risk assessment framework. Population modelling is one way to try to determine a more realistic exposure for a population and determine a more likely population level effect, over a longer period of time than is possible in traditional effect testing. This presentation will summarize our experiences in evaluating population models for mammals and conclude with our vision of the pros and cons of this model as a stand-alone and in the context of the regulatory risk assessment for birds and mammals from pesticides in the EU.

4.07.9
Aquatic risk assessment at catchment scale - case study on insecticide exposure concentrations and effects on macroinvertebrates
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Key words: exposure, effects, risk assessment, landscape Landscape modelling is meaningfull for future environmental risk assessment of aquatic ecosystems for plant protection products (PPP). By assessing the risk at landscape levels more realism can be included. The combined application of chemical fate and

ecological effect models at individual and population levels allows the spatially and temporally explicit quantification of risks. A case study will be presented about an application of the developed tool to simulate PPP concentrations and effects on macroinvertebrates in surface water within an entire landscape. A landscape model is developed which integrates various environmental modules. The hydrological model is being developed based on the hydrological programming library 'Catchment Modelling Framework (CMF)'[1,2]. In the landscape model the water flows and depths are used by modules for spray drift module (xDrift, Bub and Schad, in press), e-fate (Cascade_TOXSWA[3]), a GUTS effect module (TKTD) and an individual-based population model based on Dynamic Energy Budget (DEB) theory. The fate of the PPP can be simulated for all reaches in the catchment. The GUTS model predicts survival probabilities and margins of safety for a selected species. The population model predicts population level consequences for this target species. In this case study a strongly sorbing substance entered the water via spray drift resulting in PECs and LP50 analysed for the macroinvertebrate Asselus by Strahler category. This general effects can be calculated on the catchment scale. The result of this case study faciltates the development of a framework for landscape risk assessment. [1] Multsch S, F Krebs ,S Reichenberger, P Kraft, L Breuer, L Wipfler, W Beltman, T Schad, 2019. A novel hydrological model to support landscape-scale aquatic risk assessment. SPCXVI:Symposium on Pesticide Chemistry. [2] Kraft, P, KS Vache, L Breuer, HG Frede,. 2010. A Hydrological Programming Language Extension for Integrated Catchment Models. Environmental Modelling and Software, submitted, manuscript# ENVSOFT-S-10-00017-1. [3] Focks F, M ter Horst, E van den Berg, H Baveco, PJ van den Brink, 2014. Integrating chemical fate and population-level effect models for pesticides at landscape scale: New options for risk assessment. Ecological Modelling 280: 102-11.

Engineered Nanomaterials: Complex from Chemistry to Multi-stressor Situations

4.08.1
Biological activity of fullerenol' nanoparticles. Bioluminescence monitoring
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Fullerenols (Fs) are specific allotropic form of carbon, nanosized polyhydroxylated water-soluble derivatives of fullerenes, bioactive compounds, and perspective pharmaceutical agents. We used bioluminescence assays, cellular and enzymatic, to evaluate biological activity of Fs. A suppression of maximal bioluminescence intensity is a quantitative characteristic of General Toxicity (GT) in the environment. A particular feature of the enzymatic assay is a specificity to oxidizers, it provides monitoring of Oxidative Toxicity (OxT) of environment. We studied propertied of a series of Fs with different structure of carbon carcas and number of oxygen substituents. Toxicity of Fs solutions was evaluated with effective concentrations EC-50 using cellular and enzymatic assays. Both assays were used to evaluate GT under conditions of an oxidative stress; this stress was modeled in solutions of organic or inorganic oxidizers (1,4-benzoquinone or potassium ferricianide). Additionally, enzymatic assay was applied to evaluate OxT. Antioxidant efficiency of Fs was characterized by antioxidant coefficients DGT or DOxT. Values of DGT >1 or DOxT >1 revealed antioxidant effects of the Fs. All Fs demonstrated toxic effects at higher concentrations: from >0.01 to >0.001 g/L; their antioxidant effect was found at low and ultralow concentrations: ca 10-17-10-4 and 10-17-10-5 g/L for cell-based and enzyme-based assays, respectively. No monotonic dependencies of DGT and DOxT on concentration of Fs were found in a wide concentration range. Toxic and antioxidant effects were shown to depend on a number of oxygen substituents in the F's structure. Biological activity of Fs were compared to this of the other bioactive compounds, humic substances (HS), natural products of oxidative decomposition of organic matter in soils. In contrast to HS, the F's effects did not depend on time of exposure. The Fs demonstrated differences in toxicity and antioxidant activity. Antioxidant coefficients of Fs reached to 2.0, while these of HS did not exceed 1.5. Toxicity and high antioxidant activity of Fs were explained by ability of reversible electron acceptation, free radical neutralization, and compact Fs structure. The biological activity of Fs should be taken into consideration due to risks of Fs intensive production by biotechnological industry in future. The work was supported by the PRAN-32, grants of RFBR N18-29-19003mk, RFBR-Krasnoyarsk Regional Foundation N 18-44-240004, 18-44-242002.

4.08.2
Impact of sewage sludge-borne Ag and TiO2 nanoparticles on Eisenia fetida coelomocytes
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The majority of nanomaterials (NMs) used in commercial applications are likely to enter the wastewater stream and reach wastewater treatment plants (WWTP). Studies have shown high association of NMs with sewage sludge therefore soils can be a sink for NM pollution making terrestrial organisms vulnerable. NMs undergo transformations in different environmental matrices leading to altered behaviour, bioavailability and subsequent toxicity that can differ from the pristinepristine material. The NM transformation and the potential hazard they pose in these compartments are poorly understood. The aim of the study was to elucidate (i) the behaviour of Ag and TiO_2 NMs in sewage sludge and sludge amended-soil and (ii) the subsequent effects of transformed NMs on the coelomocytes of the earthworm *E. fetida*.Spherical polyvinylpyrrolidone (PVP)-coated Ag nanoparticles (Ag NPs, 25 nm) and uncoated TiO_2 NPs (anatase, 5 nm primary size, NM-101,JRC) were used in this study. Two types of sludge were used for the exposures, one from a municipal WWTP (Oslo, Norway), and another from a lab-scale WWTP simulating biological wastewater treatment processes continuously dosed during 5 weeks with well-characterised Ag and TiO_2 NPs. Earthworms (adults *E. fetida*) were exposed to LUFA 2.2 soil amended with sewage sludge at two application rates: 20 t ha^{-1} (maximum recommended application rate in Europe), and 3 times this application rate, *i.e.* 60 t ha^{-1} (worst-case scenario). After 12 and 39 days, coelomocytes were isolated from exposed earthworms, and effects on cell population, metabolic activity, lysosomal integrity and reactive oxygen species (ROS) formation were assessed. Characterization of NMs in the sludge amended-soil and soil elutriates, in whole earthworms and coelomocytes isolated from exposed earthworms, was carried out at the beginning (day 0), during (day 12) and the end (day 39) of the exposure period, using inductively coupled plasma-mass spectrometry (ICP-MS) and single-particle (sp)-ICP-MS. Dose and exposure time-dependent effects were observed, with an alteration in the cell composition of coelomocytes, increase in ROS formation and decrease in lysosomal membrane integrity being more pronounced at the highest exposure concentration. The importance of taking NM transformation into account and the sensitivity of the *E. fetida* coelomocytes as a model to study the effects of transformed NMs *in vitro* are discussed.

4.08.3
Host-microbiota interactions modulate the sensitivity of zebrafish larvae to silver nanoparticle toxicity
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Colonizing microbiota play a key role in human and environmental health, contributing to nutrient uptake, energy metabolism and immune responses of animals and plants. Metal-based nanoparticles that exhibit antimicrobial activity pose an emerging threat to these beneficial microbiota-host interactions, potentially altering the abundance, composition and activity of colonizing microbiota. In view of this concern, we investigated the impacts of microbiota-host interactions on the sensitivity of zebrafish larvae to antimicrobial nanoparticles. We combined established gnotobiotic techniques and standardized acute toxicity tests (OECD No. 236) to compare the sensitivity of germ-free and microbially-colonized zebrafish larvae to silver and zinc oxide nanoparticles (nAg and nZnO). We performed additional toxicity tests with silver ions (Ag+) and zinc oxide ions (Zn2+) in order to derive the particle-specific toxicity of nAg and nZnO by way of response addition modeling. In this way, we found that germ-free larvae are more sensitive to nAg than microbially-colonized larvae, whilst the sensitivity of germ-free and colonized larvae did not differ for Ag +, Zn2+ and nZnO. To the best of our knowledge, we for the first time show that colonizing microbiota offer protection against the particle-specific toxicity of nAg. This warns that the effects of nAg may be more severe to humans and the environment if these have been pre-exposed to antimicrobial agents. In this presentation, we highlight the importance of taking microbiota-host interactions into account in nanoparticle effect studies, and provide a method to screen for potential interference of microbiota-host interactions with nanoparticle toxicity.

4.08.4
Environmental Risk Assessment of nanomaterials applied in the food and feed sector
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Risk Assessment of nanomaterials is subject of numerous studies and research projects. The compilation of this body of knowledge for use in the regulatory domain is an ongoing process which often leads to guidance on how to deal with nanomaterials in commonly known risk assessment approaches. For the food and

feed chain EFSA published guidance on human and health aspects (Part 1) of the risk assessment of nanomaterials. Here we present the first overview of existing environmental risk assessment (ERA) approaches specific to the feed and food chain in relation to their applicability to nanomaterials. Next to presenting existing approaches to take into account specific behaviour of nanomaterials in the environment, remaining knowledge gaps are identified. This work will form the basis for EFSA to compile guidance on the environmental health aspect of risk assessment of nanomaterials (Part 2). The food and feed cycle contains several application types that potentially already include the use of nanomaterials, i.e. food contact materials, novel foods, food additives, feed additives and plant protection products (PPPs). Due to the nature of their application (e.g. spraying and manure application to land) feed additives and PPPs are most likely to enter the environment directly and require an environmental assessment by EFSA. The current review therefore analyses all the relevant ERA related endpoints currently applied to PPPs and feed additives, and combines this with an extensive literature search on currently available ERA approaches relevant for feed additives and PPPs. The result provides an overview of the ERA endpoints requiring an adapted approach due to nanospecific behaviour and the potential approaches to address this.

4.08.5
Poster spotlight: Bioactivities of polyligand Fe3O4 nanoparticles mediated by surface chemical states
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Environmental Risk Assessment of Pharmaceuticals: Connecting across Disciplines and Continents

4.09.1
Invasive ascidians as a tool for assessing the extent and effects of pharmaceutical contamination in marine environments
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Despite the global increase in the use of pharmaceutically active compounds (PhACs) in human and animal medicine, efficient treatment of PhACs in wastewater plants is still insufficient. This result in a chronic release of these substances into the marine environment, where their presence presents a threat to marine organisms. Measures of PhAC concentrations in marine waters and sediments are not sufficient to predict exposure concentration by marine organisms, as they provide only a snapshot in time. Additionally, it does not illustrate the harmful effects and the fate of these compounds within living organisms. We investigated the use of three solitary ascidian (Chordata, Ascidiacea) species, *Herdmania momus, Microcosmus exasperatus*, and *Styela plicata*, highly efficient filter-feeding sessile invertebrates, which are considered as cosmopolitan invasive species, as bio-indicators of three common PhACs in Israeli coastal waters. In addition, we exposed *H. momus* individuals to two environmentally relevant concentrations of Carbamazepine (CBZ) under controlled laboratory conditions, and examined their protein expression using a proteomic approach. Results show that both the Mediterranean and Red Sea coasts of Israel are contaminated with PhACs which were detected in twelve sampling sites. Four sampling sites - Haifa marina, Sdot Yam, Ashdod marina and Ashqelon marina, were contaminated with all studied PhACs. Diclofenac (DCF) was the most frequently detected compound, present in 75% of the sampling sites. The highest concentrations measured were 51.9 ng/g dry weight (dw) (of ascidian tissue) of DCF at Ashdod marina, 47.8 ng/g dw of BZF at 'Akko, and 14.3 ng/g dw of CBZ at Sdot Yam. Finally, in response to exposure of *H. momus* individuals to CBZ, differential expression was detected in 34 proteins, 3 of them are related to stress responses. By exploiting the cosmopolitan distribution of invasive ascidian species, PhAC contamination can be examined using a comparable method with the same bio-indicator species in various locations around the world. The alarming amounts of PhACs detected in ascidians along the coasts of Israel emphasize the need for future research regarding PhAC contamination, in order to provide essential data on the extant of this matter in coastal environments and revealing the possible effects of these contaminants on marine organisms.

4.09.2
The AMR Industry Alliance: Where We Are, Where We Are Going and Lessons Learned Along the Way
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The AMR Industry Alliance brings together over 100 biotech, diagnostic, generic and research-based pharmaceutical companies around the shared goal of curbing antimicrobial resistance in the world. Alliance companies are committed to contribute to and measure their efforts in fighting AMR across four key areas: research, appropriate use, access and manufacturing and the environment. Work on these efforts were initiated in 2016 to tackle specific commitments made to the United Nation's General Assembly. The AMR Alliance generic and research-based pharmaceutical companies agreed on a framework that promotes responsible antibiotic manufacturing as well as developing the first list of science-based discharge targets to guide environmental risk assessments for the manufacture of antibiotics. Specific commitments were made with progress tracked and published on a biennial basis. Highlights of the work accomplished to date, plans for the future, and key lessons learned along the way will be shared.

4.09.3
Occurrences and biological effects of emerging and legacy contaminants around a marine sewage outfall
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Marine sewage outfalls are major contributors of complex mixtures of pharmaceuticals and personal care products (PPCPs), other emerging contaminants (ECs) of concern and legacy contaminants, such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), that represent important ecological challenges in aquatic environments. Most traditional wastewater treatment plants (WWTPs) are not designed to remove PPCPs and other ECs, which are increasingly used in urban areas. Previous research in our group has shown that complex mixtures of PPCPs and ECs originating from urban runoffs and marine sewage outfalls are present in water bodies, and has also accumulated in sediments and biota. Herein, a monitoring study was performed around the marine sewage outfall from the IVAR SNJ Biological WWTP (300 000 personal equivalents), located 1.6km North West of the Stavanger peninsula in Norway. In order to evaluate the environmental impact of the sewage outfall, a monitoring study was designed for sampling and analysis of water, sediments and biota. The fate of the sewage plume was estimated by using the numerical model DREAM (Dose-related Risk and Effect Assessment Model) using data from ocean current forecasts and discharge rates for the planned sampling period. Samples for chemical and biological analyses were collected from six stations around the Stavanger peninsula at different distances from the sewage outfall. Environmental concentrations of PPCPs, ECs and legacy compounds were measured in water and ultrasonicated extracts of sediment and biota by solid phase extraction followed by liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS). Biological parameters related to neurotoxicity, genotoxicity, histopathology and general environmental stress were measured. The biota analyzed for chemical and biological parameters consisted of the Norway lobster (*Nephrops norvegicus*), the Norway king crab (*Lithodes maja*) and the common periwinkle (*Littorina littorea*). No alterations were observed in the general physiological health of organisms, indicating that the sewage plume does not seem to affect the general environmental stress conditions of the coastal area. The results from the biological tests will be discussed in depth and related to concentrations of PPCPs in environmental martices and to the sewage plume model.

4.09.4
The Influence of Manure pH on the Degradation of Selected Antibiotic Compounds
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It is well known that veterinary antibiotics are present within animal manures and as a result are often are released into the environment. Antibiotics degrade via abiotic/biotic processes within manure storage systems, and it is critical to consider the rate of dissipation within these systems to accurately predict concentrations applied to land. Currently there is large variation between the determined values for the half-lives of the same compound within animal manure, probably due to differences in manure properties such as pH. To assess the effect of manure pH, an anaerobic dissipation experiment was conducted at varying environmentally relevant pH's (5.5, 7.0 and 8.5) on a broad range of antibiotic compounds: ceftiofur, florfenicol, oxytetracycline, sulfamethoxazole and tylosin. Pig slurry pH had a dramatic effect on degradation rates, the extent of which was on a compound specific basis. Results revealed that alkaline manure conditions favoured the dissipation of florfenicol, ceftiofur and tylosin over that of neutral and acidic conditions. Acidic conditions retarded the dissipation of these compounds considerably, resulting in higher DT50 values than that of alkaline conditions 2hrs< 8.7d, 0.59hrs< 2.13hrs, and 3.52hrs< 9.75d respectively. Conversely sulfamethoxazole appeared to dissipate faster under acidic conditions over that of neutral or alkaline, although the effect was not as profound the finding is still of interest (DT50 20.6hrs< 5.12 days). Manure pH had relatively little effect on the dissipation rate of oxytetracycline, revealing this is not an important

parameter for this compound. Overall, the results suggest that manure pH is an important factor that needs to be taken into account in assessing the environmental risk of veterinary antibiotics, in order to avoid underestimation of the persistence of these compounds. Further research will investigate whether antibiotic dissipation is biotic/abiotic and whether pH-dependent differences in dissipation rates arise via different dissipation routes or from rate differences for the same route.

Environmental Risk Assessment of Polymers

4.10.1
Environmental concern of cationic polymers: a literature review
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There is increasing regulatory attention on polymers. Currently, no registration is required in many countries – e.g. within REACH in Europe. This lack of registrations is getting increasing attention and is potentially about to be reassessed. In order to implement registration of polymers in REACH, the unique testing and methodological challenges of polymers must be understood. Additional environmental effects testing may necessary. In order to prioritize polymers of interest and guide future testing, a detailed analysis of what is known is needed. Cationic polymers are widely used in industry but relatively little environmental toxicity data on polymers is available within the public domain. Hence, this review assesses the quality of the data generated in the past and the challenges we are facing in the future, the degree to which scientists report important phys/chem properties and experimental designs when testing these materials. To address this we conducted a systematic review of the environmental impact of cationic polymers, with an in depth focus on were the data exists, identifying data gaps and of what quality the published data is. The review followed Cochrane's method for a systematic review and papers identified as relevant during the initial screening were scored according to Klimisch. The result of this review gives an overview of the quality of the literature published on cationic polymers as well as bring light upon what the most tested and hence well-known compounds are. The review point toward a data gap in peer-reviewed literature on cationic polymers. Several different cationic polymers with various chemical and physical properties as well as very different application areas were identified of varying reporting quality. Information on analytical methods, effect- and fate data based on the prioritized literature from the specific search was obtained. A comparison of the different fields of interest showed a difficulty of combining knowledge, since very few compounds would be represented in all three categories. E.g. a lot of the fate data is derived from mine effluent studies, whereas some of he most in depth-effect data is published on a polymer applied in consumer products. Data is found in different areas, and the pieces of information from the published papers are not enough to conduct a full environmental hazard profile. This information should be applied in further data-generation and evaluation of cationic polymers.

4.10.2
Cationic polymers - aquatic toxicity and modelling challenges
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Polymers are currently exempt in REACH and generally not reviewed for environmental risk in most regulatory programs. They are exempt based on the assumption that their significant molecular weight and low water solubility renders them less toxicologically available. Cationic polymers are highlighted as polymers of potential environmental concern due to their aquatic toxicity and potential to exert physical impacts as a function of their charge density. The big question now is - how to assess aquatic toxicity and model this for cationic polymers in the context of REACH? This presentation will provide an overview of cationic polymers, potential toxic mechanism of action and evaluate the different toxicological descriptors with using polyquarterniums as the example. We will demonstrate what is known about the materials based on the literature such as the USEPA 1996 assessment of polymers by Boethling and Nabholz. We will demonstrate a state-of-the-art review of available data and (Q)SAR models to assess cationic polymers as an illustration of wider polymer regulatory and scientific challenges. We will present current QSAR developments for cationic polymers and assess the descriptor transferability of QSPR models to toxicity (Sanderson et al. 2019 (Springer Protocols *Ecotoxicological QSARs*)). We will analyze the (Q)SAR development needs and directions of cationic polymers by applying the OECD principles for developing (Q)SARs. Fragment based modelling approaches will be presented in light of sparse toxicity data. Key descriptors will be prioritized for fish, daphnia and algae. The scientific challenges and future needs for these materials in terms of better understanding the toxic mechanism, toxicological descriptors, and (Q)SARs are discussed in support of evolving regulatory needs.

4.10.3
What Historical Assessment of Polymers in General and Fluoropolymers Specifically Has Taught Us
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Starting in the '70s, many thousands of polymers and their physical, chemical and biological properties have been examined by various regulatory authorities around the globe to determine which properties were predictive of adverse human health or environmental effects. In the OECD and in many countries these were codified into what is now known as Polymer of Low Concern (PLC) criteria. Polymers meeting these PLC criteria have different chemical notification requirements than other substances in these coutries. In some countries, polymers were exempted altogether from chemical notification requirements. The advent of national laws requiring chemical notification before commercialization of substances within a jurisdiction necessitated collection, review and interpretation of new substances including polymers. For example, the US EPA scientists were required by statute to determine, within 90 days of receipt of the notification, whether or not the new substance "may present an unreasonable risk of injury to health or the environment". This chemical-by-chemical assessment of thousands of data packages lead to the development of tools to assist in the identification of physical-chemical properties, potential hazard, and potential exposure to assist in and expedite the chemical review and assessment process. The predictive power and reliability of these approaches were tested and refined leading to validation of tools and the development of general principles or criteria for the identification of chemicals, including polymers, with low hazard potential. About 10 years later, in 1993, the OECD Expert Group on Polymers agreed on a consensus document identifying Polymer of Low Concern criteria and the definition of a polymer, which remains in use today. These criteria are: chemical composition, molecular weight, molecular weight distribution, oligomer content, electrical charge, reactive functional groups, low molecular weight leachables (including residual monomers and additives), solubility in water and lipids and biotic/abiotic/thermal stability. By 2009, the OECD Expert Group on Polymers agreed that PLC are those deemed to have insignificant environmental and human health impacts. Using the polymeric PFAS class of fluoropolymers, these OECD PLC criteria are detailed and their relevance to human and ecological hazard explained. Through the example of fluoropolymers, these same polymer properties predictive of human and environmental hazard may be applied to the determination of what would make a polymer worthy of REACH registration.

Fate, Effects and Risk Assessment Procedures for Chemicals in Tropical and Neotropical Regions

4.11.1
Environmental risk assessment of pesticides in tropical terrestrial ecosystems: Test procedures, current status and future perspectives
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Despite the increasing use of pesticides in tropical countries, research and legislative efforts have focused on their temperate counterparts. In this study a review of the literature on environmental risk assessment of pesticides for tropical terrestrial agroecosystems was made. This was aimed at evaluating potential differences in pesticide risks between temperate and tropical regions as well as to highlight research needs for the latter. Peculiarities of pesticide risks in tropical terrestrial agroecosystems are discussed in the following subsections: 1) agricultural practices; 2) research efforts; 3) fate and exposure; 4) toxicity testing methods; and 5) sensitivity. The intensive and often inadequate pesticide application practices in tropical areas are likely to result in a relatively greater pesticide exposure. Since pesticide fate may be different under tropical conditions, tropical scenarios for models estimating predicted environmental pesticide concentrations should be developed. Sensitivity comparisons do not indicate a consistent similar, greater or lower relative sensitivity of tropical soil organisms as compared to temperate organisms. However, several methods and procedures for application in the tropics need to be developed, which include: 1) identifying and collecting natural soils to be used as reference test substrates in tests; 2) identifying and discerning the range of sensitivity of native test species to soil contaminants; 3) developing test guidelines applicable to tropical/subtropical conditions; and 4) developing methods and procedures for higher tier testing for full development and implementation of environmental risk assessment schemes.

4.11.2
Endocrine disrupting compounds in Brazilian reservoirs are affecting gonadal steroids of two fish species, Astyanax fasciatus and Hoplias malabaricus

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The aquatic environments have been affected by contaminants due to the intense anthropogenic activities. Many of these substances can be considered as endocrine disrupting compounds (EDCs) that have the potential to alter the reproduction of fish in different levels of biological organization. The aim of this study was to evaluate the impact of the water contaminants present in polluted reservoirs acting as possible EDCs on the reproductive physiology of *Astyanax fasciatus* and *Hoplias malabaricus*, neotropical teleost species with different reproductive strategies, using biomarkers at different levels of biological organization. Adult males were collected during summer and winter in three different sites of the same basin: Ponte Nova reservoir (PN), considered as a reference site, and points located in two different reservoirs subjected to a great anthropogenic impact, Bororé and Taquacetuba branches from Billings reservoir (BIL) and Embu-Guaçu and Barragem branches, and Guarapiranga reservoir (GUA). Plasma steroid level of testosterone (T), 11-ketotestosterone (11-KT) and estradiol (E2), as well as, hepatic vitellogenin (*vtgA*) and pituitary follicle stimulating hormone β subunit (*fshβ*) gene expression were used as biomarkers. The results showed that the biomarkers analyzed did not differ between the two seasons of the year for both species living in the reference reservoir (PN). However, *H. malabaricus* males from BIL and GUA reservoirs presented higher plasma levels of the androgens T and 11-KT and higher fshβ gene expression in comparison to males from PN reservoir, mainly during summer. In addition, higher values of gene expression of *vtg-A* were observed in *H. malabaricus* males living in BIL and GUA reservoirs compared to PN. Similar results were found in *A. fasciatus* males also in relation to plasma levels of T and 11-KT and *fshβ* gene expression. E2 plasma levels were also higher in both species sampled in BIL, but mainly during the winter. These data allow us to suggest that there are contaminants acting as EDCs in both reservoirs, affecting the gonadal steroid profile and increasing gene expression of *fshβ* in both species studied, while the so called exposition biomarker, *vtgA* was altered only in *H. malabaricus* males.

4.11.3

The effects of aluminum associated with acid pH and increased water temperature on the sperm quality of the neotropical freshwater teleost Astyanax altiparanae

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The introduction of metals, such as aluminum (Al), into the aquatic environment affects various physiological processes of animals, for example, reproduction. Facing the climate change, there are no data available to evaluate the seminal quality of teleosts exposed (*in vivo*) to metals in association with environmental factors, such as temperature, during the reproductive period. Thus, this study aims to evaluate the effects of subacute exposure of *Astyanax altiparanae* males to Al at environmental concentration, as well as the individual and/or synergistic actions of water temperature and acid pH on the seminal quality of this species. Sexually mature male *A. altiparanae* (n=360) were subdivided into nine groups: six groups without the addition of Al, in acid or neutral pH in three different temperatures (20, 25 and 30°C, respectively) and the other three groups containing Al in the water (0.5 $mg.L^{-1}$) at acid pH, in the same temperatures described. Before seminal collection, the animals were stimulate to spermiation with pituitary extract of common carp (5 $mg.kg^{-1}$). The fish were sampled after 24 and 96h of exposure for the evaluation of the following sperm parameters: sperm osmolality, concentration, motility, and ultrastructure. Data were analyzed by two way ANOVA and Holm-Sidak ($P < 0.05$). Al in the water at the higher temperature (30ºC) reduced ($p < 0.05$) osmolality (at 24h and 96h) and sperm concentration (at 24h). When analyzing sperm kinetics (10s post-activation), it was observed that the presence of Al caused a reduction of ~10% in sperm motility at 30°C in both exposure periods. At the sperm activation time (30s), Al triggered a reduction in the motility by more than 30% at all temperatures when animals were exposed for 24h. When this exposure time was longer (96h), both acid pH and the addition of Al in water reduced sperm motility, being more pronounced when acidity was combined with metal. Besides, the presence of Al in the water generated ultrastructural changes in the sperm head, midpiece, and flagella, regardless of water temperature. Also, it was found that the metal at 30ºC caused a reduction ($p < 0.05$) of the sperm head area and at 20ºC reduced ($p < 0.05$) the area of the midpiece. Under the experimental conditions described here, acidity influenced spermatic parameters of *A. altiparane*, but the presence of Al in the water at ambient concentrations accentuates these effects, reducing seminal quality.

4.11.4

Parhyale hawaiensis as a key species for ecotoxicity evaluation in marine tropical environments

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Parhyale hawaiensis is an epibenthic amphipod with circumtropical distribution and well recognized as a model organism in developmental, evolutionary and regenerative studies. In addition to the concise biological information available, *P. hawaiensis* is easy to handle and maintain in laboratory conditions and well distributed in the Brazilian coast. Despite the necessity of model organisms from marine ecosystem and the attributes of *P. hawaiensis*, this organism has been poorly explored in ecotoxicology. Recently we joined efforts to develop *P. hawaiensis* as a key species for ecotoxicity evaluation in marine environments. The objectives were to develop standardized culture conditions, ecotoxicity tests, a method for internal dose analysis, develop and optimize genotoxicity techniques and to explore sex-specific molecular responses after chemical exposure in *P. hawaiensis*. Successive generations have been successfully cultivated in the laboratory since organisms were collected from the field. The developed miniaturized acute toxicity test provided reliable responses with results in the same range of sensitivity reported for other amphipods. We also developed a miniaturized protocol to test sediment that was successfully applied and confirmed toxicity from samples under the influence of a sewage outfall (Santos, Brazil). Chemical analysis in the hemolymph were used to measure internal concentrations and were key in the understanding the differences on AgCl and AgNP exposures. Animals exposed via food to AgNP had a higher concentration of Ag in the hemolymph in comparison with AgCl. The comet assay did not detect DNA damage at any AgNP food concentration and exposure time evaluated. However, micronucleus frequency increased in organisms fed with AgNP in all exposure time evaluated, especially at the highest concentrations. To broaden our ecotoxicity information with *P. hawaiensis* we investigated sex-specific responses of animals exposed to AgCl and AgNP using RT-qPCR and RNA-Seq. Males presented twice the number of differentially expressed genes in comparison to females. In both sexes an increase of genes that were differentially expressed was observed for AgNP treatment. Our work demonstrated that *P. hawaiensis* is a suitable and reliable organism to be used in ecotoxicological studies. We expanded the information and tools to make *P. hawaiensis* a model organism in ecotoxicology and to be used in future studies.

Multidisciplinary Efforts to Advance Knowledge on Environmental Risk Assessment of Chemicals for Amphibians and Reptiles

4.12.1

In vitro assays as predictors for gold nanorods effects in embryos of Xenopus laevis

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Several works have been published focused on the toxicity exerted by nanomaterials (NMs) to biota. Namely, limited ecotoxicological knowledge on the effects of NMs to amphibians exist, though it is recognized as the class of vertebrates with the highest proportion of threatened species. Furthermore, there is significant interest among the toxicology and regulatory communities in moving towards a decrease of animals use for the assessment of ecotoxicity. In this context, the present study aimed to determine if *in vitro* assays may be good surrogates of the embryo toxicity teratogenesis assay *Xenopus* (FETAX) to assess the ecotoxicity of gold nanorods (AuNR) to aquatic-phases of amphibians. For this, the toxicity of AuNR (axial and long size: 10 and 45nm, respectively) was evaluated *in vivo* by performing the FETAX assay and *in vitro* by using kidney epithelial cells (A6) and fibroblast cells (XTC-2) from *X. laevis*, performing the MTT, Resazurin (RES), Neutral Red Uptake (NRU) and DCF-DA assays. The $LC_{50(96h)}$ for *X. laevis* embryos was 73 $\mu g.L^{-1}$ AuNR. Exposure to AuNR significantly reduced the hatching rates, with an $EC_{50(96h)}$ of 62 $\mu g.L^{-1}$. A significant decrease in body length (LOEC: 59 $\mu g.L^{-1}$), total body length and tail length (LOEC: 39 $\mu g.L^{-1}$ for both) of the larvae exposed to AuNR was also observed. The AuNR caused a decrease of cell viability, for cell line A6 with estimated IC_{50} of 40 $\mu g.L^{-1}$ for MTT, 354 $\mu g.L^{-1}$ for RES and 380 $\mu g.L^{1}$ for NRU (lysosome dysfunction). The AuNR decreased cell viability of XTC-2 cells with estimated IC_{50} of 286 $\mu g.L^{-1}$ for MTT, 330 $\mu g.L^{-1}$ for RES and 369 $\mu g.L^{1}$ for NRU (lysosome dysfunction). The AuNR increased the production of ROS in both cell lines, in relation to control, in concentrations ≥ 0.007 $\mu g.L^{-1}$ AuNR. The MTT assay revealed cell line A6 to be more sensitive to AuNR than XTC-2. The obtained results suggest that cell lines may be a good surrogate for the FETAX assay as they may be as sensitive as the latter assay and constitute a simple and

inexpensive methodology for early stages of risk assessment. Nevertheless, it is important to test multiple cell types originating from different organs to ensure evaluation of different effect pathways, as different chemicals may affect diverse metabolic pathways in the cells. Therefore, further knowledge must be generated for other chemicals concerning comparative studies, *in vivo vs in vitro*, with the aim to find sensitive assays and cell models along with the corresponding assessment factors to be applied.

4.12.2
Modelling the potential pesticide exposure to amphibians
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Amphibians are a taxonomic group that differ physiologically and ecologically from other vertebrates for which risk assessment schemes for pesticides already exist. While fish and tadpoles show similar sensitivity to chemicals, there is uncertainty about potential pesticides exposure and sensitivity of the terrestrial amphibian life stages. In their Scientific Opinion on amphibian and reptile risk assessment, EFSA (2018) proposes the use of population and movement modelling and presents a model of the great crested newt (*Triturus cristatus*). However, the great crested newt may not be the most relevant focal species for agricultural landscapes in Europe and there are many gaps in the knowledge about its ecology. Therefore, we selected the common toad (*Bufo bufo*) and common frog (*Rana temporaria*) as model species because they are known to be the most abundant amphibians in agricultural landscapes across Europe and their ecologies are well studied. Here we present a modular model framework that combines a dynamic landscape with modules for movement, exposure and toxicokinetic toxicodynamic (TKTD). A priority in the development of the landscape and exposure modules was to enable parameterisation with data generated in different risk assessment relevant sections (e.g. FOCUS groundwater and surface water) and using well-tested modules, such as the GUTS model. The model framework is presented with scenarios covering different landscape structures and application times. Amphibians have specific movement patterns, depending on whether they are migrating to the breeding ponds in spring or dispersing to forage summer and/or migrate back into over-wintering grounds. This difference in behaviour in combination with the vegetation cover at the time of an application significantly influences exposure and thus the risk to amphibians. Furthermore, different exposure routes contribute differently to the overall body burden and resulting endpoints depending on ecology of species, body weight, bioavailability and residue level in micro-habitats utilized by amphibians. The modular model framework offers the flexibility for expansions with other modules such as population dynamics for population relevance assessments and single modules can be adapted e.g. another TKTD module can be used to focus on chronic risk or the movement module can be re-parameterised for other species.

4.12.3
A landscape-scale population model for pesticide impact assessment for terrestrial phases of Great Crested Newts
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Individual-based models have gained acceptance as a tool in environmental risk assessment (ERA) of pesticides for scaling-up individual to population-level effects. Amphibians are not routinely included in the ERA of pesticides despite growing evidence pointing that these product would play a major role on their population declines. EFSA has recently drawn attention towards the need to provide a protective ERA for amphibians, including how to translate individual data generated in laboratory into population outcomes. We present an individual-based model, created with ALMaSS (Animal, Landscape and Man Simulatoin System), for the Great Crested Newt (*Triturus cristatus*), an amphibian species widely distributed across Europe and frequent in agricultural landscapes. ALMaSS provides a tool for simulating individual performance and crop management practices, including pesticide applications, over the representation of a real landscape. We used ten Danish landscapes with different structures and uses, and fit the model using *T. cristatus* information collected from the scientic literature. After fitting, we run three different scenarios to go through the process of incorporating individual-level effects of pestcides into population simulations. The first scenario was the establishment of a baseline for stable population size in each landscape after a 30-year simulation. The second scenario incorporated the effects of pesticide overspray, according to the management of each crop type, assuming a 5, 25, 50, 75 or 100% mortality of the oversprayed newts. For the landscape where pesticides caused greatest impact, we run additional simulations to check how population would be affected assuming that all fields would be subject to the most intensive management (i.e. that of spring barley), which resulted in a much faster and sharper reduction in population size. The third scenario analyzed how population would recover after one or 20 consequetive years of pesticide applications. Besides serving to the translation of individual mortality to population-level effects, further applications of this model shall include the incorporation of sublethal effects, or the evaluation of landscape features associated with increased resilience that could serve to develop risk mitigation measures.

Real Risks in Real Soils: Linking Exposure and Effects in a Multifaceted World

4.13.1
UNCERTAIN decisions in soil risk assessment - standing on solid ground or on feet of clay?
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Almost every decision in environmental risk assessments of chemicals (ERA) has to be taken under considerable uncertainty, be it due to incomplete knowledge, unspecific definitions, low data availability, the need for extrapolation beyond measured processes or due to the intrinsic, unavoidable variability of natural systems. There is increasing interest in quantifying the uncertainties that come with ERAs. The UNCERTAIN project approaches the problem for the soil ERA from different angles, referring to the recommendations of European Food Safety Authority (EFSA). A framework that systematically lists and classifies all sources of uncertainty is developed, based on a common language and concept. We deconstruct the soil ERA in its components (extrapolation of effects to untested situations, decisions on model parameters) and search systematically for sources of uncertainty. The comprehensive overview guides the weighting of the uncertainty sources and helps to decide which are most important and which are possibly quantifiable. Systematic literature research yielded 191 records in the core domain “uncertainties soil ERA”. The interest is constantly growing from the 1990ies. Databases holding regulatory and scientific studies (EPA ECOTOX, UBA ICS, UBA ETOX) are queried, screened for soil ecotoxicity data, and harmonized for mutual use. In total, about 15.000 datasets are available. Once identified, the individual contribution of each of the components may be quantified as far as possible. For this to be addressed, data from international ecotoxicological databases are combined with tailor-made experiments, which investigate the influence of soil properties modulating bioavailability and thus ecotoxicological values observed, using several (non-standard) test species. Data is used to populate a hierarchical random effects (Bayesian network) statistical model. The Bayesian computational approach will link all components of uncertainty, e.g. dose-response differences between laboratory and (semi-) field studies and interspecies variability. Our findings can be directly used to inform regulators on how to calibrate the tiered-risk assessment and how to derive protective uncertainty factors, with special regard to the development of new guidance for ERA of in-soil organisms. The extrapolation from results of laboratory studies to the field situation is considered of pre-dominant importance for the overall uncertainty within soil ERA.

4.13.2
Is the concentration addition model sufficient to predict toxicity of tank mixtures to non-target soil organisms?
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Tank mixtures (TMs) are defined as two or more pesticides mixed in the tank of the sprayer equipment prior to application. Despite being considered as way to reduce costs, this practice brings uncertainties on how pesticides interact and what is the effect resulting from these interactions. Whereas pesticides applied alone usually have an EC_{50} higher than the recommended dose, toxicity of TMs have several products acting simultaneously resulting in potentiated or mitigated effects, giving strenght to the need of considering the toxicity of TMs as a whole in risk assessment of PPPs. The joint toxicity of mixtures is usually estimated by the concentration addition (CA) model that assumes a similar mode of action and an addictive effect of the different components. Therefore, relevant TMs should be tested in laboratory ecotoxicological tests to understand at which point the interaction between products may interfere in the prediction of effects to non-target organisms through the CA model. With this purpose, reproduction tests with different TMs (adopted in soybean crops) were performed using soil invertebrate standard species as test organisms. Reproduction tests with the collembolan *Folsomia candida* and the predatory mite *Hypoaspis aculeifer* were carried out with a natural soil collected in an agricultural field. Ranges of increasing concentrations of relevant TMs were used in ecotoxicological tests based on toxic units estimated with effect data of *F. candida* obtained from the literature. The CA model was used to evaluate the effects of each TM. Toxicity of

TMs depended on the test organism, being *F. candida* generally the most sensitive organism except in TMs having acaricides in their composition. Effect data were highly dependent on the mixture components. There were TMs that indicated sinergistic interactions between products and others that revealed additive to antagonistic interactions. The intensity of the toxic effects in relation to the recommended doses was also highly dependent on the components of the mixture. While in some TMs the significant effects were found only above the recommended dose of the individual products, other TMs showed significant effects below the recommended dose. The use of CA model to estimate toxic effects of TMs can underestimate their risk to non-target organisms, reinforcing that risk assessment of mixtures of PPPs should take into account effect data of the mixture as a whole to protect non-target organisms.

4.13.3
FORESEE - Results of a three-day workshop on TKTD earthworm modeling in the context of environmental risk assessment
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Earthworms are important ecosystem engineers, and the assessment of the risk of plant protection products (PPPs) towards them is part of the European environmental risk assessment (RA). In the current RA scheme, exposure and effects are represented in a simplistic way and are not well integrated, resulting in uncertainty when applying the results to ecosystems. Here, modeling offers a powerful tool to potentially integrate the effects observed in lower tier laboratory studies together with the environmental conditions at which exposure is expected in the field. The presentation will be based on the results of the FORESEE Workshop ((In)Field Organism Risk modEling by coupling Soil Exposure and Effect) held January 28-30, 2020 in Düsseldorf, Germany. The FORESEE Workshop was focussed on TKTD (toxicokinetic-toxicodynamic) modeling combined with population modeling in the context of environmental risk assessment. The goal was to join scientists from different stakeholder groups (academia, regulatory authorities, industry, and contract research organizations (CROs)) to discuss the current state of soil invertebrate modeling, explore how earthworm modeling could be applied to risk assessments, and in particular how the different model outputs can be used in the tiered RA approach. In support of these goals, the workshop aimed at developing a common modeling framework for earthworms and addressing the requirements and concerns of the before-mentioned stakeholder groups in an early development phase. The workshop was based on the proposal of a modular modeling approach with four submodules to cover the most relevant processes for earthworm modeling: Environment, Behaviour (Feeding, Movement), TKTD (GUTS and DEB), and Population. Before the models are linked, each module should be tested and validated independently to ensure that the full model produces reliable results. Within the workshop, four workgroups examined different parts of the modules and focussed on various topics relevant to earthworm TKTD modeling: 1 Risk Assessment, 2 Earthworm Ecology, 3 Uptake Routes, and 4 Extrapolation and Validation. Here, we present the perspectives of each workgroup and highlight how the collaborative effort across all involved participants from multidisciplinary backgrounds helped to establish common ground. Further, we will give suggestions for how earthworm TKTD modeling might address some of the uncertainties in current risk assessments for plant protection products.

4.13.4
Developing a monitoring-concept to assess the ecological impact of pesticide use in German agriculture
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The alarming decline in biodiversity, particularly in the agricultural landscape, raises doubts if the current prospective risk assessment and registration practice of pesticides applied for plant protection purposes (i.e. plant protection products, PPPs) ensures the legally intended protection of biodiversity. The main reason cited for this is the overall intensification of agriculture, which consists of a complex of various influencing factors (e.g. use of fertiliser, monocultures). Amongst these, one important factor is the current use PPPs, which have a proven negative impact on biocoenoses in agricultural regions. The impact of PPPs on biodiversity is both direct and indirect. In addition to direct toxic effects on so-called non-target organisms, indirect effects arise e.g. by the change of the competitive balance between grasses and flowering plants in vegetation caused by herbicides. Consequently, the effects of chemical plant protection can only be proven in their entirety by retrospective ecosystem observations, i.e. monitoring approaches. As communities in the field are facing a complex of different stressors besides PPP as mentioned before, these stressors need to be also considered in a new retrospective approach. Here, we present the concept of a new, integrated monitoring scheme to assess the ecological effects of the application of PPPs in the agricultural landscape of Germany. Therefore, we propose using synergies with an established nationwide ecological area sampling site network with several, already ongoing monitoring activities (e.g. bird monitoring and high nature value farmland). The modular structure of including biodiversity and analytic endpoints, as well as a planned step-by-step implementation will be shown.

Soils as Sinks for Plastics: Analysis, Transport and Effects of Nano- and Microplastics in Terrestrial Environments

4.14.1
Solve it! Using 1,2,4-trichlorobenzene and p-xylene to make polyolefin plastic debris amenable to pyrolysis-GC/MS quantification in soil
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p margin-bottom: 0.08in; direction: ltr; color: rgb(0, 0, 0); text-align: justify; background: transparent none repeat scroll 0% 0%; break-before: auto; p.western font-family: "Arial", sans-serif; font-size: 10pt; p.cjk font-family: "Times New Roman", serif; font-size: 10pt; p.ctl font-family: "Arial", sans-serif; font-size: 12pt; a:link color: rgb(0, 0, 255); text-decoration: underline; a:visited color: rgb(128, 0, 0); text-decoration: underline; The majority of plastic is produced, used and disposed of on land, where it probably disintegrates into debris smaller than 5 mm, so-called microplastics. Until now, the lack of adequate analytical methods for the quantification of plastic debris in soil has challenged a better understanding of their occurrence and fate in the terrestrial environment. We recently developed a simple and fast method for the quantification of polyethylene (PE) and polypropylene (PP) in soil using pyrolysis-GC/MS. In order to facilitate the preparation of calibration series and to better account for the heterogeneity of soil matrix, polymers were dissolved in 1,2,4-trichlorobenzene at 120 °C. However, it was not resolved if the method was applicable to all types of PE and PP, for example with cross-linking or molar weights deviating from the initially tested analytical standards. With this follow-up study, we aimed to optimize PE and PP dissolution while further reducing matrix interferences from soil. To this end, we tested a 1:1 mixture of 1,2,4-trichlorobenzene (TCB) and *p*-xylene at 150 °C for its efficiency to dissolve a variety of different PE and PP samples. Calibration series (5–200 µg g^{-1}) were prepared in the same solvent mixture. In addition, dispersive solid phase extraction (dSPE) was evaluated for its potential to reduce interfering matrix components from reference soils. Unlike TCB at 120 °C, the TCB/*p*-xylene mixture dissolved all tested plastic samples and solutions remained stable at room temperature. Calibration series in TCB/*p*-xylene responded linearly (adj. $R^2 \geq 0.995$) with LODs of 5.7 and 3.3 µg mL^{-1} for PE and PP, respectively. Whereas matrix-induced background levels were close to the LOD of PP, reference soils without any plastic addition produced a PE equivalent of 57–145 µg g^{-1}. The dSPE cleanup reduced matrix interferences by 23–45 %. Further method development is crucial to optimize dSPE for subsequent recovery and spiking experiments. Once ready, this approach may have the potential for routine analyses and screening studies of agricultural systems.

4.14.2
Fate of microplastics in agricultural soils amended with sewage sludge
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In Europe, it is estimated that half of the generated sewage sludge is applied as fertiliser on agricultural fields, and therefore this practice has been considered as one of the main entry routes for microplastics (MPs) into the environment. The aim of this study was to assess the fate of MPs in agricultural soils amended with sewage sludge. This was done by investigating their transport to deeper soil horizons and their capacity to leave agricultural soils by water runoff by using experimental plots with the following conditions: (1) agricultural soil with no amendments (control), (2) agricultural soil amended with 5 kg/m^2 of sludge 4 years before the start of the experiment, (3) agricultural soil amended with the same amount of sludge at the beginning of the experiment. In the experimental plots, barley was planted and soil and water runoff samples were collected for one year. The results of this study show that the application of sludge and the time of application had a significant influence on the concentration of MPs in runoff water, being the highest (3 MPs/L) in the treatment amended with sludge at the start of the experiment. Based on the sludge analysis performed in this study and the amount of sewage sludge used in agriculture in Europe, it can be estimated that about 5.7 billion MPs are disposed annually to soil, and 1.750 million MPs

could be transported to surface waters via runoff annually. This study demonstrates that a small fraction of MPs leaves the agricultural soils with surface water runoff (about 0.3%), and that the application of sewage sludge to soils may contribute to long term exposure for terrestrial organisms. Therefore management options to reduce the accumulation of MPs in sewage sludge and its spread into terrestrial ecosystems are needed.

4.14.3

Quantification and modelling of nanoplastic transport in soils by advection and bioturbation

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According to first material flow estimates, plastic emissions to soils are substantially exceeding emissions to the marine environment. Nevertheless, the fate and behaviour of micro- and nanoplastics in terrestrial ecosystems is to-date poorly investigated. In order to reliably assess potential risks, processes that may modify particle concentrations in soils need to be characterized. In this study, major transport mechanisms of particulate pollutants in soils were investigated for nanoplastics, in particular advective and biologically mediated transport. For this purpose, synthesized palladium-doped polystyrene nanoplastics (256 nm diameter) were used in column leaching tests and microcosm bioturbation studies with anecic earthworms, i.e. *Lumbricus terrestris*. Plastic concentrations could be quantified indirectly by detection of palladium using ICP-MS. In column leaching tests, nanoplastic retention in disturbed saturated soil columns was relatively low compared to other transport studies on nanoparticles (mass recoveries > 66 % in leachate). Using breakthrough curves for inverse modelling in HYDRUS-1D, material-specific kinetic transport parameters were obtained. The derived average attachment efficiency was relatively low ($\alpha = 6.25 \times 10^{-4}$). Under unsaturated soil conditions as found in the bioturbation control plots, transport of plastics with percolating water could not be confirmed after four weeks of repeated water application. However, the burrowing activity of earthworms caused a significant displacement of nanoplastics into deeper soil layers, steadily increasing with exposure time. Detected spatial and temporal changes were used to determine bioturbation rates for predicting nanoplastic transport in a bioturbation model. As the model based on layer-to-layer mixing systematically underestimated concentrations in the lower layers, further differentiation of the transport modes by soil biota may be necessary. Considering that unsaturated soil conditions are more representative of prevailing field conditions, the results support that the contribution of percolating water to nanoplastic transport in soils is limited despite low observed attachment efficiencies. Consequently, bioturbation may be the dominant transport mechanism of nanoplastics under field conditions. For extrapolating the findings of this study, a wider range of types and sizes of plastics need to be included.

4.14.4

Microplastics as possible modifiers of pesticide effects in soil - The effects of polyester fibers and tire wear particles on the toxicity of chlorpyrifos

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Microplastics are found to be common not only in aquatic environments, but also in soils, which has raised concerns on the possible effects of plastic particles on soil biota and the ecosystem services they provide. Besides direct harmful effects, plastic particles can potentially have indirect effects on soil biota and functions due to microplastic-derived changes in the surrounding environment e.g. due to the release of chemical additives from the plastics or by altering the bioavailability and toxicity of chemicals present in the environment. For example bioavailability of various plant protection products used in agriculture may be changed due to their sorption to microplastics. As polyester fibers and tire wear particles are common types of microplastics in soil, knowledge on their direct and indirect effects on soil biota are urgently needed. This study investigated the effects of polyester fibers (12-2 900 µm) and tire wear particles (TWP; 0-180 µm) on the toxicity of pesticide chlorpyrifos (CPF) in soil. Springtails (*Folsomia candida*) were exposed to five concentrations of CPF (0.01-0.1 mg kg^{-1}) in Lufa2.2 soil without microplastics ("No") or spiked with fibers or TWPs in a low (0.05 %; "Low") or high concentration (0.5 %; "High") in five replicates. The microplastics were mixed in with the soil after spiking the soils with CPF and the evaporation of acetone that was used to dissolve CPF. In all microplastic treatments ("No", "Low" and "High"), also control and acetone control treatments without CPF were included. Polyester fibers did not affect the CPF toxicity to the survival (LC50) or the reproduction (EC50) of *F. candida*. Tire wear particles, in turn, affected CPF toxicity, but the effects on the survival and reproduction were divergent. Median lethal concentration (LC50) of chlorpyrifos was higher in the high-TWP treatment, indicating lowered toxicity of CPF to survival. In contrast, TWPs decreased the median effect concentration (EC50) of CPF especially in low-TWP treatment, suggesting an increased toxicity of CPF to the reproduction of *F. candida*. However, since the high concentration of TWPs overall increased the total number of juveniles, reproduction was almost constantly higher in high-TWP soils than in soils with no TWPs. Our findings indicate that the effects of microplastics on the toxicity of pesticide chlorpyrifos depend on the type and concentration of microplastics. This study is part of the project IMPASSE (WaterWorks2015, Water JPI).

Wastewater and Sludge Reuse in a Circular Economy: Benefits and Risks

4.15.1

Water reuse: the risks of Contaminants of Emerging Concern (CECs) transfer from reclaimed wastewater to agricultural products.

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Pharmaceuticals and personal care products (PPCPs) encompass a hetergoeneous group of chemicals that includes ultraviolet filters (UV filters), parabens, antibiotics, analgesics, stimulants, among others, most of which are considered compounds of emerging concern (CECs). Over the last 15 years, PPCPs consumption has notoriously increased in the European Union (EU), with its consequent greater spill in the aquatic environment. The pseudopersistence, accumulation in sediments and bioaccumulation in living organisms of PPCPs, combined with its generally poor removal in wastewater treatment plants (WWTPs), make them ubiquitous in the environment. The global good quality water scarcity, that is only expected to increase, and the growing need of feed for a growing world population make the reuse of water in agriculture a suitable strategy to face the lack of fresh water. As agriculture is one of the main sources of food for humans, this constitutes a direct entry pathway of PPCPs in our organisms, putting human's health at risk. Many studies have been focused on environmental monitoring of PPCPs, mostly in waters. However, only limited information is available on their transfer from the environment (the transference vector being the water for irrigation) to crops. This could be due, in part, to the lack of simple, sensitive and roubust methods for the determination of PPCPs in these complex matrices. The extraction method known as QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) developed to extract pesticides from vegetables [2, 3] has been optimized in the present work to extract PPCPs from different crops (lettuces, carrots and tomatoes) and its corresponding soils. All were cultivated in real conditions in gardens authorized in the area of the WWTP from where the irrigation waters obtained. The irrigation waters (reclaimed wastewater) were also analyzed following an online methodology that can be found elsewhere [4]. Analysed samples comprised two different soils (claly and sandy), two irrigation systems (drip and sprinkler) and two irrigation waters i.e (WWTP secondary treatment effluent and renaturalized water with a tertiary treatment consisting on infiltration through reactive barriers). A total of 55 PPCPs were analyzed by HPLC-ESI(QqLIT)-MS/MS.The validation and optimization of the method and the evolution and occurrence of the selected PPCPs in agricultural products, soils and irrigation water will be presented and discussed.

4.15.2

The effect of biomass addition to sewage sludge prior to pyrolysis on the polycyclic aromatic hydrocarbons persistence and bioavailability in biochar-amended soil

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Sewage sludge (SSL), as a by-product of wastewater treatment, can contain certain amounts of contaminants such as heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs) but also valuable nutrients. Very often SSL is used in agriculture as a fertilizer. However, due to toxic substances (e.g. PAHs), SSL may generate a threat when mixed with soil. One of the ways of SSL utilization can be to convert it to biochar during pyrolysis and then use it in agriculture as a soil additive. Pyrolysis is a process using high temperature (up to 900ºC) with little oxygen in the furnace. During the process undesirable components of SSL i.e. inorganic and organic contaminants as well as pathogenic elements can be transformed into less mobile forms or eliminated from the material. Unfortunately, biochar produced from SSL may still contain HMs (even concentrated compared to raw SSL) and PAHs (that can be formed during pyrolysis). For decrease of the amount of toxic substances occurring in SSL-derived biochar, it was proposed to co-pyrolyse plant biomass with SSL. This solution beside of contaminants decrease, may also improve other biochar properties i.e. increase carbon content and specific surface area and lower ash content compared to biochar produced from SSL only. Because of the biochar is planned to be applied to the soil system the research should focus on the contaminants content in biochar-amended soil. The total content of PAHs in biochar- amended soil is expected to be higher than in the clean soil and similar to SSL-amended soil. However, the content of these

pollutants in bioavailable and mobile fraction is expected to be similar to clean soil and lower than in SSL- amended soil. Since the bioavailable fraction of contaminants is the one which may pose a threat to the environment, it is expected that biochar-amended soil will be not toxic or less toxic at least than SSL-amended soil. The aim of the study was to determine the persistence of the total (C_{tot}) and bioavailable (C_{free}) PAHs in SSL-derived biochar-amended soil and in SSL/biomass-derived biochar-amended soil. The project was funded by the National Science Centre granted on the basis of the decision number DEC-2018/31/N/ST10/01588.

4.15.3
Organic pollutants as quality indicators in agricultural application of biogas digestates
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As an important prerequisite of sustainable future circular bioeconomy strategies, recycling of both non-renewable and renewable nutrients from organic residues will be important. In this context biogas production plays an ever-increasing role in providing sufficient energy for electricity, heating and transportation. Biogas production is based on carbon-rich substrate originating from sewage treatment, agricultural - household- and aquaculture wastes. As part of the propagated circular bioeconomy strategies, the application of remaining organic residues as soil amendment and fertilizer are expected to recycle the nutrients contained in the residues but may also imply a dispersal and accumulation of contaminants on agricultural soils. From soil, contaminants may be transferred into plants, ultimately resulting in animal and human exposure. The development of suitable production pathways for renewable energy production in recent years still do not take potential associated pollutant transfer sufficiently into account. Various technologies have been promoted and applied with the potential of uncontrolled emission of anthropogenic pollution. For instance, the use of biological (waste) material in anaerobic digestion, both as decentralized farm biogas plants as well as municipal plants for handling of, among others, organic household waste, has increased significantly in Europe and the North Americas. This development leads not only to an increasing amount of bioenergy produced, but also to a considerable amount of production waste to be handled properly (i.e., biogas digestate). The most attractive option to manage these digestates is to apply them as organic fertilizer to agricultural land allowing to recover nutrients, primarily nitrogen and phosphorus, and, in addition, potentially improving soil quality by adding organic matter. Unfortunately, such residues may also contain complex organic compound mixtures, salts, anthropogenic pollutants and/or pathogenic bacteria that can adversely affect terrestrial organisms and may accumulate in plants. Thus, the identification of potential pollutant sources and the development of suitable mitigation strategies are a prerequisite for the success of the currently propagated circular bioeconomy. In our project, we aim to understand the risk these biogas digestates may pose to the soil ecosystem, when used in agriculture as an organic fertilizer, by analyzing both the soil, plants and 2 various digestates in a 3-year field experiment as well as in associated experiments with composting and a degradation study in a small-scale anaerobic digestion experiment.

4.15.4
Recommendations to derive quality standards for chemical pollutants in reclaimed water intended for reuse in agricultural irrigation
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The reuse of treated municipal wastewater (herein referred to as reclaimed water) in agricultural irrigation (RWAI) as a means to alleviate water scarcity is gaining increasing policy attention, particularly in areas where water demand mitigation measures have proved insufficient. However, the use of reclaimed water in practice is lagging behind policy ambition, with on average < 2.5% of it reused in a European context. A key barrier identified as limiting its full valorisation is concern over its potential impact on human and environmental health. To address this concern, and to meet further objectives including achieving parity between current reclaimed water reuse guidelines operational in various Member States, the European Commission has proposed a regulation which identifies minimum quality requirements (MQR) for a range of microbiological and physico-chemical parameters but the inclusion of compounds of emerging concern (CECs) in terms of the determination of quality standards (QS) is missing. This work reviews the existing pertinent EU legislation in terms of identifying the need for CEC QS for RWAI, considering the scope and remit of on-going pan-European chemicals prioritisation schemes. It also evaluates opportunities to link in with the existing EQS derivation methodology under the EU WFD to address all protection targets in the environmental compartments exposed via potential pathways of RWAI. Finally, it identifies the main data gaps and research needs for terrestrial ecosystems, the removal efficiency of CECs by WWTPs and transformation products generated during the wastewater reuse cycle.

Challenges, New Approaches in Life Cycle Inventory Data Collection and Modelling

5.01.1
geoFootprint: the interactive web-application for modelling spatially explicit agricultural inventory in LCA
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Agriculture is a major contributor to GHG emissions and impacts on ecosystems. In an effort to align with sustainability goals, many companies have committed to reduce their footprint through their agricultural practices. To track progress, LCA is used as a reliable and comprehensive tool.Today, however, life cycle inventory data for agricultural production systems remain generic and incomplete. Datasets are typically defined at country level and limited to a few countries per crop. This is a significant limitation considering the spatial variability inherent to agricultural inventory flows. The geoFootprint tool presented in this work represents a new frontier in LCI data modelling. Deployed around a regionalization engine and a web-based platform, geoFootprint combines publicly available GIS geospatial data with LCI datasets provided by the World Food LCA Database (WFLDB), as well as the needed agricultural emission models and thereafter characterisation factors to compute and display regionalized footprints of crops (e.g. barley) and co-products (e.g. barley straw) for any location in the world, and across various geographical scales (10x10 km grid cells to country level). First results, demonstrate the potential of the tool to provide spatially explicit inventory, with crop-specific management practices and contextual information at the grid cell level - which is especially important for land use change (LUC) inventory modelling. Soon available as an open-access (read-only) and as a licensed version (enabling custom simulations), geoFootprint will support a variety of different actors: *private companies*, *(non-)governmental organisations*, and *the research community*.A major challenge to pursue in future work is to address combining regionalised inventory data with regionalised impact assessment. The geoFootprint tool is made possible through partnering with arxiT and the Cool Farm Alliance and supported by a network of organisations. Quantis is leading the geoFootprint project, sponsored through EIT Climate-KIC funding.

5.01.2
The Challenge of Temporal Resolution in Dynamic LCA
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The last 10 years of research in the field of life cycle assessment (LCA) have unveiled many possible pathways to consider the effects of temporal variations from supply chains on the assessment of potential environmental impacts for products, services and markets with dynamic LCA (i.e. DLCA) frameworks. In this context, defining "useful" levels of temporal resolution for the description of flows in LCA databases will require the combined expertise of system modelers and creators of impact assessment methods. We thus propose a discussion on key considerations of temporal resolution, which could bring common basic structures in DLCA. From the perspectives of system modeling and life cycle inventory (LCI) calculations, recent propositions for DLCA frameworks bring many promises. Novel approaches and tools are showing similar ideas for the computational structure. Some level of agreement is also emerging on the use of process-relative temporal distributions to calculate temporally differentiated LCI. Life cycle impact assessment (LCIA) methods for categories like climate change, photo-chemical oxidant formation, freshwater ecotoxicity and toxicity have also provided time-dependent characterization factors (CFs). Most investigations have tackled these aspects separately, which raises concerns since requirements to create links between LCI and LCIA methods can drive the data gathering efforts to provide useful temporal descriptions for different flows in LCA databases. Three observations on temporal resolution justify these concerns: 1) The use of calendar-based information to define when elementary flows are occurring during the life cycle of a system. Indeed, DLCA computation tools can now consider this data, but the relevance and necessary precision for different impact categories has not been explored. 2) The propagation of temporal resolution in LCI calculations for each path of a system brings many limitations when these resolutions vary. Evaluating the impacts of such flows will require CFs with various levels of temporal resolution bringing diverse levels of uncertainty. 3) The assessment of temporal uncertainty for CFs, which is sourced in the different levels of precision to describe the period of emissions. An assessment of potential variability of CFs over specific periods thus seems like a relevant way to evaluate the uncertainty that comes with lower temporal resolution. Nevertheless, novel development pathways can tackle these issues.

5.01.3
Predicting emission releases from mine tailings: spatially and temporally resolved life cycle assessment modelling

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Mining operations around the world have been producing tailings as wastes at huge scale. These residues, when poorly managed, would become a detrimental environmental hazard in addition to its acid mine drainage effects. Nevertheless, these tailings also contain traces of valuable metals and minerals that can be further reprocessed through sustainable metallurgical processes in the future. Directly utilizing these tailings as raw materials to produce different products, however, is not enough to justify that a process is environmentally friendly; the processes developed must be evidently better from a life cycle perspective than business as usual. It is then important to properly characterize the environmental impacts associated with tailings in life cycle assessments (LCA) using the best available data and approach.State-of-the-art mine tailings emission releases mainly involve empirical and simplified mechanisms, which are derived from landfill leachate models. The current models have some limitations, such as neglecting tailings' geo-chemical factors as well as several important site-specificity parameters such as local hydrology. For instance, some toxic species will remain in the system only after certain minerals are leached due to changes in pH value. In this work, these issues are resolved by considering tailings chemistry dynamics through geochemical simulation and by coupling this model with infiltration data at mine-site level. This framework will enable a time-dependent LCA modelling of tailings impoundments' emission releases at higher resolution on a systematic procedure. Case studies that represent tailings impoundments in different locations will be used as the basis for testing the framework.

5.01.4

Key parameters for predicting the environmental impacts of conventional geothermal technologies

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Life Cycle Assessment studies on geothermal energy are generally not abundant, and usually exhibit a large variability in the environmental impacts. In this work we deploy Global Sensitivity Analysis (GSA) to identify parameters that have little or negligible influence over the variability of the impact scores; the objective is to lay the foundations for the development of a simplified model. Here we focus on conventional geothermal technologies - although the same analysis was also carried out for enhanced geothermal plants. From a thorough literature review, we identified 16 parameters that are able to comprehensively quantify the environmental performance of electricity production from conventional geothermal technologies. We assessed the influence of these parameters on the overall variability of the impact scores by means of Sobol' total order indices. The analysis covers the latest impact categories recommended by the Joint Research Center of the European Commission. Life Cycle Assessment and uncertainty propagation analysis were carried out with the Python package Brightway2, whilst an ad-hoc Python package was developed for GSA computations. The results of the analysis show that operational atmospheric discharges of CO_2 explain nearly 100% of the variabiity in the climate change category, whilst the average gross power of the wells and their average depth account for ~80% of the variability in the remaining impact categories. The results for enhanced geothermal technologies are similar: three parameters amongst the 15 investigated explain over 80% of the variability of the results. The results of this work imply that a simplified model would need to incorporate only these three parameters - with the others being set anywhere in their range of variability - for estimating the environmental impacts of conventional geothermal technologies without significant losses in accuracy.

5.01.5

Canada's Low-Carbon Assets through Life Cycle Assessment (LCA^2) Initiative: LCI dataset development strategy

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The National Research Council of Canada (NRC)'s Low-Carbon Assets through Life Cycle Assessment (LCA^2) Initiative is a $6M four year project that will develop guidelines and benchmarks that leverage a Canadian specific life cycle inventory (LCI) database to empower Canadians to incorporate the quantification of life cycle impacts and total cost of ownership of built assets (real property, public infrastructure) into their procurement processes. To develop a pan-Canadian LCI database we have focused our scope on key requirements for buildings. Through the LCA^2 Initiative, a governance structure has been formed to ensure the Initiative will have balanced representation from public and private sector leaders. By having major material associations as key partners in LCA^2 we have found industry very willing to share industry survey data and to allow us to develop gate-to-gate industry/regional average datasets. We developed extensive lists of sector-specific products/activities that we are aiming to develop over the next four years, where we first put focus on existing datasets that have yet to be integrated with our background database of choice (ecoinvent). Non-existent LCI datasets for key products/activities are then prioritized, and data survey templates are developed where we then campaign for industry data from our broad and growing network of industry partners. One important vehicle of environmental impact information are environmental product declarations (EPDs) and we recognize EPDs as not only a valuable source of LCI data but also as an opportunity for suppliers to report their specific product's environmental profile. The ultimate goal of the LCA^2 Initiative is to incorporate LCA in the decision-making process in government procurement, and we are currently developing these specifications. By being fully transparent and open about this goal with our multi-stakeholder consortium, we have found that it spurs industry participation in sharing industry data surveys with us. Since the LCA^2 Initiative is still young (just over a year old), we have yet to implement our entire strategy. However, to date, we have already received access to industry survey data from most of the major materials associations (e.g. wood/aluminium/concrete), and we are currently underway with the development of several hundred corresponding LCI datasets based on existing industry survey data.

5.01.6

Enlarging the scope of conventional life-cycle inventories using supply chain management for Social Life Cycle Assessment

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Sustainable Development Goals encourage decision-makers to take responsible choices across the entire product system. This type of perspective is usually assumed for economic and environmental analyses, but it is not often considered when social evaluations are conducted. Social Life Cycle Assessment (SLCA) is a comprehensive methodology for the thorough evaluation and interpretation of social aspects. This methodology has usually been based on input-output (IO) analysis for the evaluation of a certain sector. However, the application of SLCA to products has additional challenges such as the need for the identification of the countries involved in the supply chain. This study presents a novel approach that enlarges the scope of conventional life cycle inventories (LCIs) in order to identify supply-chain paths within the boundaries of a product system. In particular, the proposed approach was tested for the SLCA of a bioelectricity plant in Portugal. Within this novel approach, the conventional LCI allows defining the main flows of components, materials and/or energy throughout the supply chain of a product, while the additional use of trade databases helps identify the country of origin of these flows. In this study, the databases UN COMTRADE and ecoinvent were used as sources of trade and background data. After the identification of the product's supply-chain paths, the social LCI of the product system is further ellaborated with economic and social data. In this work, the SLCI of a Portuguese bioelectricity plant was implented in OpenLCA and the corresponding social characterisation was carried out using the PSILCA database and method. The bioelectricity plant system obtained using the proposed approach encompasses more than 400 processes within seven tiers of the supply chain for SLCA. Social life cycle impact assessment results were obtained in terms of total child labour and health expenditure. More than 50% of the impacts in both indicators were found to be concentrated into four processes: ammonia production in Algeria, and petroleum extraction in Russia, Kazakhstan and Azerbaijan. Interestingly, the last three processes mean 10% of the processes included in the product's supply chain. The novel approach developed in this study proved to be feasible for the definition of the supply-chain paths and SLCI of product systems. The resultant LCIs allow analysts to conduct thorough SLCA studies, contributing to the state-of-the-art in this methodology.

Integrated Life Cycle Approaches for Decision Support towards Sustainable Development of Existing and Emerging Technologies, Products and Services

5.02.1

Exploring the effects that a non-stationary climate and dynamic electricity grid mix has on whole building life cycle assessment: a multi-city comparison

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The built environment is responsible for a significant share of greenhouse gas (GHG) emissions, for both the production and operation of buildings. Diverse initiatives are being developed and implemented to limit the release of GHGs into the atmosphere relying on the assessment, monitoring, reporting and verification of GHG emissions and removals. To ensure that actions are effective at mitigating climate change, the annual accounting of GHG flows associated with buildings should be done in a life cycle perspective. Buildings are expected to last and host people's activities for several decades. During this large time frame, the climate is expected to change, as well as the electricity mix. These parameters have combined effects on the life cycle performance of buildings. This study aims to support the design of resource- and energy-efficient buildings using a sound life

cycle assessment (LCA) methodology in the preliminary design stage. A simple procedure that can be implemented in day-to-day engineering practice for a detailed understanding of the effects of climate change and prospective electricity grid mix on building energy consumption is presented. The novelty of this study was to integrate long-term energy scenarios in an LCA approach for buildings at a high temporal resolution and taking into account various future climates and prospective electricity mixes across Canada. The research integrated the dynamic LCA capabilities directly into a Building Information Model (BIM). Such dynamic considerations as climate and energy mix increases the environmental relevance and scientific robustness of LCA indicators. The proposed method will assist users to quickly locate the correct type of building materials and components from the database as well as a clear framework that helps to define an optimized design alternative through a dynamic energy analysis and future weather forecasting simulation. An application of an actual office building is presented in order to illustrate the usefulness and capabilities of the developed integrated framework.

5.02.2
Up-scaling methods used in ex-ante life cycle assessment - a review
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Life Cycle Assessment (LCA) of emerging technologies, also called ex-ante LCA, is a challenging task as it is often carried out at an early stage of technological development (at lab-scale/pilot-scale). For such technologies industrial scale system specifications are unclear and industrial data is absent. These problems are generally addressed by using up-scaling methods and drafting most likely scenarios of up-scaled versions of the emerging technology. Here, we present a review of up-scaling methods applied in ex-ante LCA so far. The aim of the paper was to review the key characteristics and the key principles of up-scaling methods and give recommendations to LCA practitioners regarding their application. Different variations of the term 'ex-ante LCA' were searched as keywords in Web of Science, e.g. "anticipatory LCA", "prospective LCA", etc. Eventually, we selected 18 studies for the literature review. The results showed, among others, that 1) ex-ante LCA studies up-scaled different kinds of technologies from different application domains such as chemical technologies, building sector, food and waste treatment; 2) most of them did up-scaling of chemical and waste treatment technologies; 3) all studies estimated energy and material flows but some of them did not report on how they estimated emissions while those data are essential for LCA. Finally, as different types of expertise may be required for the up-scaling, we recommend that experts from different fields should be involved in ex-ante LCAs, e.g. technology developers, LCA experts and engineers.

5.02.3
A systematic approach to assess the environmental impact of new technologies: A case study for CIGS photovoltaic laminate
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Prospective LCAs, conducted at an early development stage of emerging technologies, can steer research and design efforts towards products with a lowest possible environmental impact. Furthermore, reliable estimations of future impacts enable a more fair comparison with established technologies. Available prospective LCAs take into account numerous facets in technological development, but a consistent and comprehensive approach seems to be lacking. Therefore, a systematic approach is proposed that takes into account all development processes, relevant to future environmental impacts, that were identified in literature. These were process changes, size scaling, process synergies, industrial learning, and external developments. These mechanisms are to be studied in a fixed order in three phases of prospective LCA that were observed: I – define the current development stage, II – assess experience mechanisms towards technological readiness, and III - assess internal and external developments in industrial production. The application and value of the proposed systematic approach was demonstrated with a case study of a novel method for producing CIGS (copper, indium, gallium, (di)selenide) photovoltaic laminate. The functional unit was the production of 1 kW_p of laminate. Carbon footprints (CFs) for this functional unit were calculated using the assessment method "IPCC 2013 GWP 100a". In-house data from Solliance were used to construct a life cycle inventory for the pilot production of CIGS laminate for the year 2014. The proposed systematic approach was applied to this baseline case to predict the CF of industrial laminate production in 2030. It is estimated that the CF decreases by 82.8% between 2014 and 2030 as a result of all modelled developments that were identified through the systematic approach. Dematerializations due to process changes afforded the largest decrease in CF (-73.6%). We found that empirical curves for size scaling and industrial experience could be valuable tools for these type of prospective assessment, but additional research into their synthesis and application is required. Our work provides a systematic approach to prospective LCA that allows for clear and consistent modelling of all known development mechanisms in technology development. Before, studies of prospective LCA only included a selection of developments, thus not providing a complete evaluation of all potential changes in environmental impact of future technologies.

5.02.5
Poster spotlight: Digitalisation of the supply chain to assess chemicals life cycle - First experiences in using blockchain technology for mass flow analysis
L. Zullo, My Chemical Monitoring BV
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5.02.6
Poster spotlight: Future environmental impacts of key metals and consequences for low-carbon technologies
C. Harpprecht, Leiden University / Institute of Environmental Sciences (CML)
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5.02.7
Poster spotlight: Joint use of life-cycle approaches and multi-criteria decision analysis tools for evaluation of renewable natural gas production systems
M.M. Martin-Gamboa, CESAM & University of Aveiro / Department of Environment and Planning
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5.02.8
Shared Futures: New tools for transparent and shareable scenario analysis in Prospective LCA
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The world of 2050 will be very different to the world of 2019. For emerging technologies and long-lived products, decisions made today will have a direct effect on the environmental impact which occurs in the future. While it is impossible to accurately predict what the world will look like in the future, it is possible come up with sensible assumptions representing a range of possible future scenarios. In order for the future impact of products to be estimated in a consistent and meaningful manner in LCA, the background system - most commonly the ecoinvent database - needs to be projected into the future alongside the foreground system modelled in a given study. This is not a trivial task. The latest version of the ecoinvent database contains over 18,000 interlinked processes. External factors, such as technology availability and electricity grid mixes, determined by future scenarios have the potential to affect every single one of these processes.Recent developments in advanced open-source LCA software have opened up the opportunity to apply database wide changes to modelling assumptions. This technique can be extended to alter the entire database to reflect future scenarios in a systematic way. Futura is a new piece of open-source software which allows LCA practitioners to create and, importantly, share novel background databases representing arbitrary scenarios. It allows users to import a base database and then start making targeted changes. These changes take three main forms – adding new technologies, regionalising new or existing technologies, and altering market compositions. These actions allow the creation of scenarios ranging from the simple, such as altered electricity grid mixes, to the complex, where technologies such as carbon capture and storage or electrified transportation have become commonplace.All changes made are automatically added to a 'recipe'. This recipe file contains no proprietary or licensed data and can be shared publicly. This recipe can be imported by other users, and combined with their own version of the base database to exactly recreate the modified database. The additive and transparent nature of this system means that initially simple scenarios can be built upon by others to progress towards more comprehensive scenarios in a stepwise manner.The inability to build on the work of others is a serious barrier to the progress of the LCA field. Futura goes some way to reducing this barrier in the field of prospective LCA.

5.02.9
Integrated LCA/EMA sequential evaluation: opportunities and challenges
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As the world continues to urbanize, sustainable development depends increasingly on the successful management of urban growth. In this context, management of the planet's ecological assets has become a central issue for decisions makers around the world. Circular economy is considered an opportunity to reinvent the current economic model towards more sustainable development. Commonly, a single method is applied to provide information on environmental impact assessment, however, it isn't sufficient, providing partial and sometimes even

counter productive indications. In order to provide information and support to policy makers and other stakeholders involved in decision making, this research goal faces the necessity to overlap the gaps of a single methodology evaluation, towards a more comprehensive assessment and, also, to compare LCA/emergy-based indicators and their variation, being able to understand when they can be effectively applied and how can we integrate their results. The developed framework is based on the integration and sequential application of Life Cycle Assesment (LCA) and Emergy Analyses (EMA), in three steps: (1) Ex-Ante LCA analysis - identification of the hot-spots; (2) EMA Scenario analysis, divided in: a) Business As Usual - evaluating the system as it is; b) Technology-based Efficiency Improvement - to suggest improvements of the investigated level through energy and material technological efficiency and c) Eco-Efficiency Implementation - achievable improvements of the environmental sustainability by substituting energy and material hotspots with renewable or less environmental costly input flows; and (3) Ex-Post LCA is performed for each EMA scenarios to detect their environmental burdens. It is possible to conclude that the presented framework is able to provide a multi-perspective set of indicators able to guide decision-making and management. The alternate application of LCA and EMA provides insights from the donor side (EMA) and from the consumer side (LCA), allowing a better and deeper understanding of the different investigated options and their feasibility, performances and burdens.

5.02.10
Uncertain and sensitive LCA models
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The complexity of models and the quality of inputs require LCA analysts to understand the impact of all potential sources of uncertainties on the model output, if LCA results are to be used effectively and responsibly in any decision-making process. It is clear that techniques of uncertainty analysis (UA) and sensitivity analysis (SA) are needed to elevate the quality of LCA and to increase the confidence in the results that LCA provides. In this contribution, we present a joint implementation of UA and SA that serves the needs of LCA, methodologically and practically. We will also show that care is needed to conceptually and practically implement UA and SA that are suitable to the computational and modelling needs of LCA. We will also discuss the limitations of uncertainty information used in standard UA and SA for LCA. Additional information in the extended abstract.

5.02.11
Framework for dynamic carbon accounting: development of complete carbon balances in LCA
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Renewable energy carriers and biomaterials originating from dedicated and residual forestry and agricultural biomass (e.g. energy crops, woody residues, and perennial grasses) are promoted as carbon neutral options to displace/offset fossil carbon and mitigate climate change. The exclusion of the temporal variability of biogenic carbon (Cbio) flows justifies carbon neutrality, and thus zero net CO2 emissions. The objective of this study is to incorporate the time dimension in the LCA of biomass supply chains to account for biogenic-sourced flows within dynamic LCA framework. A proposed framework, building on dynamic life cycle inventories, considers: a) upstream models for non-linear biomass growth, above- and belowground Cbio sequestration, soil organic carbon (SOC) dynamic associated to land uses, including management practices and yields; and b) downstream models for case-specific end-of-life pathways, eventually delaying emissions due to carbon storage. The dynamic models are designed to be coupled with outputs from any demand model (i.e. technical flows specifying the amount of biomass supply/use in a studied system or bioproduct) to develop complete dynamic carbon inventories (fossil + biogenic) and estimate the consequences of decision-induced changes (i.e. energy transition). The framework was tested with case studies on French energy policy, which propose alternative pathways of biomass-based (i.e. from forestry and agriculture) energy and transport. The overall results demonstrated that both Cbio sequestration and SOC dynamic vary according to the biomass-type and management practice in place (e.g. rotations, residue removal rates). Annual crops have no growth dynamic; however the residual proportion of the plant is an input to the soil relevant for SOC modelling. The mitigation results are sensitive to the model parameters (e.g. temperature), as well as to the modelling approaches undertaken, concerning the setting of the temporal boundaries (future or historic time perspective for forest carbon sequestration), shortening the rotation length, and variations in the residue removal rates. Coupling with demand models is useful for prospective evaluations and incorporation of socio-economic indicators in the assessment. Accounting for dynamic carbon flows is non-negligible as it reduces uncertainty and bias in defining actions to mitigate climate change.

5.02.12
Emerging Technologies and LCA: What LCA tells us about the status quo and future prospects
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Dye-sensitized solar cells (DSSC), organic photovoltaics (OPV), perovskite solar cells (PSC) and quantum-dot photovoltaics (QDPV) are emerging photovoltaic technologies (PVs). Due to their extremely thin-film technology and resulting material savings, we expect that they will have lower environmental impacts during their life cycle compared to commercial technologies. Based on LCA, the environmental impacts can be compared. The integration of LCA into the early-stage development is an important way to assess the current stage of development and show possible future prospects of technology development. For the case study of emerging PVs, we conduct a systematic review and harmonization to answer the questions what current LCAs can tell us about their status quo and the most influencing factors for technology development. A total number of 94 LCA datasets were considered and screened according to the key modelling assumptions (KEYAs: LCA type, technology scale), key performance parameters (KEYPs: efficiency, lifetime), and key indicators (KEYIs: GWP100). The systematic review showed that none of the reviewed LCA studies reported the technoloy scale nor the LCA type, i.e. distinguished between conventional and prospective LCAs. The harmonization of the KEYI to a consistent set of KEYPs was performed according the approach of [1]. The harmonized GWP100 describe the technology development and status quo of the four emerging PVs in comparison with benchmarks of commercial first- and second-generation PVs. This comparison confirms that the KEYIs are mostly influenced by the two KEYPs and of the assessed technology scale. Whereas OPV as the most mature PV could compete with the benchmarks, the other three PVs show higher GWP results and indicate their early-stage of development of the reviewed LCA data sets. For them, the necessary improvements of the two KEYPs and the technology scale are assessed based on a scenario analysis. The application of LCA for emerging technologies has contradictory requirements. On one hand, the room for maneuvering in terms of freedom of design is largest in the early stages of technology development. On the other hand, uncertainty of the technology alternatives compromises the usefulness of results from LCA for decision support. The developed harmonization approach for emerging PV presents a structured way not only to reduce uncertainty but also to extract significant information from the point of view of different stages of technology development.

5.02.13
Environmental impacts of emerging PV technologies: LCA meta-analysis as an early stage guidance tool
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Innovation in photovoltaics (PV) is mostly driven by the cost per kilowatt ratio, making it easy to overlook environmental impacts of technological enhancements during early research and development stages. As PV technology developers quickly introduce novel materials and manufacturing methods, the well-studied environmental profile of conventional silicon-based PV may change considerably. In this study we investigate existing trends and hotspots across different types of emerging PV technologies by conducting a systematic review and meta-analysis of life cycle assessments (LCA). In order to incorporate as many data points as possible we apply a comprehensive harmonization procedure producing over 600 impact data points for organic, perovskite, dye-sensitized, tandem, silicon and other thin film cells. We also investigate and discuss how panel and balance of system components affect environmental footprints in comparable installations. Despite the large uncertainties and variabilities in the underlying LCA data and models, the harmonized results show clear positive trends across the sector. Seven potential hotspots are identified for specific PV technologies and impact categories. The analysis offers a high-level guidance for technology developers to avoid introducing undesired environmental trade-offs as they advance to make PV more competitive in the energy markets.

LCA for Urban and Territorial Footprinting: Advancements, Trends and Applications to Promote Sustainable Consumption Patterns and Territorial Management

5.03.1
Application of LCA and building energy simulation in the early design of an urban project
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Decisions influencing the most the environmental performance of an urban project are made during early design. Applying LCA at this phase requires an appropriate methodology, e.g. combining a simplified input and the use of default values.

Impacts are highly influenced by the buildings energy performance, so that energy simulation is also useful as well as an occupants' model regarding e.g. the number of inhabitants or employees, their water consumption, use of transport, and waste management. Urban projects are complex systems, including buildings, streets, networks like water mains, electricity grid, and district heating. Specific tools have been developed to simplify LCA application, allowing e.g. automatic evaluation of materials and products quantities (e.g. tons of concrete, m2 of windows) from a graphical modeller usable by architects and urban designers. A first version of such a tool was developed in 2006, then improved and applied to various projects. This conference paper presents the method, and an example case study in Greater Paris Area. The project includes 170 000 m^2 offices, 42 000 m^2 dwelling, 5 000 m^2 hotel, 5 000 m^2 students home, 15 000 m^2 streets and 8 000 m^2 green spaces on a 5 hectares ground. The objectives of the LCA study are to reduce the environmental impacts of this project, and a first step is performed before architectural design, in order to elaborate the client's brief. The project developer asks which are the main contributors (materials, energy, water, waste, transport…) in order to identify priorities, and which requirements should be included in the brief (e.g. choice of wood or concrete structure, energy performance level). In order to answer these questions, building information models were developed for a concrete structure base case and several alternatives, e.g. considering a timber structure and varying the ratio of glazing area on facades. Thermal simulation was used, allowing heating and cooling loads as well as thermal comfort, addressed in the functional unit, to be evaluated. Overheating risk was detected, particularly for the timber structure and the highest glazing ratio. LCA was used to evaluate environmental impacts, identify main contributions, compare various alternatives with benchmark values and propose performance targets that can be required in the client's brief. Finally, perspectives are suggested for further research.

5.03.2
Environment Assessment of people's daily mobility in an urban area: coupling a Land-Use and Transport Interaction model with Life Cycle Assessment

C. Francois, Laboratoire Ville Mobilité Transport / Laboratoire Ville Mobilité Transport; J. Nicolas, Universtité de Lyon-ENTPE / Laboratoire Aménagement Économie Transports; N. Gondran, Ecole des Mines Saint-Etienne / Génie de lEnvironnement et des Organisations

Regarding the complexity of the urban system, the environmental assessment of its associated people's daily mobility needs to consider social, spatial and technological dimensions. Mobility is indeed greatly affected by both urban forms (e.g. density, accessibility, functional diversity, design …) and socio-economic characteristics of people (e.g. income, age, education, car ownership …). And associated environmental impacts are correlated with transport technologies performances including vehicles, fuels and infrastructures. Specific issues also arise from the territorial level, especially from the urban stakeholders who need more spatial approaches on both characterisation and analysis of mobility. To integrate these different parameters, the proposed method couples two tools developed in two distinct scientific fields - land-use planning and environmental sciences: with on the one hand, Land-Use and Transport Interaction models (LUTI) and, on the other hand, Life Cycle Assessment (LCA) methods. Together, these tools can account for both technological processes involved in daily mobility, as well as interactions among stakeholders and territories. The LUTI model used, SIMBAD, was developed on the Lyon urban area and it represents the daily mobility through a dynamic approach with feedback loops between land occupancy (residential, employment, commerce, leisure …) and transport performance (travel time and cost). The LCA perimeter includes all terrestrial and motorized transport modes of Lyon urban area (car, bus, train, subway, tramway) and their dedicated infrastructures. Most inventories are based on the EcoInvent database. To represent better atmospheric emissions generated by the use of vehicles, the Copert emission model was used, with spatial specifications on routes, vehicles fleets, technologies and usages. By manipulating social, spatial and technological objects, this method allows multiple interpretations of the numerous environmental impact of daily mobility. Personal cars are the main source of environmental impacts with a significant contribution of indirect processes (vehicles manufacturing, infrastructure, fuel supply). Nevertheless, spatial analysis presents a high heterogeneous distribution within households and territories. Finally, using the SIMBAD-LCA method, health impacts analysis of local pollutants can be improved by crossing emissions and density levels at fine-resolution.

5.03.3
The City Environmental Footprint: development and application of an adapted LCA method for urban footprinting

N. Mirabella, KU Leuven / Department of Architecture; k. allacker, KU Leuven / architecture; S. Sala, European Commission - Joint Research Centre / Bioeconomy unit

Cities are gaining momentum in the sustainability debate, and they are considered favorable places to take actions to promote more environmental friendly consumption practices. LCA is promising as it is considered the most robust methodology to tackle the challenge but researches show that numerous constraints and bottlenecks exist to make it applicable at city scale. Methodological advancements and research efforts are therefore necessary for each life cycle stage. In order to comply to these ambitious tasks, the City Environmental Footprint (City EF) has been developed. Being systemic and systematic, its aim is the holistic assessment of urban environmental impacts, as result of activities performed in key urban sectors (built environment, waste and water management, mobility, production and consumption sectors) by stakeholders (e.g. residents). This facilitates the identification of major hotspots and drivers in the city, and, based on these, areas of priority interventions and proposals for alternative scenarios. In the City EF, specific improvements for a comprehensive and efficacious urban footprinting are proposed, both system-related (city as object of investigation), than methodology-related (LCA as effective methodology). The firsts mainly regard the definition of appropriate system boundaries, functional unit(s) and allocation procedures (goal & scope phase), as well as attempts to solve comparability issues between cities. The seconds attempt at combining a hybrid top-down and bottom-up approach to effectively perform both life cycle inventory and interpretation phases. In order to solve the methodological limitations previously identified, the City EF addresses these issues and proposes potential solutions in five consecutive stages (four life cycle stages, plus one preliminary qualitative step complementing goal and scope phase).Furthermore a first application of City EF was performed on the city of Leuven (Belgium) to test the method, and its feasibility. The aims of the present contribution are threefold: i) an overview of the City EF, its methodological proposals for urban footprinting, and how it accomplishes to the tasks; ii) show the results of its first application to the city of Leuven; iii) based on these, providing specific insights and critical reflections for future applications, including identified strong and working points, as well as potential research outlooks.

5.03.4
Coupling Optimisation with territorial LCA to support agricultural land-use planning - Case study in Wallonia

T. Ding, Université Libre de Bruxelles; B. Steubing, Leiden University / Institute of Environmental Sciences (CML); W. Achten, Université Libre de Bruxelles / Geoscience, Environment and Society

Agricultural land use is fundamental for human life, but < pwa data-pwa-id="pwa-AF11625DB5A723EA8B5B344C3351E716" data-pwa-rule-id="PASSIVE_VOICE" data-pwa-category="style" data-pwa-hint="Passive verbs make your writing less direct. Try to use an active verb instead." data-pwa-suggestions="we also consider it~I also consider it~they also consider it" data-pwa-heavy="false" data-pwa-dictionary-word="it is also considered" class="pwa-mark pwa-mark-done">it is also considered as one of the major sources of environmental damage. The bioeconomy society emphasises the significance of land use for biomass production for different purposes (e.g. food, feed, fibre, fuel, etc). However, it faces the challenge of conflicting situations where limited land resources < pwa data-pwa-id="pwa-B071325E4C9978BFF540B297790C1FD1" data-pwa-rule-id="PASSIVE_VOICE" data-pwa-category="style" data-pwa-hint="Passive verbs make your writing less direct. Try to use an active verb instead." data-pwa-suggestions="" data-pwa-heavy="false" data-pwa-dictionary-word="are claimed" class="pwa-mark pwa-mark-done">are claimed for an increasing diversity of uses. In such a context, it is desirable to think the land allocation to the different purposes demanded, wisely to come to optimal land use plans. Proposed land use plans need to undergo the environmental assessment, to analyse the environmental performance of a plan at the specific geographical zones, to propose improvement alternatives, and < pwa data-pwa-id="pwa-16EFFC699D9D91AC0324211B5BB761C4" data-pwa-rule-id="SPLT_INFI" data-pwa-category="grammar" data-pwa-hint="Split infinitive. Some people strongly object to this type of construction, especially in formal writing. If your reader is likely to object to the split infinitive, rewrite the sentence to avoid its use." data-pwa-suggestions="" data-pwa-heavy="false" data-pwa-dictionary-word="to iteratively lead" class="pwa-mark pwa-mark-done">to iteratively lead to optimal planning. However, this iterative process might get too time and resource consuming when land-use purposes are increasingly diversifying. As an alternative, < pwa data-pwa-id="pwa-93AC6AE5E7934AE95494C3F19826292A" data-pwa-rule-id="PASSIVE_VOICE" data-pwa-category="style" data-pwa-hint="Passive verbs make your writing less direct. Try to use an active verb instead." data-pwa-suggestions="they could equip the process~we could equip the process~it could equip the process~I could equip the process" data-pwa-heavy="false" data-pwa-dictionary-word="the process could be equipped" class="pwa-mark pwa-mark-done">the process could be equipped with a combination of suitable assessment methods and optimisation models< pwa data-pwa-id="pwa-6EC13A12FE03B8A75110B44BDD10BAEF" data-pwa-rule-id="EN_DASH_SPACING" data-pwa-category="grammar" data-pwa-hint="En-dashes should never have a space on either side." data-pwa-suggestions="–~—" data-pwa-heavy="false" data-pwa-dictionary-word=" – " class="pwa-mark pwa-mark-done"> – which can exhaustively search the different land-use combinations and build up environmentally optimised land-use scenarios. This research aims at coupling "territorial LCA" (< pwa data-pwa-id="pwa-907C106DFC6898A5FF328CA540C27756" data-pwa-rule-id="CAPITALIZATION" data-pwa-category="grammar" data-pwa-hint="Unusual capitalization. Have you capitalized the wrong letter in this word?" data-pwa-suggestions="" data-pwa-heavy="false" data-pwa-dictionary-word="tLCA" class="pwa-mark pwa-mark-done">tLCA), with mixed-integer linear programming (MILP) optimization algorithms. The recently developed < pwa data-pwa-id="pwa-92FAE22901241D67F7F3F560BAEDB4E6" data-pwa-rule-id="CAPITALIZATION" data-pwa-category="grammar" data-pwa-hint="Unusual capitalization. Have you capitalized the wrong letter in this word?" data-pwa-suggestions="" data-pwa-heavy="false" data-pwa-dictionary-word="tLCA" class="pwa-mark pwa-mark-done">tLCA framework provides a fundamental platform to assess the environmental impact of current or alternative land plans at the territorial level. < pwa data-pwa-id="pwa-DA57E52A1F03BB1BCA486069456EA952" data-pwa-rule-id="PASSIVE_VOICE" data-pwa-category="style" data-pwa-hint="Passive verbs make your writing less direct. Try to use an active verb instead." data-pwa-suggestions="We have successfully applied the tLCA framework~I have successfully applied the tLCA framework" data-pwa-heavy="false" data-pwa-dictionary-word="The tLCA framework has been successfully applied" class="pwa-mark pwa-mark-done">The tLCA framework has been successfully applied to identify the impact displacements among life-cycle stages, impact categories and locations (inside and outside the territory). By linking < pwa data-pwa-id="pwa-F9361F4D75CDFEDC19BB8B1671F12A92" data-pwa-rule-id="CAPITALIZATION" data-pwa-category="grammar" data-pwa-hint="Unusual capitalization. Have you capitalized the wrong letter in this word?" data-pwa-suggestions="" data-pwa-heavy="false" data-pwa-dictionary-word="tLCA" class="pwa-mark pwa-mark-done">tLCA with MILP optimisation we aim to combine the analytical < pwa data-pwa-id="pwa-ABBC8958520D5F60787E70E9BC31788D" data-pwa-rule-id="CAPITALIZATION" data-pwa-category="grammar" data-pwa-hint="Unusual capitalization. Have you capitalized the wrong letter in this word?" data-pwa-suggestions="" data-pwa-heavy="false" data-pwa-dictionary-word="tLCA" class="pwa-mark pwa-mark-done">tLCA power at territorial level with the scenario-building power of optimisation models in dealing with large-scale planning issues. As a first 'proof of < pwa data-pwa-id="pwa-661A54119968D2600C88FB4B524DA6DB" data-pwa-rule-id="DET_CG_2" data-pwa-category="grammar" data-pwa-hint="Possible missing determiner" data-pwa-suggestions="a concept~the concept" data-pwa-heavy="false" data-pwa-dictionary-word="concept" class="pwa-mark pwa-mark-done">concept' the combined framework (< pwa data-pwa-id="pwa-AF5DCA350D9DDACDA5AEC8925E474A3D" data-pwa-rule-id="CAPITALIZATION" data-pwa-category="grammar" data-pwa-hint="Unusual capitalization. Have you capitalized the wrong letter in this word?" data-pwa-suggestions="" data-pwa-heavy="false" data-pwa-dictionary-word="tLCA" class="pwa-mark pwa-mark-done">tLCA-Optimisation) < pwa data-pwa-id="pwa-9B11A3C6BEE94D6CF2E117B57EE1380D" data-pwa-rule-id="PASSIVE_VOICE" data-pwa-category="style" data-pwa-hint="Passive verbs make your writing less direct. Try to use an active verb instead." data-pwa-suggestions="" data-pwa-heavy="false" data-pwa-dictionary-word="was applied" class="pwa-mark pwa-mark-done">was applied on a case study of land planning for < pwa data-pwa-id="pwa-41BCBECBEC0BECC2F97C0C82B5FD19E1" data-pwa-rule-id="SIMPLE_SPELLING" data-pwa-category="spelling" data-pwa-hint="Unknown word: bioenergy" data-pwa-suggestions="Bioenergy~bio-energy" data-pwa-heavy="false" data-pwa-dictionary-word="bioenergy" class="pwa-mark pwa-mark-done">bioenergy crops in Wallonia (South region in Belgium). The first result shows a distinction of in- and off-territory damages of the land use for < pwa data-pwa-id="pwa-9A4B1FF737F9C13358C7FF49107B8F7C" data-pwa-rule-id="SIMPLE_SPELLING" data-pwa-category="spelling" data-pwa-hint="Unknown word: bioenergy" data-pwa-suggestions="Bioenergy~bio-energy" data-pwa-heavy="false" data-pwa-dictionary-word="bioenergy" class="pwa-mark pwa-mark-done">bioenergy crops production. The second result presents a spatial configuration of the new crops, which will have the lowest environmental damages. These preliminary results illustrate the feasibility of < pwa data-pwa-id="pwa-367E07B7A1E72191E7C4055C450D0A5A" data-pwa-rule-id="CAPITALIZATION" data-pwa-category="grammar" data-pwa-hint="Unusual capitalization. Have you capitalized the wrong letter in this word?" data-pwa-suggestions="" data-pwa-heavy="false" data-pwa-dictionary-word="tLCA" class="pwa-mark pwa-mark-done">tLCA-Optimisation coupling and show great potential in supporting land planning issues at the territorial scale.

5.03.5
LCI regionalization for urban systems: sensitivity to parameter and scenario uncertainties
A. Tanguy, LIRIDE-CIRCERB Université de Sherbrooke / Civil Engineering; P. Blanchet, CIRCERB - Universite Laval / Department of Wood and Forest Sciences; B.M. Amor, Liride - Université de Sherbrooke / Department of Civil and Building Engineering

Inventory regionalization in LCA refers to any efforts aiming to increase the representativeness of unit processes considering a specific geography. Methods relying on impact contributions and uncertainties analyses were developed to prioritize regionalization. But while the regionalization procedure itself received a lot of attention, the type of uncertainties fed into the procedure is still less studied. Indeed, parameter uncertainty (i.e. Pedigree matrix) is generally solely considered for inventory regionalization. However, scenario uncertainty, which refers to variability due to choices, has not been addressed, whereas it was found influential in LCA results. This type of uncertainty is particularly relevant for urban systems, whose main characteristics (density, mobility) are spatially variable. It is our hypothesis that the choices related to the type of urban systems assessed can thus greatly affect regionalization prioritization. Therefore, the objective of this study is to propose a method including both parameter and scenario uncertainties in order to guide inventory regionalization. Parameters refer here to inputs that can be defined at the country level, such as impact factors of materials. Scenarios are linked to the system's spatial setting, that is more specific to the system under study than to the country. It includes different ranges of population density, for the system itself and its surroundings (affecting commuting distances and transport modal shares). In order to identify the most influential inputs, global sensitivity analyses are performed using Monte Carlo simulations for uncertainties propagation and Sobol indices to assess the contribution of each input (16 in total) to the results' total variance. Applied to the case of a neighbourhood in the Quebec Province, Canada, the first part of the analysis considers simultaneously parameter and scenario uncertainties in order to single out impact categories not sensitive to different spatial settings. As variation domains for scenarios inputs are then reduced (i.e. scenarios are fixed), the second part shows whether or not influential inputs are the same for each scenario and whether or not they are related to the national (e.g. materials' impact factors) or local contexts (e.g. commuting distances). Therefore, this study opens the discussion on the proper scale of intervention to increase the robustness of large-scale LCA results.

5.03.6
Poster spotlight: A methodology to identify sustainability indicators in small, medium and large cities in Spain
S. González-García, University of Santiago de Compostela / Department of Chemical Engineering
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5.03.7
Poster spotlight: Exploring complementarity of Life Cycle Thinking & Landscape and Urban Planning towards sustainable urban co-design Case study: a last kilometer electric tricycle-based logistic technology
G.B. Bouillass, MINES ParisTECH - OIE Research Center / Centre for Observation Impacts and Energy
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Life Cycle Impact Assessment and Ecosystem Impact Assessment: Strengthening the Link Between LCA and Ecology

5.04.2
Factoring the uncertainty of underspecified archetypes on LCA results
M. Fernandez Astudillo, 2.-0 LCA consultants / Civil Engineering

Life cycle impact assessment (LCIA) methods model different exposure situations using archetypal scenarios. Archetypes are an effective way to account for the variability of characterisation factors (CF), given the large variability of CF for the same substance in different archetypal scenarios. For example, the carcinogenic impacts of cadmium emitted to soils vary three orders of magnitude depending on whether it is emitted to agricultural or industrial soils. LCA software allows to have "unspecified" archetypes, and the CFs for these cases are sometimes defined by modeller developers other times by those who implement the model. Not specifying the archetype introduces uncertainty that is not accounted in LCA studies, potentially leading to misleading conclusions. In this presentation I propose a generic procedure to define the CF of elementary flows emitted to undefined archetypal scenarios as a probability distribution and implement the uncertain "version" of the impact category in the open-source LCA framework Brightway 2. The CF for a given substance and "compartment" and undefined archetype is logically a value within the range of CF for defined archetypes. This range is used to define the minimum and maximum of the probability distribution. The CF used in the original implementation of the method is used as an estimator of the mode. The method allows to choose between the uniform and the triangular probability distribution. The methods are tested with the ILCD 2018 method and applied to a sample of activities of ecoinvent 3.6 consequential, in an attempt to identify activities affected by *archetype uncertainty* and the extent of these effects. Of the 14 impact categories that constitute ILCD midpoint method five have uncertainty related to underspecification of archetypes. *Human health - carcinogenic effects, human health – non-carcinogenic effects* have the cfs with the largest uncertainty. The range of CF within archetypes is up to 593 times the default CF. Many activities in ecoinvent are not substantially affected by archetype uncertainty either because archetypes are properly defined or because the uncertainty range of dominant elementary flows is relatively narrow. However, for a group of activities, the effect is substantial. Acknowledging archetype uncertainty can help to prioritise inventory improvement and reduce the risk of wrong interpretation of LCA studies.

5.04.3
Towards interim characterization factors to account for the dissipation of non-energetic abiotic resources in life cycle assessment
A. Charpentier Poncelet, ISM UNIVERSITE DE BORDEAUX / Institute of Molecular Sciences ISM - The Life Cycle Group CyVi; P. Loubet, University of Bordeaux / ISM CyVi; B. Laratte, ENSAM / ENSAM I2M; G. Sonnemann, University of Bordeaux / ISM-CyVi
Life cycle assessment is a valuable tool to assess the ecological performance of a product system holistically. However, it is still an imperfect tool in which some of the impact categories especially need to be revisited. Abiotic resource use is an impact category for which much debate has been going on in the last years. Methodological choices in the existing indicators are often criticized, and the usefulness of results is of questionable relevance to decision takers in the industry or for policy makers. Also, concerns about circular economy and resource efficiency are not reflected in these assessment models. Dissipation of these resources has been identified as a promising way forward to get an intelligible method reflecting current concerns for resource use. Dynamic material flow analysis data can serve as an important basis to account for dissipated flows in a product system at different scales. The translation of data from dynamic material flow analysis into dissipation curves can give important information of a resource’s lifetime in technosphere and its potential to be recycled. This information can then be uptaken into characterization factors to account for dissipation in life cycle assessment. In this light, interim characterization factors using dynamic material flow data are proposed to account for dissipation of several abiotic resources at the global scale in life cycle assessment. This life cycle assessment method gives information concerning which resources are consumed in such a way that future recycling or reuse is not feasible, and therefore potentially provide valuable information to the various life cycle assessment users.

5.04.4
Poster spotlight: Integration of ProScale toxicity potential assessment in LCA applied to Utility Poles
T. Rydberg, IVL Swedish Environmental Research Institute
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5.04.5
Poster spotlight: Development of a parsimonious characterisation model to address space debris emission-related damages for the LCA of space systems
P. Loubet, ISM / ISM-CyVi
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5.04.6
Framework to Define Environmental Sustainability Boundaries
E.B. Vea, Technical University of Denmark / Quantitative Sustainability Assessment, Department of Technology, Management and Economics; M. Ryberg, Technical University of Denmark (DTU) / Quantitative Sustainability Assessment, Department of Management Engineering; K. Richardson, University of Copenhagen / Center for Macroecology, Evolution, and Climate, Natural History Museum of Denmark; M.Z. Hauschild, Technical University of Denmark / Quantitative Sustainability Assessment, DTU Management
Knowing the actual boundaries for environmental pressures that exist in the environment, can help us steer societal development towards environmental sustainability. Evaluating the environmental performance of current efforts in relation to environmental boundaries, allows us to answer whether actions are good enough and, thereby, support informed decision-making. However, boundaries are often communicated as objective and scientific “facts”, underplaying the subjective aspects underlying their definition and they are not always internally consistent. To facilitate application of environmental sustainability boundaries, we propose a framework for defining, communicating and adopting environmental boundaries for use in environmental assessments. The framework was elaborated by analyzing the structure and variations in methodological choices and elements across seventeen existing boundary approaches. It addresses five main aspects of defining environmental boundaries (sustainability objective, boundary principle, uncertainty principle, accepted levels of impacts, and scientific estimate), and their associated recommended practices. The framework was demonstrated by analyzing two examples of existing boundary approaches, revealing their strengths and weaknesses, and aspects to consider when adopting them in environmental sustainability assessments. For example, the Planetary Boundaries approach has an over-arching sustainability objective but is not internally consistent across its individual boundaries in terms of e.g. the applied boundary principle. While the boundaries are based on a general perception of thresholds and earth as a resilient system, some of the individual boundaries are defined according to alternative and more conservative boundary principles (e.g. according to natural levels), due to lack of knowledge. If adopting the PBs in an environmental assessment context, the practitioner should be aware of these inconsistencies. For instance, if using the environmental boundaries as normalization references in LCA, where the impacts are to be compared across impact categories, it is important that the environmental boundaries are based on the similar principles in terms of underlying science and value choices. We recommend applying the framework to enable a structured appraoch to define, critically analyze and select sustainability boundaries and, thereby, improve and facilitate the use of environmental boundaries in sustainability assessments.

5.04.7
Review of life-cycle based methods for absolute environmental sustainability assessment
A. Bjørn, John Molson School of Business, Concordia University / Department of Management; C. Chandrakumar, Massey University / New Zealand Life Cycle Management Centre; A. Boulay, CIRAIG - École Polytechnique de Montréal / Chemical engineering department; G. Doka, Doka Life Cycle Assessments; K. Fang, Leiden University / School of Public Affairs; N. Gondran, Ecole des Mines Saint-Etienne / Génie de lEnvironnement et des Organisations; M.Z. Hauschild, Technical University of Denmark / Quantitative Sustainability Assessment, DTU Management; A. Kerkhof, Navigant, a Guidehouse company; H. King, Unilever; M. Margni, CIRAIG - École Polytechnique de Montréal / Mathematical and Industrial engineering; S. McLaren, Massey University / New Zealand Life Cycle Management Centre; C. Mueller, University of York / Department of Environment and Geography; M. Owsianiak, Technical University of Denmark / Quantitative Sustainability Assessment, Department of Technology, Management and Economics; G.M. Peters, Chalmers University of Technology / Division of Environmental Systems Analysis; S. Roos, Swerea IVF AB / Chemical Engineering; S. Sala, European Commission - Joint Research Centre / Bioeconomy unit; G. Sandin, SP Technical Research Institute of Sweden; S. Sim, Unilever RD Colworth / Safety and Environmental Assurance Centre SEAC; M. Vargas-Gonzalez, Quantis; M. Ryberg, Technical University of Denmark (DTU) / Quantitative Sustainability Assessment, Department of Management Engineering
The magnitude of human induced pressure on key environmental systems is starting to exceed the carrying capacity of these systems. This has spurred development of LCA-based methods for absolute environmental sustainability assessment (AESA). The purpose of AESAs is to evaluate if an anthropogenic system can be considered environmentally sustainable relative to the environmental carrying capacities of environmental systems. With the increasing number of AESA methods, developers and users need a comprehensive overview of LCA-based AESA methods. This study presents the findings from a review of existing LCA-based AESA methods and their applications. It is intended to guide practitioners and stimulate further AESA development. A four-step review sequence was used to identify relevant LCA-based AESA studies. We identified 44 studies of which 33 present new methods and 11 apply existing methods. The impact categories covered in LCA-based AESA methods can generally be categorized as based on either the Planetary Boundaries (PB) concept or on existing impact categories used in LCA. The most frequently assessed impact category was Climate change while impact categories on land use and introduction of novel entities (e.g. chemicals) are seldom included. 11 studies include regionalized LCIA for impact categories, e.g. water use. We identified 10 distinct principles for sharing carrying capacity; the “equal per capita” sharing of carrying capacity was most often used. We provide recommendations to practitioners on selection of AESA LCIA-method (i.e. based on the PB-concept or existing LCIA indicators) and impact category coverage. Practitioners should adhere to existing best practice for compiling life-cycle inventory. However, in AESA, elementary flows should be expressed as flowrates in mass per unit of time. Practitioners should be transparent on selection and use of sharing principles and evaluate sensitivity of AESAs by testing different sharing principles. We recommend adoption of common terminology and a framework for methodological AESA development. Additionally, there is a need for further development of existing AESA LCIA-methods, for example, to improve impact coverage as well as the individual modelling of impact categories. This includes research on regionalization of AESA LCIA-methods. To increase uptake of LCA-based ASEAs, we recommend development of training materials and guides for practitioners, as well as integration of AESA methods into software.

5.04.8
The fate of evaporated water across basin boundaries - Implications for the Impact Assessment in Water Footprinting
A. Link, TU Berlin / Chair of Sustainable Engineering; R. van der Ent, Delft University of Technology; M. Berger, TU Berlin / Chair of Sustaiable Engineering; S. Eisner, Norwegian Institute of Bioeconomy Research (NIBIO) / Center for Environmental Systems Research; M. Finkbeiner, Technische Universität Berlin / Chair of Sustainable Engineering
Due to the increasing relevance of analysing water consumption along product life cycles, the method of Water Footprinting has gained rising attention in the last years. In this context, the water accounting and vulnerability evaluation model (WAVE+) was developed. The model provides the water depletion index (WDI) as an effect factor which describes the vulnerability of basins to freshwater depletion based on a consumption-to-availability ratio derived from the

hydrological model WaterGap3. Additionally, it is the first model which includes a fate factor describing atmospheric moisture recycling within basins. This basin internal evaporation recycling (BIER) can significantly reduce the water consumption volumes within specific drainage basins. The WAVE+ method combines BIER and WDI into an integrated characterization factor available for more than 8.000 basins. However, the model does not yet include evaporation recycling patterns across basin boundaries. Thus, the aim of this research is to provide fate factors describing the basin external evaporation recycling (BEER) and to integrate them into the impact assessment of the WAVE+ model. Besides the provision of results on a basin scale, results on a country level are targeted. Fate factors with regards to the re-precipitation of evaporated water were determined with the numerical moisture tracking model WAM-2layers. The tracking was conducted on a 1.5° * 1.5° grid and was based on reanalysis data from the ERA-Interim database. With regards to evaporation and precipitation data, the Era-Interim data were replaced by data of the hydrological model WaterGap3 to ensure consistency with the WAVE+ model. Gained results on a grid cell basis were aggregated to basin and country scales. In order to integrate the new fate factors into the Wave+ model, the old characterisation model was modified while considering cross-boundary patterns of re-precipitation. As a result of this research, new fate factors for the re-precipitation of evaporated water were successfully developed for 8223 basins and 265 countries. Their implementation into the Wave+ method will lead to characterisation factors for the vulnerability of basins to cross-boundary freshwater depletion. This will create new perspectives with regards to the evaluation of water consumption along product life cycles and will enable to make statements on both – the potential impacts of water consumption within and outside of the considered region.

5.04.9
Improvement of the water footprint AWARE model

A. Hélias, Irstea / ITAP ELSA-PACT; P. Roux, INRAE - Elsa-Pact Chair / ITAP ELSA-PACT

The Available WAter REmaining (AWARE) model highlights the importance of considering consumption rather than withdrawal and takes into account spatial variability. It results from a massive and collective effort on behalf of the Water Use in LCA (WULCA) working group. The AWARE model provides a consensual, operational and recommended indicator for addressing and comparing water impacts, and fully succeeds in this purpose.The present work discusses the shape of the model, as well as associated limitations on its range of validity, which do not distinguish between regions that are more degraded than fair. A subsequent improvement is then proposed. This improvement is based on the ratio of the demand (requested by the ecosystem) to availability (minus the effective human appropriation), more simply named the Demand-To-Remaining (DTR). It provides useful and straightforward information representing the current state. follows the common practice in LCIA by (1) the definition of a relationship modelling the impact according to human intervention and (2) the use of marginal approach for determining the characterisation factor (CF). The significance of the approach is addressed by the sensitivity of the CFs according to the components of the model. As expected, the CFs of the AWARE and DTR models increase linearly with the area, in the same manner. In both situations, an increase in C_H *(the human water consumption)* produces the same result as a decrease in A *(availability)*. The increase grows faster when the AWARE model upper boundary is being reached, and when the complete human appropriation of water ($C_H = A$) is being attained for the DTR model. This implies that the relationships present similar features but at different intervals, without any discontinuities for DTR.AWARE consensus model brings a major benefit to the community by proposing a shared standard. However, AWARE relationship is only defined when human consumption has spared sufficient water for an ecosystem in fair condition and loses its validity for more severe situations. By defining impact as the fraction of ecosystem demand on what is left by human activity, the DTR model proposed in the present work makes it possible to overcome this limitation. This improvement is mathematically sound, all the while satisfying the same expectations as the AWARE model.

5.04.10
Guiding the interpretation of toxicity-related impacts in the Environmental Footprint 3.0: a case study on the consumption of EU citizens

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Life Cycle Assessment (LCA) aims to estimate all potential impacts on ecosystems quality and human health of products or services, during all phases of their life cycle: from the extraction of raw materials to their disposal. Toxicity related impacts on human health (cancer and non-cancer) and on freshwater are quantified by means of models, accounting not only the intrinsic toxicity of substances but also their distribution in the environment and bioavailability. In 2018, the Joint Research Centre of the European Commission (EC-JRC) released a new set of toxicity Characterisation Factors (CFs) in the version 3.0 of the Environmental Footprint (EF). The new CFs have been calculated using new sources of physico-chemical and toxicological data (REACH, EFSA and PPDB) to broaden the number of characterised flows and to ensure the coverage of chemicals on the EU market. Regarding freshwater ecotoxicity, the impact model has implemented the new Pellston recommendations, including the adoption of chronic data and HC20 for the effect factor. The goal of the study is to guide EF 3.0 users in the interpretation of toxicity results by (a) detailing the differences between EF2.0 (based on USEtox®) and EF3.0, and (b) applying the two versions of the method to a case study towards observing main differences and potential limitations. When comparing the EF 3.0 results with the previous version (EF 2.0), a larger number of chemicals can be characterised due to the new data sources employed. This allows noticing contributions of flows that would be otherwise neglected, such as aluminium. As well, the top contributors and their ranking change between the two methods. The five areas of consumption were analyzed separately by considering the Basket of representative Products (BoP) for each of them. Analysing the principal contributors in common to all BoPs offers an idea of the possible background noise present in all processes, such as petroleum extraction in the freshwater ecotoxicity impact category. In conclusion, the larger set of CFs available in EF 3.0 offers a more complete description of the toxicity impacts and the use of a robustness factor effectively reduce the overall contribution of metals. For a correct interpretation, it is recommended to analyse organics, inorganics and metals separately and to consider aspects regarding the inventory data and the characterisation factor.

5.04.11
Development of Eco-factors for the European Union based on the Ecological Scarcity Method

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Recent methodological developments have given new momentum to weighting as an optional element in life cycle impact assessment (LCIA). The Ecological Scarcity Method (ESM), published and developed in Switzerland, is one method for Distance-to-Target (DtT) weighting. It is based on the ratio of desired policy targets to current environmental situations. The purpose of this study is the application of the ESM to the European Union (EU) as well as its application in a case study. Therefore a set of eco-factors was determined, including normalization and weighting factors for a wide range of emissions and resource uses. The eco-factors consider the current specific environmental situation and policy targets of the EU for each substance. Data collection for a wide range of emissions and resource uses was carried out, as well as the identification of corresponding binding and non-binding policy targets. As a result, a set of 123 eco-factors for various emissions and resource uses was developed and applied to the current environmental situation of the EU in a case study. The aggregated results showed a high relative importance for the environmental issues climate change (28%) and main air pollutants (30%). The sensitivity analysis demonstrated lower results when considering only short-term or binding targets. The ESM was transferred to the EU as a DtT weighting method for LCA practitioners. Besides the promotion of forward-looking actions in the EU, future developments could aim at other countries and regions. Not all substances could have been covered due to the lack of quantitative policy targets and should be integrated in future methodological developments.

5.04.12
Biodiversity impact of fisheries

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Fisheries modified all the marine ecosystems. If Life Cycle Assessment (LCA) successes to quantify the land use its consequences on the environment (the ecosystem quality area of protection (AoP)) the impact of sea use on ecosystems appears poorly assessed by LCA community. To our knowledge, there is no approach assessing ecosystem impact of fisheries (the withdrawal of fish) which would be compliant with the current guideline. This lack of indicators is highlighted for comparison between sea- and agricultural-based products: the impacts are not expressed in the same unit and are not comparable. With the current LCIA possibilities, the causal effect on ecosystem quality of fishing cannot be represented, that means its impact equals zero. The aim of the present work is to solve this situation proposing operational CFs for global fisheries. The impacts leading to ecosystem quality are often addressed with CF = FF ×EF. For a given intervention, the characterization factor (CF) is the product of the fate factor (FF) with the effect factor (EF). FF allows the representation of the time period during which the effect occurs and the second gives the associated effect. In a recent work, we defined CFs for biotic resources (natural resource AoP) based on population dynamic model and marginal approach. This approach is based on the disappeared fraction of the stock (the given species in its habitat) and is used here as EF. Most impacts leading to ecosystem quality result from substance emissions. In this context the FF represents the persistence of the involved substance in the media. For a given compartment, it can be assimilated to the inverse of the sum of the removal rates or to a residence time. The FF for an impact on the ecosystem of fisheries is reversed since it results from a resource withdrawal, but the principle remains the same. By analogy, we defined the fate factor as the inverse of the growth rate of the fish stock. We have calculated CFs for almost 5000 fish stocks identified by FAO, using both marginal and average approaches and considering vulnerability scores to convert regional PDF to global PDF. CFs are spread over ten orders of magnitude but with the interquartile over less than two. The global CFs vary over 13 orders of magnitude but here again the interquartile is much more compact with two orders of magnitude. As illustration, four contrasted fisheries are presented and compared to livestock production.

5.04.13

Land use impact assessment for marine constructions in the Mediterranean Sea - are biodiversity data available enough?

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In a context a sea artificialization projects and of biodiversity erosion due to changes in land and sea use at a world scale, it is important that impacts of marine infrastructures on biodiversity could be addressed by land use in Life Cycle Impact Assessment (LCIA). Recently, UNEP-SETAC recommended a method based on Species Area Relationship (SAR), using species richness as indicator and proxy of biodiversity for land impact assessment. To our knowledge there is no proposal of characterization factors for the impact assessment of marine infrastructure on biodiversity. Their compatibility with the framework recommended by UNEP-SETAC would imply adaptations because of marine ecosystems specifities. In particular, it implies to identify a relevant classification of ecoregions and to identify taxonomic groups being both relevant as a bio-indicator of ecological damage and having biodiversity data available (mostly species richness data, SAR and distribution maps by species). The main difficulty remains in data collection, because marine biodiversity is a lot less known than for terrestrial ones. The goal of this study is to explore original new data sources allowing the future calculation of characterization factors for marine infrastructure in the Mediterranean Sea. The present study provides possible solutions for data sources. It appears that most of the necessary biodiversity data are well known for fishes. For this group, some SAR are available per type of habitat in the Mediterranean Sea, as well as distribution maps for 635 specie, based on complete species richness data sets. Thus, it can already be a good base to assess impacts of marine constructions. However, there is a strong limit of data availability for benthic taxonomic groups, although they are the main taxonomic biota impacted by marine constructions and having the most diverse species richness (such as Crustacea, Mollusca and Annelida), especially in coastal area and shallow waters. The lack of data is even stronger for rarer taxonomic groups, although most of them have high rates of endemism. In conclusion, we recommand the use of this kind of data to solve the issue of biodiversity due to marine habitat destruction in land use impact assessment, at least for fishes in a first step. Another perspective is the integration of the benefits on biodiversity due to recolonization along the infrastructure border, with a net ecological gain, because it is a new available hard substratum.

5.04.14

An interdisciplinary approach to improving LCIA methodologies for freshwater use impacts on terrestrial ecosystem quality

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Advances have been made toward more robust, regional specific water footprinting methods for life cycle impact assessment. However, the link between surface or groundwater consumption and impacts on terrestrial ecosystems (which use water stored as soil moisture) is unclear. This challenging exist in part because terrestrial ecosystems vary in their acquisition of freshwater depending on biotic attributes specific to the ecosystem as well as on temporal variation in water availability. Therefore, we propose an interdisciplinary approach to resolving this challenge. The aim of this literature review was to identify challenges that exist in developing impact pathways of freshwater use on terrestrial ecosystems in LCIA and what approaches, potential pathways, and models outside of LCIA can be employed to address these challenges. A review was performed to identify complete, partial, and potential damage pathways of anthropogenic freshwater consumption as it impacts terrestrial ecosystem quality for both the midpoint and endpoint levels. The literature search revealed the challenge in modelling terrestrial ecosystems due to their variable acquisition of freshwater depending on biome-specific attributes. We therefore propose defining unique water use biomes (UWUBs) to serve as a framework for developing environmentally relevant, more robust, and less uncertain impact pathways for freshwater use on terrestrial ecosystems. A subsequent review of eco-hydrology, evaporation shed and water vapour dynamics was undertaken to assemble a non-exhaustive initial list of four biomes which utilize water distinctly from each other (UWUBs). The four UWUBs identified were drylands and semi-arid biomes, freshwater wetlands, woodlands and biomes adjacent to lakes and rivers. In addition to climatic, vegetative and soil attributes, UWUB identification was based on biome attributes at the interface of the hydrological cycle, which incorporated acquisition location (atmosphere, groundwater compartments, etc.); extraction mechanism (i.e. root uptake, canopy runoff, etc.); recycling water within the biome (to account for feedback systems); and intra-annual variability. UWUBs can serve as the framework for developing the characterization factors for more robust, less uncertain, and more environmentally relevant freshwater use impact pathways on terrestrial ecosystem quality.

5.04.15

Biodiversity impact assessment of low-pesticide scenarios for the Swiss food sector: How can we broaden our vision to include impacts abroad when addressing domestic food consumption?

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Low-input farming as a measure to reduce environmental impacts within a country will most likely increase the negative effect of agriculture in other countries, as lower yields require more import products. Regarding biodiversity, it is essential to consider the area under farming and the origin of the imported agricultural products. We analyze the environmental impacts of different regulations for pesticide use and animal husbandry within Switzerland as well as the consequences abroad caused by increased amounts of imported food. We combined two biodiversity assessment methods: The method SALCA-biodiversity to account for the effect of changes in the agricultural practices of pesticide-free farming in Switzerland, and the method Chaudhary & Brooks to cover the species loss potential due to imports. Low-input farming had a twofold effect. Within Switzerland, cultivation without pesticides reduced the pressure on biodiversity by 10 to 20 % in individual crops. However, as Switzerland has a comparatively low share of cropland in the overall agricultural land, this effect would be relatively weak considering the whole basket of product needed to supply food to the population. As second effect, a higher amount of imported products increased pressure on biodiversity abroad by 40 to 60 %. Above all, imported animal-based foods led to a higher pressure on biodiversity. The highest impact came from pasture land occupation for beef production in Brazil and Uruguay, although less than 20 % of imported beef actually came from these countries. This confirmed the fact that small amounts of imports from world regions with a high species loss potential can determine the outcome of the results. The combined use of both methods showed that an improvement of biodiversity can be achieved within a country when low-input farming is promoted, but the decrease of crop and animal yields can lead to a higher pressure on biodiversity abroad. Measures aiming at lowering the biodiversity impact of domestic agriculture can only lead to an overall positive effect, if they are combined with other leverages such as changing the population's diet or paying attention to the provenance of each imported product and its impact in the production country. Combining two assessment methods offers a promising way to solve the dilemma of global applicability vs. detailed and specific local assessment.

5.04.16

Biodiversity Footprinting for Financial Institutions: A Case study for ASN Bank

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ASN Bank wants to assess its footprint in order to determine what responsibility the bank holds with regard to biodiversity. ASN needs a metric that provides insight in the differences in impact between different investment opportunities. Our method is using the environmentally extended input-output database Exiobase in combination with the LCA impact assessment method ReCiPe2016 to quantify the biodiversity impact of investments. The first step is understanding the investment. In this step we take a closer look at the economic activities a company is involved in. The information on the companies economic activities is linked to the sectors in Exiobase. The environmental pressures of the entire supply chain of a company is translated to impact on biodiversity using the impact assessment method ReCiPe2016. Besides investments in listed equity, the biodiversity footprint of investments in government bonds, local government and specific projects are also calculated. This is done for renewable energy projects, (agro)forestry, mortgages, and investments in infrastructure. For renewable energy projects, positive impacts can be achieved as the renewable sources are assumed to replace electricity average grid mix in the countries where these projects are located. For (agro)forestry projects, positive impact can be achieved if degraded lands are converted to (agro)forestry sites with more biodiversity. The result is a biodiversity footprint showing the impact per invested euro for different types of investments. Calculating a footprint for the approximately 4000 different investments of ASN bank does not allow a detailed site specific biodiversity impact assessment of each company or project. Therefore, the results should be interpreted with care and the quantitative analysis is accompanied by a qualitative assessment that addresses the shortcomings. Although there is still room for improving in the way that biodiversity is addressed in impact assessment methods, the combination of environmentally extended input-output databases and impact assessment methods is a promising way to measure the impact of investment portfolios of financial institutions. The BFFI can identify hotspots in a portfolio, provide a tool to monitor the progress towards sustainability targets, and serve as a compass to guide positive change in the financial sector.

5.04.17

Poster spotlight: Product Biodiversity Footprint - A case study on salmon

A. Asselin, Sayari

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5.04.18
Poster spotlight: Developping a life cycle impact assessment model based on functionnality to assess competition between users
C. Chabas, Ciraig UQAM / CIRAIG
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5.04.19
Poster spotlight: Domestic freshwater use in Life Cycle Assessment: enhancing the characterization of Human Health impacts
L. Debarre, CIRAIG - École Polytechnique de Montréal / Mathematical and Industrial engineering
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Quantifying Life Cycle Emissions and Environmental Impacts of Agricultural Practices Related to Pesticides and Fertilisers

5.05.1
Emission and toxicity modelling for pesticides: operationalising the pesticide consensus
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Current emission modelling and toxicity characterisation of pesticides suffers from several shortcomings. General guidelines on how to align the LCI and LCIA phases for pesticide emission modelling and toxicity assessment in agricultural LCA have been developed in the pesticide consensus effort. The OLCA-Pest project ("Operationalising Life Cycle Assessment for Pesticides", 2017-2020, funded by ADEME) aims at operationalising and harmonising the emission quantification and impact characterisation of pesticides. Based on the analysis of potential gaps and overlaps between the PestLCI Consensus model (PLCM) for pesticide emission modelling, the dynamiCROP plant uptake model for human exposure and toxicity characterisation with special focus on pesticide residues in food crops, and the USEtox scientific consensus model for human toxicity and ecotoxicity characterisation, we propose solutions for the integration of pesticide emissions into LCI databases.The initial emission distribution fractions from PLCM to air, off-field surfaces and field soil can be directly used as inputs to dynamiCROP, in order to calculate the crop residues and related human toxicity impact factors. The emission fractions can be linked to the emission compartments in USEtox rural air, agricultural soil, and the emissions to off-field surfaces should be distributed between freshwater, agricultural soil, and natural soil. The emissions to the crop will be linked to residue-related human toxicity characterisation factors from dynamiCROP. For LCI databases and background datasets, the emitted amount of pesticide should be divided into the compartments air/low population density, soil/agricultural, water/river & lake, soil/forest (or soil/natural), and a newly defined compartment "crop". The latter should be further subdivided into 18 archetype crop classes, by distinguishing "food" and “non-food" uses. For an easy integration, default site-generic emissions fractions for different standard application situations (crop classes and pesticide target classes) will be provided. Furthermore, customised emission fractions can be easily calculated by the PestLCI Consensus web tool.Operationalising the pesticide consensus, the proposed linking of PLCM, dynamiCROP and the toxicity impact assessment by USEtox or other methods provides a consistent framework for the assessment of pesticide emissions and related toxicity impacts for use in agricultural LCA.

5.05.2
New insights for PestLCI Consensus model: sensitivity and scenario analyses for pesticide emissions and impacts
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Life Cycle Assessment of agricultural products requires the consideration of pesticide emissions to quantify their (eco-)toxicity. PestLCI model estimates pesticide emissions in various environmental compartments. Recently designed as a web-tool, it was initially developed for temperate conditions and its adaptation to tropical conditions is ongoing. To test PestLCI’s validity in tropical conditions and to identify the key input variables, a sensitivity and scenario analyses were carried out at the impact level. This allowed to assess the relative contribution of the different environmental compartments to the total impact and a comparison of impacts using either primary or secondary distribution. The mean absolute deviation method was selected to evaluate qualitative and quantitative variables, which is an index of the change induced by the variation of inputs, with a baseline scenario of insecticide application on tomato in tropical conditions. Overall, 282 scenarios were simulated, changing the value of one input variable at a time. To calculate impacts, the emission fractions to each USEtox compartment were multiplied by the corresponding characterization factors, for freshwater ecotoxicity and human non-cancer toxicity. The most influential variables are the application method and its drift reduction, field width, fraction intercepted by leaf, tillage type and pesticide. The main compartments contributing to the impacts are agricultural soil and surface water in USEtox, linked to off-field surfaces, groundwater and field soil in PestLCI. Related variables should therefore be determined precisely. For the freshwater ecotoxicity, the impacts from the secondary emissions are significantly higher than that from the primary distribution. For the human non-cancer toxicity, results are similar with both distributions. This study should be completed by including impacts due to direct ingestion of crop residues. These results reveal the importance of having accurate data adapted to the agricultural context studied, especially for the most influential variables. Adaptations to the tropical conditions must be made in priority on the most influential variables. However, the validity of PestLCI equations for tropical conditions is being further analyzed and will be specifically discussed. Indeed, we concluded that soil and climate data were probably insufficiently accounted for in PestLCI in relation to the low sensitivity of the impacts to their variations.

5.05.3
Characterizing ecotoxicity impacts on insect pollinators in life cycle impact assessment
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The use of pesticides is a main contributor to global decline of insect pollinators. Pesticides transfer and accumulate in various crop parts, including nectar and pollen. As most insect pol-linators collect nectar and pollen also from field crops, they can be exposed to pesticide resi-dues via nectar intake, or via dermal contact with nectar, pollen or both. This can lead to pesti-cide-related ecotoxicological impacts on pollinators, an impact pathway that is currently miss-ing in life cycle impact assessment (LCIA). However, ignoring insect pollinator impacts in LCIA and hence in evaluating the environmental performance of product systems in a life cycle per-spective might lead to unjustified decisions, wherever the use of pesticides is involved. To address this gap, we develop a framework for characterizing ecotoxicity impacts on honey-bees, representing the most important insect pollinator species worldwide. We define bee in-take fractions and bee dermal contact fractions as novel exposure metrics respectively for oral and dermal exposure of different bee types. We test our framework in a proof-of-concept case study on the insecticide λ-cyhalothrin and the fungicide boscalid, both authorized for applica-tion on field crops in the EU. We observe that exposure varies across types of bees, depending on pesticide properties and count of bees. Highest bee dermal contact fractions are found in nectar foragers for λ-cyhalothrin and in nectar-pollen foragers for boscalid, while highest oral intake fractions are found in nectar foragers for both pesticides. Hive oral exposure is up to 100 times higher than forager oral exposure, which is due to both the large number of bees exposed, and the nectar and pollen loads carried to the hive. Combining exposure with ecotoxicological effect data for bees, we yield characterization factors, which are three orders of magnitude higher for the in-secticide. The two pesticides affect 1,500-150,000 bees per kg applied, mainly attributable to the use of λ-cyhalothrin. Overall, nectar foragers are the most affected forager type for both pesticides, dominated by oral exposure. Our framework constitutes a first step toward integrating ecotoxicity impacts on insect pollina-tors into LCIA, and is consistent with other toxicity-related impact pathways. Our framework needs to be extended to address also other pesticides, field crops and pollinator species that are relevant for assessing environmental impacts in a life cycle perspective.

5.05.4
Indigo-N v.3 - a model for estimating direct field nitrogen emissions under contrasting agricultural situations
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Agricultural systems differ in terms of the type of produced crops (e.g. field crops, vegetable gardening, perennials), underlying pedoclimatic conditions (e.g. temperate, tropical and sub-tropical conditions; predominantly sandy, silty or clayey soils), and agricultural practices adopted (e.g. crop rotations/sequences, fertilisation strategies and irrigation). This variety of management and pedoclimatic conditions can be labelled as contrasting agricultural situations. Indigo-N v.3 (I-N3) was designed to estimate N losses from agriculture systems under contrasting agricultural situations. It is an operational model simple enough to be used in a LCA context by practitioners that are not necessarily agronomy or agriculture experts. I-N3 is based on the following principles, objectives and

functionalities: i) it calculates all N emissions at the field scale, with a focus on nitrate leaching, identified as the main N agricultural emission (in quantity) and the most complex one to assess; ii) it represents a wide variety of field crops, vegetables and grasslands, under various pedoclimatic conditions,; iii) it provides worldwide default values for pedoclimatic and agricultural parameters, to keep the user data demand as low as possible; and iv) it assesses single crops, sequences and crop rotations. The model combines different existing approaches to estimate NH3, NOx and N2O emissions and proposes a new formalism to estimate NO3 losses, based on calculation of balance during drainage periods that integrates N absorption by crops, mineralisation kinetics of organic N sources, drainage regimes based on water inputs and outputs, and the effects of previous crops in the rotation. I-N3 was implemented as an Excel spreadsheet tool, and tested with several global datasets of agricultural itineraries, depicting contrasting agricultural situations. Results (i.e. predicted emissions associated with crop rotations or sequences) were validated against measured emissions, results from mechanistic simulation models, and by expert opinion. The model is supported by a database containing global data pertaining to crop characteristics and monthly pedoclimatic conditions. Crop data are available for most economically relevant crops, worldwide, with the exception (in the current version) of rice, oil palm, grapewines and perennial trees. A few cereal/legume mixtures are included. Pedoclimatic data are available for each intersection of country and FAO Global Agro-ecological Zones.

5.05.5
AGEC-LCI: an open access tool for calculating emissions from fertilizers and metal-based fungicides applications
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One methodological issue related to the application of life cycle assessment to agricultural systems is the lack of standardization in the modelling of emissions at inventory level, even though some guidelines for inventory generation have been provided. In a practical perspective, the modelling of agricultural systems generally involves a great amount of data, including the amount of fertilizers and pesticides applied, as well as the resulting emissions from the application of these agricultural inputs. Subsequently, the LCA practitioner must enter the foreground data into an LCA software, which is time consuming and susceptible of an inconsistent linking of LCI data and life cycle impact assessment methods. The aim of this study is to provide the LCA practitioner with an open access, transparent, customizable and scientific-based tool for generating inventories of agricultural emissions resulting from the application of fertilizers, soil amendments and metal-based fungicides, and to reduce the risk of a mismatch between LCI data and characterization models by generating reports that can be directly imported into LCA software. A state of the art analysis of the models for computing direct field emission from fertilizers, pesticides and soil-amendments applications was carried out. Acknowledging that agricultural emissions are site- and time dependent, a parsimonious approach was considered for the selection of the emission models. The selected models for calculating direct emissions from agricultural inputs were integrated in a Visual Basic for Application (VBA) tool hosted in Excel, called AGEC-LCI, which stands for Agricultural Emissions Calculator for life cycle inventory. AGEC-LCI allows the user to select inputs from a database composed of 25 crops, 42 fertilizers, 6 metal-based fungicides, 2 soil amendments and the pedo-climatic characteristics of 5 French regions according to data from AGRIBALYSE. The aim of AGEC-LCI is to contribute to an "Open Science for Enhanced Global Environmental Protection", by providing transparent calculations and reports of agricultural emissions. Furthermore, this tool aims to facilitate the modelling of the foreground process of agricultural systems and to avoid the potential inconsistent linking of LCI data to LCIA.

5.05.6
Poster spotlight: Comparison of operational N direct field emissions models used in LCA - suitability for contrasting agricultural situations
A. Avadi, CIRAD / UPR Recyclage et risque
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Quantifying the Environmental Impacts of Marine Litter: Interdisciplinary Knowledge towards Addressing Marine Litter in LCA

5.06.1
Drawing a framework to assess marine plastic litter impacts in life cycle impact assessment: the MarILCA project
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Environmental impact pathways from marine litter, namely plastics, are yet to be fully covered in life cycle impact assessment (LCIA) methods. This currently implies a methodological drawback, since it limits the holistic nature of Life Cycle Assessment (LCA), hindering its robustness in policy-support. In this context, the MarILCA (MARine Impacts in LCA) group is positioned to close this methodological gap. MarILCA, which operates under the UN Environment Life Cycle Initiative and with the Forum for Sustainability through Life Cycle Innovation (FSLCI) as a communication partner, aims at setting out a framework to support coordinated research progress towards the development of harmonized pathways to account for impacts of plastic litter, specifically to the marine environment. The framework includes an overview of life cycle inventory requirements (leakage to the environment; a focus of other research efforts), and a detailed description of possible marine litter impact pathways, modelling approaches and data requirements. A detailed mapping of impact pathways is provided, with guidance on those relevant from an inventory perspective. The framework links waste generation with potential damage to several areas of protection (AoP), namely ecosystem quality and human health, by describing mechanistic steps along impact pathways. We recommend development of material-, spatially- and size-differentiated LCIA models including details on fate, exposure and effect processes. Fate processes include those related to waste management, transport to and within the marine environment, and material fragmentation and degradation. Moreover, we include exposure processes when relevant, describing the potential interaction between litter and human populations and species in ecosystems. Effect pathways are then specific to the litter classes, which are defined according to material, shape and size, with the degree of damage also influenced by the sensitivity of species or human populations to exposure. Overall, in the framework, we identify the gaps and building blocks that form a foundation for guiding coordinated and harmonized, and therefore efficient, LCIA model developments for ultimately having characterization factors for wastes leading to marine litter and the associated environmental damage. Here, at SETAC Europe, we present this framework, highlighting specific contributions of the MarILCA Scientific Committee to individual building blocks in the overall framework.

5.06.2
The Plastic Leak Project: A pre-competitive initiative to harmonize plastic metrics
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Global plastic leakage is estimated to be in the order of magnitude of **10 Mt/year**, with different authors presenting yearly values from 4.8 Mt/year to 12.7 Mt/year, focused on leakage into the ocean. The Plastic Leak Project (PLP) was completed in 2019 and delivered a first methodology and metrics to assess plastic leakage within the life cycle assessment (LCA) framework. The project was split into a strategic committee, an advisory board and industrial partners. This project provides specific guidance for calculating and reporting estimates of micro- and macroplastic leakage quantities, at each life cycle stage and at both product and corporate levels, and ultimately to include them in LCAs. The developed methodology is then expanded to provide sector-specific guidance to assess the plastic leakage for four leakage routes in particular: transport (due to tyre abrasion), textiles (due to textile washing), plastic products and packaging (due to waste mismanagement) and pellets production (pellets spilt and not cleaned up at any point in the plastics value chain). The global methodological guidelines include guidance on the choice of functional units, inventory of leakage sources for key industries, default system loss rates and environmental release rates for a set of pathways, meta-data and key sources of data for different regional archetypes. All leakage routes are modelled according to a common framework, taking into account **loss** as well as **release** through a **transfer and redistribution pathway.** The developed approach is tested in a case study to evaluate the plastic leakage of Arla Foods corporate value chain. The plastic leakage assessment of the Arla Foods corporate value chain showed that out of **99'466 t macroplastics** generated in 2017 (including plastics used at farm and packagings), **3'814 t** leaked into the environment. In addition, **41 t microplastics** leaked from pellets lost during the plastics production and during tyre abrasion related to logistic transport. The PLP defines methodological guidelines to quantify plastic leakage as a global reference, supported by recognized experts and top international organizations. PLP provides a framework that allows for a holistic approach to the plastic leakages to close the tap while avoiding shifts of environmental burdens. This inventory can also be used as a basis for plastic leakage impact assessment.

5.06.3
Modelling the plastic footprint of EU consumption: knowing the flows and stock as the basis for estimating plastic marine litter emissions
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Cattolica / Directorate D - Sustainable resources, Bioeconomy Unit; C. Caldeira, EC JRC / Directorate D - Sustainable resources, Bioeconomy Unit; V. Kouloumpis, X. Yan, University of Exeter / College of Engineering, Mathematics and Physical Sciences; S. Sala, European Commission - Joint Research Centre / Bioeconomy unit
The emission of plastic waste to the marine environment has recently gained relevance as an emerging environmental pressure. While the effects on the marine ecosystem has become visible (e.g. fauna entanglement), the potential effects on human health are still uncertain. With estimations of the global amount of land-based plastic waste entering the ocean ranging between 4.8 and 12.7 million tons a year, marine litter emissions are expected to increase due to the relevance of plastic in the current society and its presence in consumed goods. To evaluate the importance of marine litter impacts, there is a need to operationalize a holistic and comprehensive approach to estimate the potential impacts associated to marine litter. In this context, the integration of marine litter impacts into life cycle assessment (LCA) can set the basis to estimate the impacts for products and systems, while contextualizing their relevance against other environmental impacts (e.g. climate change, acidification). For this purpose, the first step is to define how to compile marine litter emissions in an inventory, to which impact assessment models can be then applied. For compiling this inventory, there is a need to understand and quantify the plastic flows taking place in the current economy, which can be susceptible to become marine litter emissions. The goal of this study is to model and quantify the plastic flows and stocks in the EU consumption for the reference year 2010 and determine the plastic footprint of an average EU citizen, by polymer. The plastic footprint will set the basis to estimate the marine litter generation based on potential litter rates and available data on river and beach littering. For this exercise, the Consumption Footprint indicator will be employed as the reference system. The Consumption Footprint assesses the environmental impacts of EU consumption by considering the process-based LCA of around 150 representative products of five areas of consumption: food, mobility, housing, household goods, and appliances. The Consumption Footprint considers the domestic production and the trade of goods (imports and exports) by quantifying the consumption intensity of products with Eurostat statistics (Prodcom). Available transport and impact assessment models will be evaluated to be employed in the life cycle impact assessment phase towards estimating the impact of marine litter emissions due to EU consumption.

5.06.4
Approach to Consider Plastic Emissions in Life Cycle Assessment
D. Maga, Fraunhofer UMSICHT / Sustainability and Resources Management; N. Thonemann, Fraunhofer UMSICHT; J. Bertling, Fraunhofer Institute for Environmental, Safety, and Energy Technology UMSICHT
There is an increasing public, political and scientific concern over the presence of plastics in the environment. In particular, due to possible negative effects on marine organisms but also due to its appearance, plastic emissions are seen as a relevant environmental issue. However, existing life cycle assessment (LCA) studies are not able to address these impacts (Sonnemann and Valdivia 2017). In many cases, there is a clear contradiction between the apparent positive LCA of a plastic product compared to an alternative and the negative perception when this product is found in the environment. Current works mainly focus on plastic footprints which account for plastic emissions of products in their life cycle and do not consider related environmental impacts. One example is the marine plastic footprint currently under development by the International Union for Conservation of Nature (IUCN). In addition, there are present studies on losses of plastics across their value chains (Ryberg et al. 2019). While the quantification of emissions gives a first idea about the dimension of the problem, it does not allow for evaluating the damages that the plastics may cause to ecosystems and human health. Although there are first proposals for such an impact assessment starting from plastic emissions, through fate over exposure and ending with damages to ecosystems and humans (Woods et al. 2016), there is no consistent framework at all. Current studies such as Koelmans et al. (2017) exclusively address environmental fate pathways, other studies exclusively address damages such as the proposed entanglement factor (Woods et al. 2019). The goal of this work, which is linked to the BMBF funded project PlastikBudget[1], is to present a methodology to consider possible risks of plastic based on the persistence of plastics. Following the idea that the endangerment of plastic emissions is mainly driven by the quantity and persistence of plastics in the environment, our methodology focuses on the fate and degradation rates of plastics in soil and sea. The fate considers the location of a plastic emission as well as transmission factors. Degradation rates are derived from an expert survey that uses data from a comprehensive literature on degradation of plastics as input. [1] https://www.plastikbudget.de/english/

5.06.5
Aquatic Micro- and Nano- Plastics in LCA: Development of an ecotoxic effect factor
J. Lavoie, UQAM / ISE - Institut des sciences de l'environnement; A. Boulay, CIRAIG - École Polytechnique de Montréal / Chemical engineering department; C. Bulle, Université du Québec à Montréal / CIRAIG, École des Sciences de la gestion
Plastic litter of all sizes has been acknowledged as a serious threat to biodiversity, especially in the marine environment. The fact that LCA does not properly consider these issues is a serious problem for the aspirations of LCA in the public sphere. Micro- (MP) and nano- (NP) sized plastics could have a large impact on ecosystem quality due to their increased bioavailability and capacity to affect a greater range of species, it is therefore of paramount importance to address these issues. The data regarding MPs and NPs' ecotoxicity was extracted from the academic litterature. Transformations and adaptations were conducted so that we end up with EC50s (Effect Concentration affecting 50% of tests organisms) allowing for the calculation of an $HC50_{EC50}$ (Hazardous Concentration where 50% of the species tested are affected above their EC50) as needed to generate the Effect Factor (EF) in the USEtox model. Statistical analysis were performed on the 111 data points to bring forward existing relations between toxicity and different parameters such as particle size, polymer type and particle shape. Data concerning *Daphnia magna* data was selected to conduct these analysis since it is the only species that was the focus of a sufficient amount of studies. No significant difference could be observed between the toxicity of the different polymers represented in our data set (polyethylene, polystyrene and all others combined). Toxicity was also the same between MPs and NPs, and for the different shapes of particles used in the experiments. A Species Sensivity Distribution was constructed for all data combined since analysis did not show any difference in ecotoxicity between the different subsamples tested. An interim EF is proposed and recommended for the moment since MP and NP research is still recent and suffers from great irregularities, which bring inconsistent results and conclusions. Next steps should focus on the potential ability of these polymers to adsorb and transfer other pollutants in the ecosystems which has been highlighted has a potentially harmful toxicity pathway and a menace for aquatic biota.

5.06.6
Reliable test methods to evaluate the biodegradation of plastic materials in the marine environment
M. Weber, A. Eich, HYDRA Marine Sciences GmbH; K. Schlegel, C. Becker, E. Biedermann, BASF SE; M. Lasut, UNSRAT Sam Ratulangi University; C. Lott, HYDRA Marine Sciences GmbH
Current life cycle analysis methodologies lack indicators to assess the biodegradation of plastic materials introduced to the natural environment. If ending up in the sea, some plastics are assumed to be less persistent than conventional ones. However, reliable methods and data on the biodegradation of any plastic material in the ocean are not systematically available. We applied a 3-tier test scheme of reliable and environmentally relevant methods in laboratory, mesocosm and field testing to assess the intrinsic biodegradation as well as the disintegration performance of several plastics including certified compostable materials under real environmental conditions in coastal habitats of Southern Europe and SE Asia. The mathematical modeling of the experimental results and the calculation of a specific half-life of a plastic material under specific environmental conditions show that the persistence is highly depending where a certain plastic item is ending up. The specific half-life as a material property thus allows for the numerical comparison of different environmental scenarios and can be fed into LCA models.

Sustainability and Risk Assessments and Circularity Indicators for the Transition to a Circular Economy

5.07.1
Calculation of technical substitutability coefficients in waste management LCA studies
l. rigamonti, Politecnico di Milano; S. Taelman, Gent University / Department of Sustainable Organic Chemistry and Technology; S. Huysveld, Ghent University / Sustainale Organic Chemistry and Technology; S. Sfez, K. Ragaert, Ghent University; J. Dewulf, Ghent University / Department of Sustainable Organic Chemistry and Technology
Life Cycle Assessment (LCA) is a widespread tool used to guide decision-makers towards optimal strategic choices for sustainable growth. A key aspect of LCA studies of waste management systems where recycling activities are present is to account for resource recovery and the related substitution effects. When the LCA analysist wants to apply the approach called "system expansion with substitution" or "avoided burden method", he/she has to identify and model mono-functional processes external to the system under study, which yield products or functions that are equivalent to those of the co-products of the considered multi-functional process. These inventories are commonly subtracted from the inventory of the original multi-functional process in order to estimate the inventory associated with the co-function of interest. However, quantifying the extent to which products are functionally equivalent and intersubstitutable is a difficult task. Although multiple scientific papers assume a 1:1 substitution ratio between similar materials/products, this is often incorrect as the actual ratio is likely to vary. The focus of this presentation is on the calculation of the substitutability coefficient for secondary materials based on technical characteristics. A state of the art literature review showed that many different calculation procedures were applied, which led

to a wide variety of substitutability coefficients. In this perspective, the objective of this presentation is to provide guidelines on the procedure to be followed to calculate the substitutability coefficient for secondary materials, based on the learnings from previous studies. In particular, departing from the framework proposed by Vadenbo et al. in 2016, the focus in this presentation is on the technical functionality of the materials as a basis for calculating the substitution coefficient. These guidelines are then applied to two waste management case studies. In total, sixteen technical substitutability coefficients are given for ten secondary materials, based on state of the art and presented case studies. The research thus represents a step forward in quantifying the substitutability of secondary materials in waste management LCA studies. The guidelines presented may allow other case studies to enrich the list of coefficients, useful for all LCA practitioners to evaluate in a more correct and harmonized way the environmental impacts associated with recycling activities.

5.07.2
Measuring the performance of more circular complex product supply chains
E. Bracquene, J. Duflou, W. Dewulf, KU Leuven / Department of Mechanical Engineering
Although a number of circularity indicators have already been proposed in literature, none was found to properly describe the product system taking into account the 'tightness' of the material cycles and the relationship with other product systems such as the use or supply of recycled material. Therefore, a new Product Circularity Indictor (PCI) is developed in this paper. The new indicator is applied and tested in a case study for Washing Machines (WM). The case study results show that the proposed PCI is a useful indicator to quantify the effectiveness of different circular economy (CE) strategies. A shift to CE presents the challenge of recirculating material flows in a manner that can promote eco-effectiveness. Therefore the potential trade-off between increasing circularity and minimising the environmental burden of the WM is investigated using Life Cycle Assessment (LCA) to quantify the potential environmental impact of the product system.

5.07.3
Return of the milkman: using combination of circularity and life cycle metrics for decision making in context of circular models for fast moving consumer goods (FMCG)
M. Vieira, PRe Sustainability; E. Valencia Martinez, PRé; J. Dewaele, Procter&Gamble / Brussels Innovation Center; B. Keteleer, Procter&Gamble
Procter & Gamble (P&G) is a partner of LOOP, which is piloting a new system offering products to consumers in durable and re-usable packaging that is returned and refilled after use. That aims to reduce the single-use plastic use and resulting packaging waste that is delivered to consumers via e-commerce and brick and mortar (B&M). As LOOP brings back the old 'milkman' model, it is clear that this model could bring other system changes with it that go well beyond the use of different packaging materials and weights. To better understand the environmental performance of this system as a whole, and specifically in its relation to traditional fulfillment options (e-commerce and B&M), PRé is conducting an LCA study comparing these three options. As an initiative that is being regarded to support a more circular economy, the study is further complemented with material circularity assessment using Ellen McArthur's methodology. The study analyses two products each focused on the specific settings of the pilot being conducted, namely Ariel laundry detergent 3-in-1 pods in Ile-de-France (the region in north-central France surrounding Paris) and Pantene shampoo in the New York region. However, the study hopes to generate conclusions from this analysis that can be taken to other settings and thus help set direction to minimize environmental impacts of this new model in all regions. When using the best available data from P&G and TerraCycle, the results point out important differences in contribution from packaging materials and transportation across the different models. In Loop - when operated as a stand-alone model - the environmental impact is dominated by multiple transport steps. On the other hand, in e-commerce and B&M, the production of packaging is more prominent, whilst other forms of transport continue to play important roles too. Material circularity results for products in reusable packaging shipped via LOOP model are confirmed to be much better than those of products using disposable packaging delivered via e-commerce and B&M, thus living up to the circularity promise. The following steps of the project will be to 1) refine the model using the data from the pilot which will be completed by May 2020, and 2) to determine what the conditions are for Loop to be better than e-commerce and B&M. More specifically, what the maximum distance allowed for the reverse logistics is. This is scheduled to be completed in the first quarter of 2020.

5.07.4
Confronting challenges of combining and comparing Material Circularity Indicator with Life Cycle Assessment indicators: a case of alkaline batteries
E. Glogic, University of Bordeaux / Institut des Sciences Moléculaires - CyVi; S.B. Young, University of Waterloo / SEED; G. Sonnemann, University of Bordeaux / ISM-CyVi
Product-level assessment indicators are developed to incorporate circular economy ideas of resource minimization and cycling in the product design and management. Indicators applied to improve product circularity are only plausible if they can be combined with assessment frameworks such as life cycle assessment so that possible environmental trade-offs can be identified. Furthermore, indicators need to be plausible to be applied across array of circularity strategies and incorporate the complexities of benefits and limitations inherent to those strategies. In the current work, we investigate the trade-offs between the prominent circularity indicator Material Circularity Indicator (MCI) and indicators of Life Cycle Assessment (LCA) in order to understand the scope and extent of their combined use. The indicators are calculated for several scenarios for design and management of single-use alkaline batteries involving strategies of recycling, use of recycled content, end-of-life collection, and improved utility. We investigate how the choice of recycling alternatives influence the trade-offs since recycling is fairly crudely assessed for MCI. In addition, the trade-offs are observed under changing boundary assumptions in order to determine how the lack of secondary material characterization (byproducts of recycling) typical to MCI, affects the robustness of the dual analysis. Results suggest that trade-offs between MCI and LCA indicators could be significant given the choice of a recycling route when considering use of recycled content in battery manufacture (i.e., using 10% of recycled content improves circularity by 9% but also increases the impacts by up to 6.85 %). The robustness of the results is notably affected under truncation of system boundaries to exclude manganese slag as a potential substitute to clinker cement in which case, the MCI is more significantly affected than indicators in LCA (by 30% in comparison to 1-3% change of LCA indicator values). We offer a new approach to visualize and identify the trade-offs between indicators and offer recommendations for further indicator use and development.

Increasing the Utility of Non-standard Studies in Weight of Evidence Evaluations

6.01.1
Enhance the impact and regulatory use of your data
C. Ajao, ECHA-European Chemicals Agency
Every day thousands of peer-reviewed articles are published in scientific journals. Many of them are performed in line with 'good' or 'robust' science, e.g. suitable study design, appropriate statistics, correct data analysis, and transparent, structured and correct reporting of the experiment. However, unfortunately, not all reported studies provide reliable, relevant and reproducible analysis, and therefore do not really contribute significantly to scientific and regulatory processes for the end goal of improving environmental quality. 'Quality and credibility of science' is one out of the eight strategic objectives for SETAC Europe. To raise awareness on its importance, a training was organised in SETAC Helsinki 2019 entitled 'conduct and report reliable, relevant and reproducible environmental studies'. In this presentation, we will give tips on how to enhance the impact and regulatory use of your data to allow it to be used in a weight of evidence approach in regulation. The tips will be based on the categories outlined in the SETAC Technical Issue Paper - Recommended minimum reporting information for environmental toxicity studies. Further, the presentation will provide a case example of how several studies were evaluated for a substance proposed for identification as a Substance of Very High Concern (SVHC) under REACH. Here, data from open literature was used as evidence for the identification however some data was assessed differently by decision makers in the decision making process at the Member State Committee of ECHA, leading to uncertainty.

6.01.2
Examination of key studies used during REACH restriction process
F. Borchert, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; A. Beronius, Karolinska Institutet; M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry
The European chemicals regulation Nr. 1907/2006 on the Registration, Evaluation, Restriction and Authorisation of Chemicals (REACH) came into force in 2007. While producers or importers are required to submit a registration dossier for substances produced at or above 1 tonne/year, the European Chemicals Agency (ECHA) is in managerial position of the substance evaluation process to identify and to further initiate the implementation of regulatory risk management options for substances of concern. Substance evaluation itself is done by a Member States Competent Authority (MSCA). In case the assessment concludes that a substance or its mixture is of concern to the environment or human health, this substance can be subject to restriction and inclusion to Annex XVII of the REACH regulation. A restriction can either entail a ban or a limitation to manufacture, use or place a substance on the market. The Committee for Risk Assessment at ECHA is in charge of evaluating the eligibility of the proposed restrictions to reduce a given hazard or risk to the environment and human health. In traditional hazard and risk assessment, key studies are often studies conducted according to internationally validated and standardized test guidelines, but can also be non-standard studies. However, non-standard studies have historically been given less weight than standard studies in regulatory risk assessment if standard studies have been available. Doubt of being sufficiently reliable, relevant or reproducible has been expressed by involved parties. Nevertheless, minimum

reporting requirements are increasingly demanded irrespective of the study type to promote the value for policy-making. Within REACH, opinions of the Committee for Risk Assessment on restriction proposals are based on one or more (eco)toxicological key studies. Thus, the scope of the present study is to scrutinize the restriction intention, justification and opinion of the Committee for Risk Assessment as well as to characterise the selected key studies on which a decision is based. Preliminary results show that non-standard studies from academia are primarily used as key studies and, in most cases, restrictions address concerns which effect human health and not the environment. Possible reasons for a lack of restriction proposals addressing adverse effects in the environment will be discussed. Moreover, some restriction justifications are deficient in transparency and clarity, thereby disabling full understanding of the reasoning behind the decisions. This study will give insight into the scientific basis for decision-making in the REACH regulation, as well as provide overall knowledge about the transparency and clarity of risk assessment and risk management decisions.

6.01.3

Weighing evidence of effects of pesticide mixtures in bees: how to deal with uncertainties

H. Serra, M.C. Hansson, Lund University / Centre for Environmental and Climate Research (CEC); U. Sahlin, Lund University / Centre for Environmental and Climate Research; M. Rundlöf, Lund University / Department of Biology

Bees are essential pollinators of crops and wild plants. They can be exposed to many different pesticides in agricultural landscapes when providing their pollination services. Current standard protocols for assessing toxicity of pesticides on bees focus mostly on acute effects of single compounds in honeybees, neglecting long-term effects, especially on wild bees. While the evaluation of all pesticide mixtures is not possible, the additivity assumption (i.e. lack of interaction) is suggested as a conservative approach to predict and estimate effects of chemical mixtures. Nevertheless, a comprehensive analysis of empirical knowledge coming from both standard and non-standard tests is currently missing. We performed a systematic review to test the hypothesis that mixtures of pesticides with different modes of action have additive effects in bees. After evaluating data quality, the results will be grouped by mode of action and combined into lines of evidence using a weight-of-evidence approach. 42 of 690 peer-reviewed articles were included in the systematic review after title, abstract and full text screening performed in October 2019. Most of them reported from laboratory-based studies (36) and fewer from semi-field and field studies (7). Apis mellifera was the most investigated species, and 14 studies assessed contact toxicity and 24 oral toxicity. The neonicotinoid and pyrethroid insecticides, and triazole and strobilurin fungicides were the most investigated pesticides. While most studies focused on mortality, several investigated non-standardised endpoints such as colony growth, behaviour or histological changes. The evaluation of study quality based on relevance and reliability is ongoing. A preliminary evaluation revealed that only 8 of 36 peer-reviewed articles used a mathematical approach to predict combined effects of chemicals, shedding light on possible misleading use of "additive" and "synergistic" terms. Furthermore, we observed that there was an effect of pesticide mixtures on the food intake in 7 of 12 studies monitoring food consumption, resulting in different exposure levels in single and mixture treatment groups, potentially leading to erroneous interpretation of mixture effects. The results and the challenges and benefits to combine standard and non-standard data will be discussed from a scientific and regulatory perspective, to provide a rigorous and up-to-date evaluation of knowledge of effects of pesticide mixtures in bees.

6.01.5

Could ecotoxicology pre-publish methods as do clinical studies?

S. Owen, AstraZeneca / Global Sustainability; S.K. Maynard, AstraZeneca / Safety Health and the Environment; J. Snape, AstraZeneca UK Ltd. / Global Sustainability

The quality of academic ecotoxicology reporting is often questioned, and this had led to difficulty in using the data in a regulatory context. But all ecotoxicologists fundamentally want to understand pollution and protect the environment. How our community moves towards better incorporating available information in future is critical in sustainably protecting the environment. We need to establish a better structure for incorporating non-standard studies into environmental risk assessment. One solution might be to borrow from how clinical trials of new medicines work. Before any work is conducted we could pre-publish the methods in collaboration with academics, regulators and industry. This may lead to more robust hypothesis driven studies of reported quality sufficient for regulators to make decisions to better protect the environment. There are risks to academic, regulator and industry parties, but on balance such a step might be a workable path towards greater collaboration and more importantly better protection of the environment. This presentation will highlight the case of Metformin, a WHO essential medicine for diabetes and its controversial potential to impact wildlife.

Integrated Effect Assessments to Enable a Sustainable Future for the Marine and Coastal Environment

6.02.1

Biological effect monitoring in the context of the Marine Strategy Framework Directive (MSFD)

V. Tornero, EC JRC; G. Hanke, European Commission, Joint Research Centre (JRC) / ISPRA

The MSFD is the policy framework for protection of the European Seas. MSFD Descriptor 8 describes protection against pollution by chemical contaminants, where criterion D8C1 regards concentrations of chemical substances in different marine matrices, and D8C2 includes provisions for assessing the effects of contaminants on the health of species and condition of habitats. EU Member States (MS) are due to submit every six years updates on MSFD Articles 8 (assessment of their marine waters), 9 (determination of Good Environmental Status, GES), and 10 (setting of environmental targets). The Joint Research Centre (JRC) is reviewing the latest submissions in order to analyze comparability between MS and marine regions and provide recommendations for improving assessment approaches in view of reaching/maintaining GES in EU.Analysis of D8C2 shows that most MS have specific monitoring programs for biological effects but, except for imposex, there is high variability among them. Harmonized and agreed methodologies are required for threshold setting and assessment methods. The application of biological effect methods in integrated assessments and the QA/QC for compliance checking are still challenges. The MSFD Expert Network on contaminants, coordinated by JRC and consisting of MS experts, Regional Sea Conventions (RSC) and other stakeholders, is exchanging information and discussing MS practices in order to agree on D8C2 implementation, considering also the need for consistency with the Water Framework Directive (WFD) and RSC as well as current scientific developments. Outcomes are expected to help establish a common set of biological effects that can be agreed at (sub)regional level, along with guidance for its application in integrated assessments. It is a particular challenge to embed biological effect monitoring in a holistic and cost effective strategy, which allows a complete assessment of risks through chemicals and the identification of pollution sources with the final goal to reduce contaminant input wherever possible. Biological effect based methods play a specific role in such assessments, as the number of measured chemicals is increasing and there is need to close assessment gaps, consider potential synergetic effects and alert on substances not included in current monitoring programmes. In that context, assessments by biological effect measurements need to be comparable, so there is need to improve harmonization for the next phase of MSFD implementation.

6.02.2

JPI Oceans Knowledge Hub: Integrated Assessment of New Pollutants

I. Schulz, JPI Oceans; A. Lillicrap, NIVA Norwegian Institute for Water Research / Ecotoxicology and Risk Assessment; A. Trujillo, Spanish State Research Agency; H. Lee Behrens, Research Council of Norway

The Water and Marine Strategy Framework Directives (WFD and MSFD) have established mechanisms to evaluate 'chemical quality' such as Environmental Quality Standards (EQS) for chemical hazardous substances. The 'substance by substance' approach of EQS requires a sound basis in relevant ecotoxicological data. However, even for known contaminants, sometimes assessment is very onerous due to e.g. the need to compare with very low limit values. Member States have generally identified that better tools are required to address efficiently the monitoring and evaluation of chemical pollution especially in view of the new pollutants reaching the marine environment. The currently available effect-based assessment systems suffer from scarcity of marine, effect-related data in general and from ecotoxicological data related to target monitoring matrices such as sediment and biota in particular. In 2019, the Joint Programming Initiative for Healthy and Productive Seas and Oceans (JPI Oceans) launched a knowledge hub on integrated assessment of new pollutants. The network consists of selected experts from JPI member countries; it aims to understand what is needed to improve the methodological basis for marine chemical status assessment. As an outcome of this networking and information exchange, the JPI Oceans Knowledge Hub will generate a report presenting the currently most appropriate methodology(-ies) for integrated assessments of effects of new pollutants, taking account of the above rationale for a knowledge hub. Further an overview of relevant improvements and refinements of existing methodology(-ies) will be given. This includes also research found necessary for improved effect/hazard studies, monitoring and sampling on the level of new and emerging pollutants. It is anticipated that a set of political, economic and social dimensions will be included as the source and impact of pollutants are utterly linked with society. The report should provide specific recommendations to actions that can be taken by JPI Oceans. This presentation will present the preliminary results of the activities of the Knowledge Hub on New Pollutants.

6.02.3

Integrated biological effects assessment of an aluminium smelter discharge in a Norwegian fjord

S. Brooks, T. Gomez, NIVA / Ecotoxicology and Risk Assessment; K. Petersen, NIVA - Norwegian Institute for Water Research / Ecotoxicology and Risk Assessment; A. Macken, NIVA / marine pollution

An integrated biological effects monitoring approach using field transposed mussels was used to determine the potential biological effects of the effluent discharge from an aluminium smelter into a fjord recipient. Concentrations of PAH and metals have been previously monitored in sediment, water and biota within the Sunndals fjord, Norway over the last 20 years and have been considered to be the main contaminant threat to marine life. However, this is the first time that the biological effects of mussels exposed within the water column to the discharge plume have been used. Mussels were positioned approximately 1, 2, 5, 10 and 20 km downstream from the smelter discharge outlet for an exposure period of 6 weeks. At the end of the exposure duration, the mussels were measured for a suite of PAH (40) and metal concentrations as well as ten biological effects measurements at subcellular, tissue and whole organism response levels. A time zero (t0) group for chemical and biological assessment of the mussel population prior to field deployment was used for comparison. The initial biomarker responses indicate effects on mussel health with proximity to the aluminium smelter. With the full complement of chemical and biomarker data, an holistic biological effects assessment to determine the relationship between chemical concentrations and biological response in relation to distance from the aluminium smelter will be possible. The Integrated biological response index and the principle component analysis followed by redundancy analysis will be used to integrate the data and determine if the discharge from the aluminium smelter is impacting organisms that live within the water column of the fjord recipient.

6.02.4

Unravelling the composition-influence on toxicity of offshore produced water using high-resolution gas and liquid chromatography - mass spectrometry

L. Sørensen, SINTEF Ocean / Environment and New Resources; P. McCormack, University of Plymouth; J. Farkas, SINTEF Materials and Chemistry / Environment and New Resources; P. Sutton, University of Plymouth; B. Hansen, SINTEF Ocean / Environment and New Resources

The toxicity of produced water (PW) discharges has been repeatedly demonstrated. However, the causative mechanisms of toxicity remain poorly understood. While PW composition is very complex, regular monitoring of effluent composition still only focuses on a limited set of chemical classes, most of them petrogenic or degradation products of petrogenic compounds. Early life stages (ELS) of fish have been shown to be particularly sensitive to pollution and are expected to be vulnerable also to PW compounds. In the current study, we apply a novel functionality-based fractionation method to separate twelve discrete fractions of a PW collected at the point-of-release from a North Sea oil production facility. A battery of standard, non-standard and high-resolution chemical characterization techniques are applied to each fraction to allow the to date most comprehensive investigation of PW composition described. The toxicity of the PW and its fractions to ELS of the model fish species *Danio rerio* (zebrafish) is seen in light of the chemical composition of each fraction. The bulk composition of the PW in question comprised aliphatic and aromatic hydrocarbons, while phenols and organic acids also constituted a significant part of the total. A range of production chemicals were identified in separate fractions of the PW. In terms of toxicity, the produced water extract caused effects on hatching success, cardiac function and development of the zebrafish embryos. No individual fraction was able to provoke the same level of effects as the total PW, but the "hydrocarbon" fractions in combinations (oil constituents) were considered the most potent mixture. The potential for synergistic effects between different fractions is investigated.

6.02.5

Source to Outcome Pathways - a way forward in integrating marine exposure and impact assessment?

C.L. Eastabrook, Newcastle University / School of Natural and Enviornamental Sciences; E. Jarosz, Norwegian University of Life Sciences (NMBU) / Environmental Chemistry; K. Petersen, NIVA - Norwegian Institute for Water Research / Ecotoxicology and Risk Assessment; Y. Song, Norwegian Institute for Water Research (NIVA) / Ecotoxicology and Risk Assessment; R. Wolf, NIVA Norwegian Institute for Water Research; G.S. Caldwell, Newcastle University; H. Teien, Norwegian University of Life Sciences NMBU / Centre for Environmental Radioactivity (CERAD); K. Tollefsen, NIVA - Norwegian Institute for Water Research / Ecotoxicology and Risk Assessment

Coastal environments are often contaminated with chemicals, mostly from land-based industrial and domestic point sources or more diffuse pollution sources that collectively give rise to adverse effects in marine organisms. The potential impact of complex chemical mixtures are often unknown, as are the links between exposure metrics and biological effects. This is a particular problem for chemicals originating from different sources, with different environmental properties and displaying different toxicities to target organisms on their own or when present in complex mixtures. Linking concepts such as Aggregate Exposure Pathways (AEPs) to Adverse Outcome Pathways (AOPs) has been advocated as solutions to facilitate considerations spanning the source to outcome continuum, but has not been thoroughly applied for marine environments yet. The aims of the present study were (1) to characterise the potential environmental risk based on exposure and existing effect information for metals in a marine exposure scenario (Kaldvellfjord); (2) to characterise the effects of the most relevant trace metals to populate a set of AOPs; and (3) to carry out a combined toxicity assessment of the most relevant trace metals for marine exposure scenarios. Data were used to evaluate the utility of a Source to Outcome Pathway (STOP) for metal exposure. Kaldvellfjord is heavily contaminated with trace metals. Different sources and environmental concentrations of trace metals were determined, alongside a preliminary cumulative hazard and risk assessment performed with the NIVA Risk Assessment database (RAdb). Copper, zinc and nickel were identified as risk drivers. *Tisbe battagliai* is a widely used marine copepod and was a suitable experimental model for a marine effect assessment of metals in single and combined exposures and AOP development. The antagonistic effects observed for the ternary mixture of copper, nickel and zinc suggest that interactions are occurring either at the toxicokinetic (i.e. AEP) and/or toxicodynamic (i.e. AOP) part of the source to outcome continuum. This study advocates the utility of STOP frameworks to assist lab to field extrapolations, and emphasises the importance of STOP when assessing complex exposure scenarios with metal mixtures.

Marine and Freshwater Pelagic and Benthic Harmful Algal Blooms: Toxins Production, Detection, Fate, Effects, Monitoring and Management

6.03.1

Comparison of four analytical methods for the detection of "rare" or novel freshwater paralytic shellfish toxins in New York

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Paralytic shellfish toxins (PSTs) are of increasing concern in freshwater ecosystems. While there are several analytical methods developed for the analysis of PSTs in marine systems, the analysis of PSTs produced by freshwater cyanobacteria has primarily used STX-ELISA and/or LC-MS/MS, methods that were originally intended for the detection of marine PST congeners. Freshwater cyanobacteria can produce congener profiles that are different from their marine dinoflagellate counterparts. The ability of ELISA or LC-MS/MS methods to detect freshwater cyanobacteria toxins, such as the Lyngbya wollei toxins or other novel cyanobacterial PSTs, has not been adequately evaluated. Samples from 245 lakes in New York State were initially screened for PSTs by HPLC fluorescence with post column chemical oxidation (PCOX). A subset of samples with high levels of PSTs measured by PCOX were reanalyzed by three methods: LC-MS/MS, STX-ELISA, and the STX receptor-binding assay to detect PSTs in blooms. Agreement between the methods was poor, with some samples negative by ELISA exhibiting a strong response in the PCOX method and some samples positive by ELISA showing little to no response in the PCOX and LC-MS/MS methods. LC-MS/MS methods run by different laboratories often gave conflicting results for the presence or absence of particular or novel PSTs. Analysis of freshwater PSTs clearly presents a significant analytical challenge. We recommend using a combination of methods for detecting freshwater PSTs as this reduced the likelihood of reporting falsely negative results and best protects human health.

6.03.2

Cylindrospermopsin transformation products formed by manganese-oxidizing bacteria show reduced toxicity in human cell line models

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Cylindrospermopsin (CYN) is a highly persistent alkaloid cyanotoxin with hepatotoxic and neurotoxic properties. Changes in climatic conditions favour the occurrence of toxigenic cyanobacterial blooms, thus, the risk of cyanotoxins in freshwater and marine systems is highly increased. Therefore, it is important to search for strategies to remove CYN from aquatic systems and to understand its fate in the environment. Manganese-oxidizing bacteria (MOB) have been proposed as a biological tool to treat water for the removal of diverse pollutants. The removal of CYN by MOB isolated from natural and technical systems, has been reported. However, so far it was not known if and what kind of transformation products are formed in biological transformation processes and if they are less toxic than CYN itself. So far, only transformation products formed after treatment of CYN with different chemical procedures like photocatalysis and advanced oxidation processes have been reported. Therefore, we investigated the formation of transformation products by MOB and analysed their cytotoxicity with human cell line models. All the tested MOB remove 10 mg L^{-1} of CYN at rates ranging from 0.5-3 mg L^{-1} day^{-1}. The rates obtained in the present study are higher than those reported for other CYN degraders. Eight transformation products were detected and identified based on a targeted screening of single ions, using an inclusion list for mass spectrometric analysis (LC-MS/MS) and further analysis of their fragmentation patterns. For all tested MOB the same eight transformation products were detected, regardless of their origin or phylogenetic association, suggesting a general transformation mechanism catalyzed in the presence of MOB. The molecular mass of the transformation products indicates that oxidation was the most important reaction pathway. The uracil moiety of CYN was the part most susceptible to modifications of the CYN molecule. The cytotoxicity of the mixture of transformation products in two hepatic human cell lines was almost completely removed. Together, our results suggest MOB could be used for water treatment to remove CYN and might contribute to the natural removal of CYN in freshwater systems.

6.03.4

Seasonal geosmin production from benthic cyanobacteria in a freshwater canal, with implications for drinking water supplies.

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We present and discuss a seasonal episode of geosmin occurrence in the drinking water supplies of Athens, which was attributed to benthic cyanobacteria in a part of a canal transferring surface water to treatment plants (WTPs). The case started with only a few (2) consumers noticing an unfavorable "earthy" odor in drinking water. Samples were analyzed immediately in EYDAP's laboratories with targeted and non-targeted GC-MS methods, to confirm the presence of geosmin at low levels (< 10 ng/L). A broad forensic investigation was initiated to locate the source of geosmin in the aqueduct. Results showed that geosmin was not produced in any of the water reservoirs but in a part of the canal transferring water to all four WTPs. *Gloeotrichia* spp. were identified as geosmin producers in benthic mats on the walls of the canal, probably among other mixed communities of benthic cyanobacteria. Cyanotoxin analysis (LC-MS/MS, PPIA) and molecular methods for toxin genes (qPCR) proved the absence of cyanotoxins/genes (microcystins, anatoxin-a, cylindrospermopsin) in untreated, treated water and in samples from canal walls. Since geosmin at such low concentrations is not effectively removed by common disinfection (chlorination) and flocculation followed by rapid sand filtration, efforts were made to remove the benthic mats from the canal by mechanical scrubbing and by copper sulfate dosing. None of those measures was effective in completely removing the geosmin producers, although cell lysis was evident during copper dosing, resulting in geosmin spikes. We present results and conclusions derived from this case with the aim to raise awareness and encourage more research on harmful benthic cyanobacteria that are by far less studied, especially with regards to their management and control.

6.03.5

Combined hydrogen peroxide-MlrA treatment – possible solution to cyanobacterial blooms and cyanotoxin degradation

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Treatment with hydrogen peroxide (H_2O_2) is one of the contemporary proposals for effective control of cyanobacterial bloom formation. However, there are contradictory opinions on how this kind of treatment influences the actual concentration of cyanotoxins such as microcystins (MC). Fast reduction of MC concentration by H_2O_2 in blooming water bodies has been questioned because the physiological response may include enhanced MC production. Further, field experiments have provided other arguments that H_2O_2 used alone is not effective in fast MC elimination. Moreover, different responses have been observed among cyanobacteria taxa indicating a possible species-specific adaptation to H_2O_2. These examples indicate the need to combine H_2O_2 treatment with other techniques which enable the reduction of MC concentration efficiently. The recent research [1] provides an indication that the recombinantly produced MlrA should be considered a very effective agent which hydrolyses and detoxifies different MC variants. Such an enzyme-based bioremediation represents a simple, fast and ecologically acceptable approach [2]. The aim of the present study has been to perform preliminary mesocosm experiments of the combined H_2O_2 + MlrA treatment in environmental water (Lake Ludoš, Serbia). Field work allows formulating the following conclusion: (a) the H_2O_2 treatment decreased the abundance of the dominant cyanobacterial taxa *Limnothrix* sp., *Aphanizomenon flos-aquae* and *Planktothrix agardhii*; (b) the intracellular concentration of MC was reduced/eliminated by H_2O_2 but the reduction of the extracellular MC could only be accomplished by supplementation with MlrA. This new concept, of the combined treatment with both H_2O_2 and MlrA enzyme, is being proposed to be applied in conditions where cyanobacterial biomass with a high MC concentration needs to be rapidly and efficiently reduced. Key words: toxic cyanobacteria, microcystis, MlrA, hydrogen peroxide [1] D, Maksylewicz A, Maroszek M, Marek S. 2018. Combined treatment of toxic cyanobacteria *Microcystis aeruginosa* with hydrogen peroxide and microcystin biodegradation agents results in quick toxin elimination. Acta Biochim Pol 65: 133–140. [2] Sharma B, Dangi AK, Shukla P. 2018. Contemporary enzyme based technologies for bioremediation: A review. J Environ Manage 210: 10–22.

Modern Approaches to Assessment and Management of PFAS: A Science-Policy Dialogue

6.04.1

Identification and occurrence of novel cyclic and polymeric perfluoroalkyl ethers (PFECAs) downstream of the fluoropolymer manufacturing plants

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The ban on perfluorooctanoic acid (PFOA) led to the production and use of alternative fluorinated compounds used as processing aid in the manufacture of fluoropolymers. During the transition, most of the producers developed their own alternatives. Among the well-known replacements used in fluoropolymer manufacture there are HFPO-DA ammonium salt, commercialized by DuPont/Chemours under the tradename of Gen-X, and ADONA from 3M/Dyneon which have already been detected downstream of the effluent discharge from fluorochemical or fluoropolymer manufacturing plants. Little information is available on the processing aids currently used by other fluoropolymer producers. In this study high resolution mass spectrometry was used to investigate the occurrence and identity of novel fluorochemicals in the rivers receiving discharge from two Solvay fluoropolymer manufactures, located in Italy and the USA. One cyclic perfluoroalkyl ether carboxylic acid, cPFECA (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), with trade names F-DIOX Acid or cC6O4 Acid and a mixture of Cl-PFECAs (C3F6ClO-[CF2CF(CF3)O]n-[CF(CF3)O]m-CF2COOH, n=0-2, m=0-2, CAS No. 329238-24-6) have been identified. These compounds were detected in the rivers downstream of the discharges but also upstream of the industrial facilities. This suggests that these PFECAs are not only emitted into the river water but also to the air and they contaminate the area around the productive facilities via atmospheric deposition. Moreover, almost 300 km far from the Italian fluoropolymer manufactory plant, the drinking water produced by river water that received the discharges was polluted with one of the PFECAs. Retrospective study of LC-HRMS chromatographic analyses carried out on Italian river samples collected in previous years allowed to detect these compounds leastwise since 2012. Standards are not available for most compounds making proper quantification very difficult; however, chromatographic peak areas of some of these compounds in the rivers downstream of the productive facilities are similar, or greater, than those of PFOA, raising some concern. Further investigations are ongoing to identify and quantify more compounds and to assess the ecological and health risks of these newly identified substances.

6.04.2

Distribution of legacy and emerging per- and polyfluoroalkyl substances (PFAS) in seabirds and fish from Atlantic offshore and coastal environments

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Long-chain per- and polyfluoroalkyl substances (PFAS) demonstrate remarkable environmental persistence, bioaccumulative capacity, and have been found globally in surface water and biota, including birds from diverse habitats. Public health concerns and regulatory attention have caused a shift in PFAS production, towards short-chain analogues or structurally diverse compounds with variable functional groups or fluorination patterns. Seabirds are ideal sentinels to assess the occurrence of both legacy and emerging PFASs in food webs, as their upper trophic level position allows them to assimilate resources and related biological, physical, and chemical conditions across multiple ecosystems and temporal scales. Here, PFAS were measured in multiple tissues from juvenile Atlantic seabirds collected in 2017 and 2018, along with forage fish from Massachusetts Bay. Seabird species sampled included deceased immature or juvenile herring gulls, great shearwaters, terns, and pelicans from Narragansett Bay in Rhode Island, Massachusetts Bay off the coast of Massachusetts, and the Cape Fear River Estuary (CFRE) in southeastern North Carolina. Multiple tissues were analyzed for legacy and emerging PFAS using liquid chromatography/high resolution mass spectrometry, employing both targeted and suspect screening methods. Sand lance, a key forage fish in Massachusetts Bay were also measured for PFAS via LC-MS/MS. PFOS dominated all liver samples across all individuals and habitats, making up 58% of concentrations observed across all habitats (range: 16 - 280 ng/g). Chicks hatched downstream of a fluoropolymer production site contained significant concentrations of a perfluorinated ether sulfonic acid (Nafion byproduct-2; range: < LOD – 110 ng/g in liver) and two perfluorinated ether carboxylic acids (PFO4DA and PFO5DoDA; PFO5DoDA range: < LOD – 30 ng/g in liver). These perfluoroalkyl ether acids were likewise detected in multiple tissues from CFRE chicks, with high inter-individual variability. Emerging compound Nafion byproduct-2 was detected at concentrations similar to or exceeding legacy PFOS in brain.

6.04.3

High-throughput assessment of PFAS mixtures in environmental samples by combining bioassays and Effect-Directed Analysis

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Effect-directed analysis (EDA) is a process where extracts are fractionized, and sub-fractions are tested in bioassays. Chemical identification can then be limited to fractions showing a response in the bioassay. PFAS have been reported to have the ability to displace thyroid hormone thyroxine (T4) from its plasma transporter protein transthyretin (TTR), and TTR binding assays can be used to assess the potential of environmental mixtures to displace T4 from TTR.In this work, the use of bioassays and EDA in an automated liquid handling system for high-throughput screening of environmental PFAS mixtures potency on relevant biological targets, and identification of suspect compounds for the observed onset of a molecular initiating event (MIE) is demonstrated.A freshwater lake in Norway, lake Tyrifjorden, was chosen as case study site. The main source of PFAS-contamination to the lake is considered to be residues from a closed-down factory area for paper industry.Standards of individual compounds were acquired for bioassay testing. Field sample extracts (biota and sediments) were used for EDA and bioassay. Each extract was injected on a liquid chromatography system and fractionized with high-resolution in 10 second fractions into a microtiter plate. TTR binding potencies of individual compounds, sample extracts, and sample extract fractions were tested.Targeted chemical analyses showed that PFAS profiles in biota are dominated by PFAAs while sediments are dominated by compounds which are precursors to PFAAs. However, analyses of the total extractable organic fluorine showed that a large fraction remains unexplained by the targeted analyses.Precursor compounds were generally less potent in the TTR assay than the corresponding PFAA end product. Biota extracts showed higher potencies compared to sediment extracts, which might be due to the higher PFAA fractions in biota extracts. Further, potencies were limited to specific extract fractions. The combination of bioassay, EDA and further chemical analyses will be used on these fractions to identify suspect compounds, and to prioritize compounds found in the environment for further effect studies.

6.04.4

Per- and Polyfluoroalkyl Substances (PFAS) under REACH and CLP

J. Peltola-Thies, European Chemicals Agency - ECHA / Risk Management; I. GONZALEZ RODRIGUEZ, ECHA / Evaluation (C3); C. Ajao, ECHA-European Chemicals Agency; P. Karamertzanis, ECHA; J. Caley, European Chemicals Agency - ECHA / Evaluation; Z. Kloslova, ECHA / Evaluation; S. Lapenna, F. Broeckart, N. Andersson, K. Kivelä, ECHA; D. Mottet, ECHA / Risk Management Implementation Unit

The European Chemicals Agency (ECHA) Integrated Regulatory Strategy aims to ensure a coherent implementation of the regulatory processes and supports authorities in addressing substances of concern. With the support from the EU Members States, ECHA has moved from a substance-by-substance assessment to assessment of groups of structurally and/or functionally similar substances. Per- and polyfluoroalkyl substances (PFASs) constitute a very large group of substances used in diverse consumer and industrial applications. Many PFASs have recently been subject of regulatory activities in Europe. The main PFASs groups covered by assessment, testing and/or risk management until today are, C6-C14 -PFCAs, C4-, C6- and C8- PFSAs and some PFECAs, their salts and related substances. However, a high number of other PFASs have not yet been assessed. ECHA identified in a recent mapping study 471 substances registered under REACH that have at least one constituent (>10% w/w in the registered substance) containing a CF bond in their composition and an additional ~1900 substances that have been notified in the C&L inventory . ECHA is refining the algorithms for the allocation of the identified structures to subgroups. An assessment and testing strategy, where relevant, needs to be developed for the PFASs groups not yet assessed. Part of the work aims at gaining understanding on whether in addition to persistence also other properties are common among the remaining PFASs and which PFAS subgroups could be assessed together. A focus on the properties of a common ultimate degradation product (‘arrowhead’) within each subgroup provides a good starting point for the work based on the experience gained by today.

6.04.5

Strategies for grouping per- and polyfluoroalkyl substances

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It is increasingly apparent that to effectively protect the public and environment from the wide range of possible PFAS-related effects, strategies should be sought to group PFAS for regulation rather than to regulate them one-by-one. For example, in the recent Zurich Statement on PFAS, the authors recommended that, “actions need to address groups of PFAS rather than individual chemicals and that such a grouping approach needs to be scientifically sound”. There are, however, several possible motivations to group PFAS and each of these motivations requires a different strategy. For example, in the Madrid Statement on PFAS a large group of scientists argued for treating PFAS as a single class for regulatory action. Regulatory authorities, on the other hand, may have more immediate practical needs, for example, to develop scientifically sound approaches for considering mixture toxicity resulting from drinking water exposure to multiple perfluoroalkyl acids (PFAAs). The aims of this presentation are to discuss 1) past and future grouping approaches for PFAS, 2) outline challenges and opportunities and 3) present a way forward for advancing grouping approaches.

6.04.6

Poster spotlight: Environmental Sources, Analyses, Chemistry, Fate & Transport of Per- and Polyfluoroalkyl Substances - Workgroup Summary, Output, Conclusions and Implications from the 2019 SETAC Focused Topic Meeting

S. Korzeniowski, FluoroCouncil

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6.04.7

Poster spotlight: A comparison of perfluorooctanoic acid (PFOA) and hexafluoropropylene oxide dimer acid (HFPO-DA, or GenX) effects in target tissues of exposed mice.

S. Fenton, NTP Labs/NIEHS / National Toxicology Program

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6.04.8

Poster spotlight: Biosorption and permeabilities of PFAAs and four of their alternatives and the effects on toxicokinetic behaviour

F. Allendorf, Helmholtz centre for environmental research - UFZ

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Nanotechnology Regulatory and Assessment Frameworks and Nanoremediation Approaches

6.05.1

A Framework for Grouping and Read-Across of Nanomaterials- Supporting Innovation and Risk Assessment

T.F. Fernandes, Heriot-Watt University / Centre for Marine Biodiversity and Biotechnology, Institute of Life and Earth Sciences

Nanomaterials can exist in a variety of nanoforms characterised by differences in properties such as size, shape or coating. Assessing the potential risks of each nanoform can be laborious and expensive. According to legislation, grouping can streamline data generation for the risk assessment of substances. The H2020 GRACIOUS project Framework aims to support the grouping of nanomaterials or nanoforms (NFs), in a regulatory context and to support innovation. This includes using grouping to support read-across from source(s), for which data and information exist, to a similar target NF where information is lacking. The Framework provides an initial set of hypotheses for grouping of NFs, which take into account the identity and use(s) of the NFs, as well as the purpose of grouping. Initial collection of basic information allows selection of an appropriate pre-defined grouping hypothesis and a tailored Integrated Approach to Testing and Assessment (IATA), designed to allow the user to accept or reject the hypothesis. Users needing to develop their own case-specific hypothesis (and IATA) are also supported by the Framework. In addition, the IATA guides acquisition of the information needed for read-across justification. This approach gathers information leading to effective and efficient risk assessments, including the reduction of the use of test animals. Examples of IATAs will be presented and implications discussed in the context of risk assessment.

6.05.2

Regulatory Risk Assessment Framework Development for nanomaterials in Canada

M. Sauve, Environment and Climate Change Canada
Nanotechnologies offer great possibilities due to their properties. However, these same properties may also generate risk to the environment or to human health. Canada has been conducting risk assessment on nanomaterials for the past 10 years adapting existing conventional chemicals methodologies and tools. This presentation will take the lessons learned from years of knowledge and focus on the feasibility of using these existing methodologies and tools for risk assessment of existing nanomaterials. It will also introduce our risk assessment framework, and identify the major challenges that Canada is experiencing, and some options considered to overcome these challenges. The Government of Canada is involved with various stakeholders on both domestic and international fronts and closely follows and fosters nanomaterials' scientific research, method development and data generation. Furthermore, advanced materials characterization, and the need to continuously anticipate emerging issues resulting from new innovative materials that are being developed. It is clear from a number of studies that there are going to be many future developments involving more advanced nanomaterials.

PBT and Other Substances of (Very High) Concern: Policies, Practices and Challenges

6.06.1
A strategy on how to identify criteria for chemicals of global concern
T. Backhaus, A. Arrhenius, University of Gothenburg / Department of Biological and Environmental Sciences; S. Ubl-Kägi, M. Scheringer, ETH Zurich / Dep. of Environmental Systems Science
The Strategic Approach to International Chemicals Management (SAICM) is a policy framework with the aim to "ensure that, by the year 2020, chemicals will be produced and used in ways that minimize significant adverse impacts on the environment and human health". As we are far from achieving that goal a process is mandated to adopt recommendations to respond to the national and international challenges of chemicals and waste after 2020. The recommendations are planned to be adopted in October 2020. One of the currently discussed objectives is to identify and tackle "Issues of international concern", which includes hazardous substances or groups of substances, globally traded in products and articles. In this presentation we will outline criteria for identifying chemicals of global concern from the tens of thousands of chemicals used for commercial purposes globally. The suggested tiered approach aims to avoid "paralysis by analysis" and acknowledge the fact that information on chemicals is extremly diverse. It is designed to make optimum use of existing information from national and regional regulations (i.a. REACH, TSCA) as well as international conventions, such as the Stockholm and Rotterdam conventions. Furthermore, the approach employs a set of hazard- and exposure based *in silico* assessments. These include PBT/vPvB, PMT/vPvM criteria as well as the application of the TTC/ecoTTC concept.

6.06.2
Towards better guidance on the analytical monitoring of environmental fate studies: an essential issue for data quality and regulatory usability
A. Catherine, ECHA (European Chemicals Agency) / Hazard Assessment; I. Costea, R. Demi, European Chemicals Agency (ECHA) / Prioritisation and Integration; A. Kapanen, European Chemicals Agency (ECHA) / Hazard Assessment; m. skowron, European Chemicals Agency (ECHA) / Prioritisation and Integration; M. Sobanska, J. Weber, European Chemicals Agency (ECHA) / Hazard Assessment
There is currently a lack of clarity on the minimum requirements in terms of analytical detection and quantification of substances that can be reasonably expected from environmental fate studies. This unclarity originates partly from a misinterpretation of the limit of 0.1% (w/w) for constituents, impurities and additives to be considered relevant for the PBT/vPvB assessment (ECHA Guidance R.11) and to the technical limitations inherent to the conduct of biodegradation simulation tests (i.e. EU C.23/OECD TG 307, EU C.24/OECD TG 308 and EU C.25/OECD TG 309). In practice, the level of information that may reasonably be obtained from biodegradation simulation studies will depend on the substance considered but also on its interaction with the matrix being analysed. Therefore the selected analytical chemistry methodology will have a paramount impact on the quality and reliability of the information being generated. This presentation will summarize the outcome of an ongoing ECHA project to clarify expectations for regulatory acceptance of biodegradation simulation studies and to develop better guidance to industry and contract research laboratories (CROs) responsible for the generation of environmental fate studies. The project includes the following elements: a comparative analysis of the legal requirements of relevant European regulatory regimes including the REACH Regulation (EC No 1907/2006), the BPR (EC No 528/2012), the PPP Regulation (EC No 1107/2009), the medicinal products regulation (EC No 726/2004) and the Stockholm Convention on Persistent Organic Pollutants (POPs);a critical literature review of analytical methods (and their limitations) applicable to environmental fate studies and focussed on relevant methodological aspects to identify and quantify parent and transformation/degradation products;the outcome of a consultation of relevant CROs with regards to the main issues faced in the conduct of biodegradation simulation studies;a review of recent biodegradation simulation studies submitted by industry with a focus on the main methodological deficiencies impacting the reliability of study results. Preliminary guidance will be proposed for discussion with scientists, regulators and other stakeholders.

6.06.3
Multi-laboratory validation of a new marine biodegradation test for persistence screening
A. Ott, T. Martin, Newcastle University / School of Engineering; D. Lyon, Shell Oil Company, Shell Health – Americas / Shell Health Risk Science Team; C. Malin, N. Robinson, EOSCA; B. Rowles, Cefas Lowestoft Laboratory; J. Snape, AstraZeneca UK Ltd. / Global Sustainability; I. Still, EOSCA; G. Whale, Whale Environmental Consultancy Limited; R. Davenport, Newcastle University / School of Engineering
Exponential growth of the human population has been accompanied by increased manufacture and use of chemical compounds. To classify the fate and behaviour of these compounds in the environment, a series of international standardised biodegradation screening tests were developed over 25 years ago. In recent years, regulatory emphasis (e.g. REACH) has shifted from measuring biodegradation towards prioritisations based on chemical persistence. Current biodegradation screening tests are not specifically designed for persistence assessment, often show high inter- and intra-test variability, and often give false negative biodegradation results. Based on previous studies and recommendations, an international ring test involving 13 laboratories validated a new test method in 2017 for marine biodegradation with a focus on improving the reliability of persistence screening. The new method incorporated increased bacterial cell numbers to better represent the microbial diversity inherent in the sampled environments and ran beyond 60 days, which is the half-life threshold for persistence in the marine environment. The new test provided a more reliable and less variable characterization of the biodegradation behavior of five reference chemicals (sodium benzoate, triethanolamine, 4-nitrophenol, anionic polyacrylamide, pentachlorophenol), with respect to REACH and OSPAR persistence thresholds, than the current OECD 306 test. The proposed new method provides a cost effective screening test for persistence that could streamline chemical regulation and reduce the cost and animal welfare implications of further unnecessary higher tier testing. There was a positive relationship between total cell counts and biodegradation potential. However, the greatest rates and extents of degradation were not necessarily observed at the laboratories with the highest cell concentrations, suggesting that cell concentration is not the only factor influencing the degradation potential of an environmental sample. The poster presentation "Inside the black box: microbial community profiling in marine biodegradation screening tests" investigates to what extend microbial community analysis of seawater samples (based on 16S rDNA gene sequencing) can explain and predict observed biodegradation rates.

6.06.4
Moving persistence (P) assessments into the 21st Century: Developing a new paradigm to assess degradation potential of chemicals
A.D. Redman, Exxon Mobil Biomedical Sciences, Inc. / Toxicology and Environment Science Division; J. Bietz, Clariant; P.K. Curtis-Jackson, Environment Agency (England and Wales) / Chemical Assessment Unit; P. Dalkmann, Bayer AG Crop Science Division / Environmental Safety; R. Davenport, Newcastle University / School of Engineering; J. Davies, Lynondell Basel; J.W. Davis, Dow Chemical Company / Toxicology and Environmental Research and Consulting; K. Fenner, Eawag / Department of Chemistry; T. Gant, King's College London; L. Hand, Syngenta Limited / Product Metabolism; D. Lyon, Shell Oil Company, Shell Health – Americas / Shell Health Risk Science Team; P. Mayer, Technical University of Denmark / Department of Environmental Engineering; K. McDonough, Procter & Gamble Company / Global Product Stewardship; A. Ott, Newcastle University / School of Engineering; J.C. Otte, BASF SE; F. Palais, SOLVAY; J. Parsons, University of Amsterdam / Institute for Biodiversity and Ecosystem Dynamics; A. Schaeffer, RWTH Aachen University / Institute for Environmental Research (Biology V); C. Sweetlove, lOREAL SA / RECHERCHE ENVIRONNEMENTALE; J. Tolls, Henkel AG & Co. KGaA
Understanding the degradation potential of organic chemicals is important in supporting risk assessment and management. Persistence has become a key evaluation metric within European hazard assessment frameworks (e.g. PBT). However, P criteria are based on dated scientific concepts that reflect a narrow range of chemistry that was studied extensively for more than 30 years. Newer chemistry does not fit neatly into that older paradigm and scientific principles and experimental techniques have been developed that are changing the way science evaluates persistence. ECETOC is a collaborative space for scientists from industry, academia and governments to develop and promote practical, trusted and sustainable solutions to scientific challenges related to chemical safety assessments. ECETOC has formed a task force (TF) whose objective is to develop an improved framework and best practices for persistence and degradation assessments, based on progress in the scientific understanding of the underlying processes. The terms of reference for this TF and a description of the planned work products will be discussed. In addition there are two posters that provide more details into the two main themes being studied in this TF: 1) Weight of

Evidence Framework, and 2) Guidance to improve experimental methods.

6.06.5
Comparative ranking of impact potentials of substances of concern
M. Nendza, Analytisches Laboratorium; S. Hahn, Fraunhofer ITEM / Chemical Risk Assessment; M. Klein, Fraunhofer IME / Ecological chemistry; S. Gabbert, Wageningen University / Social Sciences
The (in)acceptance of hazards posed by PBT/vPvB and other substances of concern may be assessed by socio-economic analyses (SEA) to support viable risk management options (RMO)). Chemical substances differ with regard to exposure dynamics in water, sediment, soil and air, transport to remote regions, persistence, bioaccumulation and effects on human health and the environment. Based on these differences, comparative ranking of the relative impacts allows to identify better or worse alternatives among different substances with similar uses ((regrettable) substitution alternatives). The comparative ranking is based on an impact scoring approach consisting of scores to account for expected environmental stocks and possible effects on the environment and on human health via the environment (toxicological benchmarks). The impact scores are combined with concern scores that cover uncertainties related to yet unknown long-term effects due to extended persistence. The resulting pattern of individual scores (fingerprints) and the overall scores can indicate what kinds of damages may happen to either lesser or greater extents. For example, two substances may have similar total scores but different fingerprints that indicate which substance might be less (or more) critical. The comparative ranking is illustrated with three case studies regarding different intrinsic properties (D4/D5), different use and emission pattern (brominated flame retardants) and unacceptable alternatives (PFOS, PFOA). The present scoring scheme needs further refinement and political decisions are needed for appraisal of inacceptable effects. Substantial progress would be the availability of representative reference substances that could be used like internal standards in analytical chemistry. With stock and impact scores agreed by the competent authorities, such reference substances would make it much easier to classify the impact potentials of substances with poor data status.

6.06.6
A Concern-based Approach to Socio-economic Analysis of PBT/vPvB Substances Using Dynamic Stock Pollution Modelling
S. Gabbert, Wageningen University / Social Sciences; J. Klein, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Ecotoxicology; M. Nendza, Analytisches Laboratorium; S. Hahn, Fraunhofer ITEM / Chemical Risk Assessment; M. Klein, Fraunhofer IME / Ecological chemistry
Substances with persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) properties can accumulate in environmental media and biota, leading to an increasing pollution burden over time. The (unpredictable) long-term effects are therefore a key concern that have to be addressed in regulatory decision-making. The European chemicals legislation REACH adopts two main regulatory instruments for PBT/vPvB substances, i.e. authorisation and restriction. Both regulatory procedures use socio-economic analysis (SEA) for assessing and comparing different kinds of impacts in order to identify most suitable risk management measures. For PBT/vPvB substances, however, there is wide agreement that a quantitative impact assessment is not possible due to lacking information about safe concentration levels. Hence, the question is how SEA can still allow for a concern-based, comparative evaluation of risk management options for these chemicals. This study suggests an approach that estimates the time-dependent accumulation of a chemical (i.e. the stock) in different environmental compartments, and to use information on the resulting 'time path of pollution' arising from PBT/vPvB use as a surrogate of their expected long-term impact potential. The approach is applied to hexabromocyclododecane (HBCDD) as an illustrative example, assuming an assessment period of $t = 12$ years. Furthermore, we distinguish different spatial scales (continental/regional), different modes of entry of emissions (equal release fractions, 100% to water, 100% to air), and policy scenarios (emission stop, linear emission reduction). Our results demonstrate that the time path of pollution of HBCDD differs considerably across compartments. For the selected case compound, the stock accumulates predominantly in sediment. Furthermore, if emissions stop, the degradation process stretches over several years, depending on the compartment specific elimination rates. This underlines that risk management measures are particularly urgent for PBT/vPvB substances for which environmental stocks are high (i.e. at or close to steady state) or can be expected to become high in one or several compartments with a continued use. Finally, the approach offers transparent insight how uncertainties regarding, for example, data on PBT/vPvB properties, may impact cost-effective risk management measures. While not being equivalent to standard impact assessment, the dynamics of environmental stocks can be regarded a key component of the regulatory concern related to PBT/vPvB substances and should, therefore, be a building block of SEA.

Science and Risk Communication in an Ever-changing World: How Could It Be Done to Build Trust?

6.07.1
How to communicate scientific uncertainty while ensuring trust among stakeholders - mission impossible?
S. Fiack, German Federal Institute for Risk Assessment BfR / Risk Communication
1. Introduction Uncertainty is an integral part of science, but how should public scientific institutions, especially in times of crisis, deal with scientific uncertainty? Most risk communication guidelines recommend communicating uncertainty although there is only limited scientific literature about the effects of communicating uncertainty on cognition, affect, trust and decision-making. Whether greater transparency in the communication of uncertainty will enhance credibility and public trust is still an open scientific question. The scientific literature shows that different audiences, and even individuals within those audiences, are likely to have different reactions to various presentations of uncertainty. 2. Materials and methods Using examples (e.g.fipronil in eggs, glyphosate in breastmilk, *E. coli* in sprouts) from the work of the German Federal Institute for Risk Assessment (BfR), some of the challenges associated with the communication of risks are presented. Risk perception is studied with the BfR consumer monitor, a representative survey of over 1,000 persons. The BfR Consumer Monitor provides answers at 6-month intervals to the question of how the population perceives topics from the field of consumer health protection. 3. Results and discussion The communication of risks not only involves the conveyance of substantiated knowledge but also how to deal with what is not known and how to communicate uncertainty. A clear understanding of how risks are perceived and what factors influence risk perception are indispensable for adequate risk communication. *Table 1 could look like this* The results of the BfR Consumer Monitor can be used to design risk communication processes effectively - including the communication of uncertainty. Public institutions should address uncertainty as this is the basis for transparency and openness. **The challenge is to provide independent, quality-assured and transparent assessments of scientific findings, for politicians need sound advice to ensure knowledge-based decisions. It also means communicating the degree of scientific uncertainty and openly dealing with knowledge gaps and even scientific divergence.** Scientific advice needs to be a transparent and impartial process, with a clear mandate to ensure that science is separate from politics. Consumers expect easy-to-follow tips and clear information that are helpful to them in their everyday life [3]. A summary that includes concrete recommendations, written in language that the general public can understand is recommendend. In addition, a risk profile has been developed which summarises the key points of the opinions in the form of a graph, thereby visualising the described risk. The information includes the validity of the data which is one aspect of uncertainty.

6.07.2
Exploring disagreements about EDC evaluation
B. McIlroy-Young, University of British Columbia / Resources, Environment and Sustainability; G. Oberg, University of British Columbia / IRES; A. Leopold, Calidris Environment BV / Calidris Environment BV; J. Durant, University of British Columbia. / RES; D. Steel, University of British Columbia
Endocrine disrupting chemicals (EDCs) are substances in the environment, food and consumer products that can impact the hormonal system and lead to adverse effects. There are an increasing number of studies indicating that a diversity of health issues in wildlife and humans may be tied to exposure to mounting levels of EDCs in the environment. As such, regulating EDCs presents a formidable challenge and different jurisdictions take different approaches to their evaluation. A complicating factor is that consensus is lacking among scientists as to how to best evaluate the possible threat posed by EDCs: some support a hazard-based approach, based on a chemicals' potential to cause an adverse health effect. Others support a risk-based approach, which assesses the likelihood of real-world exposure at a hazardous dose. Scientists on each side accuse the other of being unscientific. Previous studies have shown that the way scientists present and discuss (frame) a topic is related to how they position themselves in policy-relevant debates such as climate change and GMOs. Assuming that this also holds true for the EDC controversy, the aim of this exploratory study is to analyze how challenges posed by EDCs are framed by scientists supporting a hazard-based versus risk-based approach. We do this by carrying out two focus groups with experienced scientists, aiming to have one group with supporters of a hazard approach and the other supporting a risk approach. Our findings reveal fundamental differences in how the two groups view uncertainties about the potential harm caused by EDCs. The risk assessment group holds that the potential harm posed by EDCs is blown out of proportion: these substances have become the source of widespread public anxiety despite there being no more cause for concern than for many other contaminants. In contrast, the hazard group holds that the potential harm posed by EDCs is highly uncertain but potentially tied to many human health and environmental concerns. These differences seem to colour how the two groups frame scientific consensus around EDCs, the influence of inappropriate scientific advocacy, and the drivers of EDC regulation. This result aligns with other studies showing that differences in framing often underlie scientific debate in policy-relevant fields. Our study suggests a need for research to increase transparency around differences in framing to improve the conduct and

communication of research for regulation around EDCs.

6.07.3
Effective tools for communicating science: storytelling and improvisational theatre

J. Muncke, Food Packaging Forum Foundation / General Management; K. Bschir, Yes and Science klg

The use of storytelling techniques is often suggested as a way to better engage the audience in science communication. But it is often not clear what "storytelling" exactly means in this context and how it should be applied in scientific publications or presentations. In this presentation, we explain the ABT framework, developed by Dr. Randy Olson, that can be used to develop narratives for scientific writing and presentations. We also discuss other aspects that are important for live presentations, based on our own experience as scientists, science communicators and communication coaches. We describe our method for training scientists to do effective live presentations, using techniques from improvisational theatre, and discuss why people trust some scientists more than others.

6.07.4
Grassroots Organizing Strategies for Co-Developed Research in Environmental Justice Communities

M.E. Guyader, Sierra Club

Science communication is often undermined by a lack of trust amongst stakeholders. Top-down organizational management, common throughout the home institutions of SETAC's membership, contributes to assumptions of an individual's interest in collaboration. Personal fears then contort these assumptions and drive wedges into potential relationships across stakeholder groups. Grassroots movements provide an alternative model of social organization that attempts to remedy this predicament. Community organizers have developed a set of strategies that facilitate relationship building amongst individuals with mutual self-interest, and sustain the quality of their own, independent relational network. The presenter will share a case study in applying these tactics while engaging in co-developed research with environmental justice communities in Denver, Colorado.

6.07.5
Science-Informed Decision Making: Holistic Frameworks to Support Societal Values

S.E. Apitz, SEA Environmental Decisions Ltd

Managing environmental and social impacts requires new approaches to communication and collaboration. Such approaches raise a number of questions: What attributes or conditions does a decision aim to sustain or restore? Who benefits? For how long? At what cost (to whom?) and Who decides? These are normative questions, rooted in societal values. To address them requires broad stakeholder engagement. Consensus-based environmental decision making (CBED), which promotes the empowerment of stakeholders to guide the decision process (ASTM 2017), calls for frameworks and indicators to evaluate the range of potential effects to the environment associated with decision making. To achieve this, environmental scientists must ensure that the science they generate is relevant to and translated in terms of societal values and objectives, to facilitate its incorporation into local, national, and international decision-making processes. A range of multi-criteria frameworks for evaluating and communicating the trade-offs inherent in ecosystem management and restoration translate impact measures in terms of, for example, ES, regional risks, sustainability (or sustainable development goals), stakeholder (including regulatory) objectives, habitat, or monetary units. The development of such frameworks to address a specific question follows a similar conceptual pathway: 1) Identifying stakeholders and their objectives; 2) identifying alternatives; 3) identifying criteria and indicators; 4) developing metrics of impact on indicators; 5) (quantitatively or qualitatively) scoring impacts on indicators based on scientific metrics; 6) weighting indicators based on stakeholder (including regulator) priorities; and 7) evaluating trade-offs to inform decisions. In all cases, the purpose is to build a clear and unbroken chain between what we can measure as scientists and what we hope to achieve as a society. This paper will describe how such frameworks can be built and used to support trade-off evaluation, policy development and decision-making. A number of examples, developed to inform decisions such as habitat restoration, landscape regulation to protect soil/sediment balance, coastal protection, dredged material management and sustainable remediation strategies, will be used to illustrate framework development and application. Strengths, weaknesses and embedded assumptions behind various approaches, and a path forward to better support science-informed CBED, will be discussed.

6.07.6
Reprise - Communicating Science-based Information is Hard Work

L. Kapustka, LK Consultancy

Effective transmission of science-based information requires alignment of communication objectives and the beliefs and goals of the intended audience. Goals can range from simply sharing the gee-whiz excitement of an observation to informing citizenry about a pressing societal issue. Often one hears a refrain that goes something like "if only they knew what I know ..." But the reality is that they may already understand the issue and are persuaded by other perspectives or beliefs. The complexity of social-ecological systems present challenges for communications about the ways the systems work. Dumbing down the information results in lost credibility; infusing the communication with jargon obscures the message. There is always much we do not know and much that is unknowable about these systems, which leads to the special challenge of balancing what we know (certitude) versus what we don't know (uncertainty). Finally, to be viewed as an honest broker of science-based information, we must work to avoid the pitfalls of normative science and steal advocacy. Audiences come with varied beliefs and persuasions. Three groups have been identified: moderns, cultural creatives, and tranditionals. Effective messaging must be compatible with their beliefs before they will accept the information and act upon it. Risk communication often fails as a result of misalignment of beliefs and goals.

The Role of Science in Derivation of Site-specific Environmental Quality Benchmarks and Management of Water Quality

6.08.1
Development of a risk assessment framework for nickel in South East Asia and Melanesia

G. Merrington, WCA Environment Limited; A. Peters, wca; C.E. Schlekat, NiPERA Inc; E.R. Garman, E.T. Middleton, NiPERA Inc / Ecotoxicologist

Over two-thirds of the world's nickel deposits are in tropical regions, and just under half of these are in South-East Asia and Melanesia (SEAM). It is expected that over the next five years production of nickel, specifically in the form of lateritic ores, is likely to considerably increase. Nickel exposures in this region are from nickel mining, smelting and refining. From these operations, dissolved and particulate nickel can be released via site run-off, seepage from waste storage facilities, runoff and seepage from slag disposal/storage sites, via solids/slurry from the bag house or as dust from stack and fugitive emissions. Our focus was initially upon the freshwater aquatic environments. The use of scenario-based approaches, i.e. focussing upon ecosystems for which data and understanding are available, represents an appropriate starting position and fits with the premise of the risk assessment paradigm and specifically focusses efforts of refinement. A generic assessment has been performed for sites processing various quantities of nickel per year, and with varying degrees of dilution available for discharges into the local aquatic environment. It is possible to considerably improve assessments which are based largely upon precautionary default assumptions even with limited amounts of site-specific data. Targeted monitoring provides a valuable means of improving the relevance of the risk assessment to a specific site, this could include measurement of the degree of dilution of discharges into local water courses or measurement of exposure levels of nickel in the receiving environment. Drinking water guidelines represent a locally relevant threshold but are not relevant to the protection of ecosystems and ecological communities. These assessments focus solely on the potential risks posed by nickel and do not take account of any additional stressors which may be present such as other metals, suspended solids, or acidity which can also have important implications for human and ecological health.

6.08.2
Evaluating the relationship between sediment quality cleanup objectives and protecting biological receptors: Lessons learned from 11 case studies of contaminated sediment remedy effectiveness

J. Stern, King County Department of Natural Resources and Parks / Department of Natural Resources and Parks; C. Patmont, B. Henry, Anchor QEA, LLC; M. Mills, U.S. Environmental Protection Agency / Office of Research & Development; S. Nadeau, Sediment Management Work Group; R. Johnston, Applied Ecological Solutions

In a recent collaborative workshop, we reviewed case studies of cleanup at 11 large contaminated sediment sites to ascertain lessons learned regarding remedy effectiveness. The sites were located in the Pacific Northwest (6), Upper Midwest (2), South (1) and Northeast (2) regions of the United States. Remedial actions included dredging, capping, cover, and monitored natural recovery. Common contaminants of concern included PCBs, PAHs (including NAPL), mercury, and creosote constituents. Each site was reviewed with respect to remedial objectives and risk drivers, a summary of early and follow-on remedial actions, significant remedy scope or schedule deviations, timing and effectiveness of source control, primary pre-and post-remedy monitoring elements, achievement of short- and long-term remediation objectives for surface sediment, whether the remedy was on track to achieve long-term remediation objectives for water and/or biota, and key take home messages. Post-remediation monitoring generally focused on chemical concentrations in sediment, surface water, fish, and shellfish as well as porewater and cap integrity in the case of capping remedies. Complexity in the linkages between sediment and fish tissue concentrations were identified, including variations in external inputs from point (industrial and municipal) and non-point sources (stormwater), releases from navigational and remedial dredging projects, and recirculation of contamination within the food web. Robust, long-term monitoring data are needed to demonstrate the exposure linkages affected by sediment cleanup. Overall, several themes emerged from the case study review. While remedial efforts were fairly successful at reducing surface sediment concentrations, the effectiveness of reducing contaminant concentrations in biota was mixed with some sites showing reductions consistent with expectations while others did not. The latter was generally attributed to an incomplete understanding of what controls contaminant concentrations in biota, particularly the link between sediment and tissue concentrations. Source control at the site and within the watershed was of primary importance and early or interim actions often resulted in significant progress toward meeting remedial objectives. At several sites, adaptive management in various forms was an effective way to deal with an evolving conceptual site model or changing site conditions.

6.08.3
A removal requirement index for assessing source water quality for purification to drinking water
T.E. Pronk, KWR Water Research Institue / Chemical water quality; G. Stroomberg, RIWA River Water Works; S. Kools, KWR Water Research Institute
The European Water Framework Directive (WFD) Article 7.3 states “Member States shall ensure the necessary protection for the bodies of water identified with the aim of avoiding deterioration in their quality in order to reduce the level of purification treatment required in the production of drinking water.” However, no measurement or parameter has been defined to quantify or assess the success or failure in achieving the goal of reducing the purification treatment level required in the production of drinking water. In the past, many indices have been developed to evaluate water quality by different definitions, by summarizing this in a single measure [1][2]. These water quality indices are for instance targeted to risk evaluation, as duration and scope of parameter concentrations are incorporated, or to ecological status, or water quality in a more general sense. We propose a new water quality index, the water quality index for removal requirement (WQI-RR) to specifically summarize and evaluate the removal requirement for chemical micro pollutants. When applied to locations along the river Rhine the index indicates that no improvement in required removal has occurred.

6.08.4
Poster spotlight: SIMONI 2.0: future developments in smart integrated monitoring of the water quality
R. van der Oost, Waternet / Onderzoek en Advies
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Addressing the Challenges of Applying New Approaches Supporting Chemical Safety Assessment and Regulatory Decision Making

7.01.1
Grouping and read-across under REACH: principles, shortcomings and positive examples
M. Sobanska, European Chemicals Agency (ECHA) / Hazard Assessment; A. Pirovano, European Chemicals Agency (ECHA); R. Cesnaitis, European Chemicals Agency; J. Laitinen, European Chemical Agency ECHA; A. Karjalainen, ECHA-European Chemicals Agency; B. Versonnen, European Chemicals Agency - ECHA / Evaluation; D. Knight, ECHA-European Chemicals Agency; W. De Coen, European Chemicals Agency - ECHA
Grouping of substances and application of read-across approach is one of the most commonly used alternative approaches for filling data gaps in registrations submitted under the REACH Regulation. Read-across is regarded as a technique for predicting endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s), (source substance(s)). If the grouping and read-across approach is applied correctly, experimental testing can be reduced as not every substance within the group would need to be tested. ECHA developed a Read-Across Assessment Framework (RAAF) designed for regulators assessing read-across cases developed under REACH. The framework to assess human health hazards was published in 2015 and the environmental hazard and fate part in early 2017. The main principles and approaches of the RAAF are based on sound science and are therefore also very useful for registrants and scientists developing read-across cases and are not limited to cases developed under REACH but more widely applicable. For a read-across approach to be correctly applied, the following read-across principles must be followed: (1) it must be based on structural similarity of the target and source substance(s), (2) a well-defined hypothesis must explain why properties of structurally similar substances can be predicted despite their structural differences, (3) target and source substance(s) must be sufficiently characterised and (4) adequate and reliable data (including bridging studies) are required to support the evidence for grouping and read-across approach. In the past 10 years the European Chemicals Agency (ECHA) has checked over 1750 dossiers for compliance. The use of grouping and read-across approach was found in approximately 75% of cases. In the vast majority of the checked dossiers (86 %), ECHA concluded that the non-compliances found were severe enough to require further action and generation of new information. Moreover a relatively high failure rate in using of the grouping and read-across approach under REACH was detected. Nevertheless, there is a number of positive examples where the approach developed and documented by the registrants was accepted. In this presentation we will explain the main principles of the RAAF and illustrate some examples of the main shortcomings of read-across approaches with a focus on environmental endpoints. We will also present examples of acceptable read-across predictions.

7.01.2
Machine Learning Algorithms in Risk Assessment - Predictions and Understanding
T.E. Allen, University of Cambridge / MRC Toxicology Unit; A.J. Wedlake, University of Cambridge / Department of Chemistry; M. Folia, S. Piechota, Unilever / Safety and Environmental Assurance Centre; E. Gelžinytė, J.M. Goodman, University of Cambridge / Department of Chemistry; S. Gutsell, Unilever / SEAC; P. Kukic, P. Russell, Unilever / Safety and Environmental Assurance Centre
Molecular Initiating Events (MIEs) are the initial chemical-biological interaction within an organism that starts an Adverse Outcome Pathway (AOP). MIEs are good targets for computational modelling, as they represent direct points of entry to potential toxicity effects, shedding light on the mechanisms underlying the responses. There is also growing recognition that an improved prediction of chemical interactions and potentially-affected molecular targets within the context of the AOP framework will allow for a more informed extrapolation to unravel impacts across chemicals and species and, ultimately, catalyse the shift toward the adoption of Next-Generation Risk Assessment approaches both in the human and environmental arena. A number of computational approaches based on the chemistry of chemical binders have been recently developed to make predictions at pharmacologically important human MIEs. Open source receptor-binding data was obtained from ChEMBl and ToxCast for a variety of biological targets, including G-protein coupled receptors, nuclear receptors, enzymes, ion channels and transporters. Two machine learning algorithms have been used to build models to predict MIEs - random forests and neural networks. The random forest models were constructed using sklearn in RDKit, with 200 physicochemical descriptors as the input. The neural networks used chemical fingerprints and were developed in Python 3 using TensorFlow. Several network architectures and combinations of hyperparameters were considered to give the highest level of statistical performance. These approaches were combined with previously constructed structural alerts using a maximal common substructure algorithm. All three computational approaches consistently provide models with over 90% accuracy against test data. While the developed computational models provide high levels of performance, we have combined their predictions to give increased predictivity and steps have been taken to aid interpretation in risk assessment. This has included developing a network similarity algorithm to interpret which examples the neural network “thinks” about in the same way and identifying how well new chemicals fit within the training data domain. These models have also been assessed on truly external biological data to test their effectiveness. This allows us to assess the usefulness of computational models outside the training and test data and hence their exploitation in risk assessment practices.

7.01.3
Predicting key events and key event relationships - a knowledge based deep learning approach
S. Kraemer, J. Schor, Helmholtz-Centre for Environmental Research- UFZ GmbH / Molecular Systems Biology; J. Hackermüller, Helmholtz centre for environmental research - UFZ; D. Martinovic-Weigelt, University of St. Thomas / Biology; C. Lai, University of St. Thomas / School of Engieenering
p margin-bottom: 0.1in; direction: ltr; color: rgb(0, 0, 0); line-height: 115%; p.western font-family: "Liberation Serif", "Times New Roman", serif; font-size: 12pt; p.cjk font-family: "AR PL SungtiL GB"; font-size: 12pt; p.ctl font-family: "Lohit Devanagari"; font-size: 12pt; a:link color: rgb(0, 0, 255); An adverse outcome pathway (AOP) is a directed and structured sequential “chain” that starts with molecular initiating event (MIE), and progresses through a series of causally linked biological key events (KEs), culminating in an adverse outcome (AO). The structural nature of the AOP supersedes the utility of past risk-assessment tools; it was developed to rely on collaborative expert-sourcing, and it allows for modular data deposition in a Wiki knowledge base. In spite of extensive buy-in from the research and regulatory community to utilize the AOPs to guide risk assessment at present there are only circa 280 AOPs representing only a subset of current biological knowledge. Machine learning and artificial intelligence approaches can be applied to rapidly predict new AOPs and extend existing AOP networks. A novel workflow, which predicts potential KEs and KE relationships is presented. The conserved knowledge in terms of literature abstracts within the U.S. National Library of Medicine was parsed for subject-to-subject-relationships. Using the Unified Medical Language System (UMLS), subjects were transformed to biological concepts. Based on semantic similarity, we embedded these concepts in a multidimensional space. Semantic UMLS concepts were assigned based on the levels of biological order in the AOP-framework. Present work considered two endpoints of an AOP: 1) MIE represented as concept ‘biomolecule’, and 2) AO represented as concept ‘disease’. For this combination of concepts, we considered the respective relationships and trained an artificial neural network to predict

relationships. The multidimensional word-embedding vectors of concepts were used to train a prediction model with MIE concepts as input and AO concepts as output . We present promising results for the validation performance of our deep learning model and demonstrate its capabilities to predict AOs based on the external data from AOP-Wiki. Predicted key events and relationships might help with development and evaluation of new putative AOPs in future.

7.01.4

Development of Adverse Outcome Pathway based on ToxCast™ and deep learning models combined approach

J. Jeong, University of Seoul / School of Environmental Engineering; J. Choi, University of Seoul / Environmental Engineering

Adverse outcome pathway (AOP) is a framework that organizes existing knowledge about linkage between molecular-level perturbation and an adverse outcome. The AOP framework allows the use of molecular level experimental data for hazard assessment. ToxCast™ project was launched in 2006 by the US EPA to generate toxicity information on large number of environmental chemicals at the molecular level. Deep learning has attracted researchers' attention due to its efficient feature extraction and prediction properties. Deep learning may be particularly well-suited for toxicology because the approach deals with complex pattern and "big data". It integrates information and data from a variety of sources to develop mathematical and computer-based models to better understand and predict adverse health effects caused by environmental chemicals. In this study, development of AOP was attempted based on molecular mechanism data obtained from ToxCast and Deep learning combined approach. The hit call data were collected from the selected 339 assays with intended target information in the ToxCast database. The intended targets belonged to 17 intended target families, mainly nuclear receptors, cytokines, DNA binding, GPCR, cell adhesion molecules, etc. We built 339 chemical activity classification models for the assays. The accuracy of the models ranged from 48.82 to 99.85. In order to improve the reliability of the analysis results, the models with accuracy less than 90 were excluded. As a result, 218 models were selected. Assay activities of chemicals were classified using ToxCast and deep learning models. Through this analysis, we have provided potential insights into the toxicity mechanism of environmental chemicals. By incorporating toxicity pathways information from ToxCast and deep learning models and in vivo toxicity data, we tried to develop the AOP relevant to environmental chemicals. In this study, we proposed an intergrated approach using ToxCast database and deep learning models for mechanism-based screening and development of AOP. Since the coverage of experimental data is limited in ToxCast database, it is possible to use deep learning technique for prediction of missing data. This could be used as the screening step in enabling mechanistic-based assessment and could also be used in development of AOP.Acknowledgement - This work was supported by a grant from the Korean Ministry of Environment through 'Environmental Health R&D Program' (2017001370001).

7.01.5

Automated feature recognition in zebrafish embryos

S. Scholz, Helmholtz Centre for Environmental Research / Bioanalytical Ecotoxicology; E. Teixidó, Unit of Experimental Toxicology and Ecotoxicology (UTOX) Barcelona Science Park; T. Kießling, Scientific Software Solutions; W.R. Oesterheld, Hemholtz Centre for Environmental Research - UFZ / Dept.Bioanalytical Ecotoxicology; C. Lai, University of St. Thomas / School of Engieenering

The zebrafish embryo represents a small scale model used to to measure diverse endpoints for research and to identify hazards in ecotoxicology and human toxicology. The assessment of morphological alterations provoked by chemicals or environmental samples is one of the most often used endpoints typically conducted by visual assessment by an experienced observer using a microcope. Visual assessment can be biased by the experience and potential fatigue of the observer. Furthermore, subtle changes may not be recognised. Particularly if the data would be used for subsequent pattern analysis and a diagnostic purpose a more quantitative approach for the assessment would be required. Using an automated assessment based on the FishInspector software (Teixido et al. 2019, Tox. Sci., 167, 438–449.) we obtained EC50 patterns of morphological alterations for 26 different test compounds. Similarity was observed between compounds that shared similar mode of actions such as interference with retinoic acid metabolism or ACCase inhibition. However, variable responses were observed within the same MoA group as well. The variability may be explained by discrepancies between the pharmacological mode of action used for grouping of chemicals and the MoA provoking the developmental phenotype. Initially the morphological feature recognition was based on standard image segmentation approaches. The detection occasionally required user correction and the number of features that could be detected was limited. Therefore, the previously detected features were used to train feature-specific models using Matlab Computer Vision Toolbox and the VGG-19 pretrained Convolutional Neural Network. Some features such as body contour or yolk revealed very high recognition superior to the previously used segmentation based approach. Given the versatility of the model-based approach new features can be trained in step wise approach using initially a small number of manually labeled image with subsequent rounds of automated recognition and gradually reduced the need for user interaction. This approch allows to train images from various orientation of embryos and is more flexible regarding different image characteristics.

7.01.6

Poster spotlight: Recent updates to the MechoA scheme, a tool to support mechanistically-driven read-across approaches.

F. Bauer, KREATiS

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7.01.7

Poster spotlight: The development of a Molecular Initiating Event (MIE) based profiler for chemical classification and read-across

D. Ebbrell, Liverpool John Moores University / School of Pharmacy and Biomolecular Sciences

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7.01.8

Poster spotlight: Training a molecular dissimilarity measure for target specific activities

C. Garcia-Hernandez, Universitat Rovira i Virgili / Departamento de Ingeniería Química

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Exploring the Potential of Wastewater-based Epidemiology to Monitor Human Exposure to Pollutants and Public Health

7.02.1

Do we know how to best develop new wastewater-based epidemiology applications?

S. Castiglioni, Mario Negri Inst. / Environmental Health Sciences; E. Zuccato, Istituto Mario Negri / Environmental Health Sciences; B. Kasprzyk-Hordern, University of Bath / Department of Chemistry

Wastewater-based epidemiology (WBE) is an innovative methodology enabling the retrieval of epidemiological information from urban wastewater via the chemical analysis of specific human metabolic excretion products (biomarkers). The first WBE application was developed to estimate illicit drugs use, and it is now recognised as an additional drug use indicator among the existing epidemiological indicators. When WBE started to be applied by several groups in different countries, it was very soon clear that improving, coordinating and harmonising its application was necessary. A cross-sectoral and transdisciplinary network (Sewage analysis CORE group – SCORE; https://score-cost.eu/) was established in 2010 with the aim to develop a common protocol of action and perform international studies. The first best practice protocol established covered the main critical steps to perform WBE studies: i.e. wastewater sampling, biomarkers selection, chemical analysis, back-calculation of drug use and data normalization. More recently, several studies have focused on optimising the specific steps necessary to apply reliably a WBE approach. These findings go beyond the estimation of drug use are nowadays particularly valuable for developing novel WBE applications. The SCORE network is currently working to update the protocol of best practice previously developed, and this presentation will give an overview of the main essential requirements as highlighted by the recent literature. For instance, technical guidelines are now available on how to perform sampling of wastewater and select suitable biomarkers. Since WBE is based on the chemical analysis of specific substances, it is also essential to develop and validate proper analytical methods, and interlaboratory exercises may help to check the quality of measurements among different laboratories. Finally, ethical issues has to be carefully considered when dealing with small communities (i.e. workplaces, schools, prisons, city districts, entertainment venues) in order to avoid any kind of stigmatization of a specific group of persons. WBE is a very promising new approach to obtain a wide range of information on the health status of a population, but it is essential to follow common and standardised guidelines in order to allow comparisons among different studies and obtain reliable complementary information to epidemiological studies.

7.02.2

Assessment of the Spanish population exposure to phthalate plasticizers as obtained by wastewater-based epidemiology

I. Gonzalez-Mariño, Universidade de Santiago de Compostela / University of Salamanca; R. Rodil, University of Santiago de Compostela; R. Montes, University of Santiago de Compostela / IIAA Institute for Food Analysis and Research; L. Ares, Universidade de Santiago de Compostela; V. Andreu, CIDE CSIC UV GV; L. Bijlsma, University Jaume I / Research Institute for Pesticides and Water; N. Etxebarria, University of the Basque Country UPV/EHU / Plentzia Marine Station (PiE-UPV/EHU) & Dep Analytical Chemistry; F. Hernandez, University Jaume I / Research Institute for Pesticides and Water; M. López de Alda, Institute of Environmental Assessment and Water Research (IDAEA CSIC) / Environmental and Food Chemistry (ENFOCHEM); E. López-García, IDAEA-CSIC / Department of Environmental Chemistry; R. Marce, Universitat Rovira i

Virgili; M. Miró, Universitat de les Illes Balears / Department of Chemistry; Y. Pico, University of Valencia / Environmental Quality and Soil; E. Pocurull, Universitat Rovira i Virgili; C. Postigo, IDAEA, CID-CSIC / Environmental Chemistry; A. Rico, IMDEA Water Institute; Y. Valcárcel, Universidad Rey Juan Carlos / Department of Medical Specialties and Public Health,; J. Quintana, University of Santiago de Compostela

Phthalate diesters are high-production-volume chemicals that have been widely used in the manufacturing and processing of plastics for more than 80 years. Recently, they have been included in the priority lists of dangerous substances in most of the industrialized countries. Ingestion is considered the major route of exposure to phthalates, either by consuming contaminated food, accidental ingestion of contaminated dust and soil, or licking of products in which they are contained. Once in the human body, phthalates are hydrolysed to their corresponding monoesters and further oxidized or conjugated into glucuronide complexes and, finally, excreted. Wastewater-based epidemiology (WBE) is a complementary approach to human biomonitoring to estimate the level of exposure to a substance through the analysis of its metabolic residues in urban wastewater [1], considering that raw wastewater is a highly diluted urine sample representing an entire community. The objectives of this study were to analyse [2] the metabolites of 6 phthalate diesters in wastewaters from different locations in Spain and to assess the exposure to phthalate diesters in the investigated cities. Raw wastewater samples from 17 wastewater treatment plants, serving a total population of ca. 6 million inhabitants (13% of the Spanish population), were analysed. The results show that the highest population-weighted exposure loads were obtained for diethyl phthalate, followed by dimethyl phthalate and the isomers di-i-butyl phthalate and di-n-butyl phthalate. *Acknowledgements* - The authors acknowledge support by Xunta de Galicia (refs. ED431C2017/36 and IGM postdoctoral contract, Plan Galego I2C-Modalidade B, ED481D 2017/003), the Spanish Research Agency-AEI (ref. CTM2016-81935-REDT/AEI and CTM2017-84763-C3-R-2) and FEDER/ERDF. References [1] Zuccato, E.; Chiabrando, C.; Castiglioni, S.; Bagnati, R.; Fanelli, R. Environ. Health Perspect. 2008, 116 (8) 1027– 32 [2] I. González-Mariño, R. Rodil, I. Barrio, R. Cela, J. B. Quintana. Environ. Sci. Technol. 2017, 51, 3902−3910

7.02.3
Estimation of community-wide exposure to chemicals via water fingerprinting and high resolution tandem quadrupole time-of-flight mass spectrometry
B. Kasprzyk-Hordern, University of Bath / Department of Chemistry
Timely and comprehensive assessment of community-wide public exposure to harmful and toxic chemicals is essential for the prevention, control or mitigation of exposure risks and for improving health. There is growing, evidence of association between man-made chemicals present in industrial and household products and public health outcomes. Current human monitoring approaches are associated with high cost, are the restricted size of study groups and inability to gather comprehensive information on combined spatiotemporal exposure to mixtures of stressors, and their effects. More efficient approaches are critically needed to identify cause-effect linkages between harmful chemicals and human health. Wastewater-based epidemiology (WBE) via water fingerprinting provides a timely alternative to traditional approaches. This presentation will discuss the development and application of WBE for spatiotemporal estimation of community-wide exposure to several indutrial and household derived chemicals including endocrine disruptors such as bisphenol A, and pesticides (e.g. atrazine) or biocides (e.g. triclosan).

From Ecology to Land Management via Regulation to Protect Biodiversity in Agricultural Landscapes

8.01.1
FAO's Engagement in the Post-2020 Global Biodiversity Framework: Mainstreaming Biodiversity Across Agricultural Sector
P. Lourenço Dias Nunes, FAO
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8.01.4
Measuring biodiversity status – national action taken to protect biodiversity in the agricultural landscape in Switzerland
K. Knauer, Federal Office for Agriculture / WBF
.

8.01.5
A farmer's perspective on biodiversity
A. Bergin, Andrew Bergin - Regenerative Farmer
.

8.01.6
Case study on pesticides: generating data to assess effects and risks to biodiversity through a risk assessment
V. Ducrot, Bayer Ag / Environmental Safety Ecotoxicology; M. Miles, Bayer CropScience UK / Environmental Safety; E. Pilling, Corteva Agroscience / REgulatory Sciences; A. Alix, Corteva Agrisciences / Risk Management
.

8.01.7
Case study on pesticides: Risk mitigation measures to protect biodiversity from side effects of pesticides
A. Alix, Corteva Agrisciences / Risk Management; E. Pilling, Corteva Agroscience / REgulatory Sciences; M. Miles, Bayer CropScience UK / Environmental Safety; V. Ducrot, Bayer Ag / Environmental Safety Ecotoxicology
In the context of EC Regulation No. 1107/2009 on the placing on the market of Plant Protection Products (pesticides) in the agricultural area, risk mitigation measures may be defined to ensure that the "high level of protection" goal to human health and the environment, including biodiversity, is reached. These measures are defined at country level and are tailored based on the outcome of the risk assessment. They were inventoried in the proceedings of the SETAC workshop MAgPIE. The MAgPIE toolbox complements the set of farm management measures listed in the Common Agriculture Policy (CAP) that include field margins, hedges, trees, buffer strips, or conservation areas. Although the two sets of measures may take different forms, they are aiming at bringing a common benefit on biodiversity. This benefit could be assessed in a number of studies dedicated to the evaluation of the efficacy of farmland management plans at improving biodiversity in cultivated areas. This presentation will illustrate the effect of risk mitigation measures derived from both the MAgPIE and CAP toolboxes, as measured in field and monitoring studies, on metrics to evaluated biodiversity in cultivated areas.

8.01.8
Ecosystem services and biodiversity; towards a holistic approach to risk assessment
L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences
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Gender and other Forms of Bias. How Do We Achieve Diversity and Equal Opportunity in Scientific Research?

8.02.3
The SETAC award for scientists returning to research after a maternity break
M. Bloor, Scotlands Rural College
.

Open Science in Regulatory Environmental Risk Assessment

8.03.1
Introduction
T. Brock, Wageningen Environmental Research / Environmental Risk Assessment Team; Y. Devos, EFSA; P. Dohmen, BASF SE / Environmental and Consumer Safety, Ecotoxicology
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8.03.2
Open Science in Regulatory Environmental Risk Assessment: An Overview
K. Elliott, Michigan State University / Lyman Briggs College
This talk will introduce the session on "Open Science in Regulatory Environmental Risk Assessment" by providing an overview of key objectives, initiatives, barriers, and opportunities. First, it will discuss the major goals associated with the open science movement as a whole, including efforts to generate more rapid innovation, promote more reproducible science, and make information available to a wide array of stakeholders. Second, it will highlight the range of initiatives currently being implemented or considered as part of the open science movement, including open access publications, data repositories, registries of study results, guidelines such as the FAIR and TOP principles, open peer review processes, sharing of materials and computer code, and strategies for reporting study results in "real time." Third, it will discuss barriers to achieving full transparency in regulatory science, such as the privacy concerns that have complicated the U.S. Environmental Protection Agency's recently proposed policy associated with data availability. Finally, it will discuss major opportunities for achieving greater levels of openness in regulatory environmental risk assessment, including strategies for reaching a wider range of stakeholders with the forms of information that are most relevant to them.

8.03.3
SETAC perspectives to Open Science - past, current and future activities to make it an integral part of environmental research
T. Seiler, RWTH Aachen University / Ecosystem Analysis
Open Science is more than simply making our findings accessible to everyone. It

is a measure in building trust among society that our research is of high quality. With openly sharing our data we show confidence in what we do. From the perspective of fairness theory Open Science can be seen as a measure towards distributive, informational and interpersonal justice: outcomes are transparent and accessible, and thus people feel well-treated. As environmental issues often affect daily life, the potential impact of environmental science on improving environmental health and human safety is deeply dependent on trust. On our mission to ensure and improve environmental quality through science, Open Science can be seen as a key tool to make the best use of all the data generated by SETAC members. The better we are able to build our research on what has already been done, the more relevant any future work can be. Thus, Open Science highly resonates with SETAC's strategic goals of advancing science and science-based decision making. It is also included as a key aspect in SETAC Europe's strategic goal „Quality and Credibility of Science". This presentation will give an overview of the past, current and future activities in SETAC that are aimed at fostering Open Science to make it an integral part of SETAC research. It will further discuss the reasons why SETAC researchers should participate in Open Science and try to convince everyone in the room to start sharing their findings the FAIR way.

8.03.4
Problem formulation enhances the transparency of regulatory environmental risk assessments
J. Romeis, Agroscope; M. Meissle, Agroscope ART / Biosafety
In the context of regulatory Environmental Risk Assessments (ERA), the goal of open science is transparency about the data/information that was used and on the way it was used to support decisions on the potential release of a regulated stressor. Problem formulation frames the ERA process. It is the phase where policy goals are identified and specific protection goals are defined that need to be addressed. This includes defining what requires protection and what impact is regarded as harmful. Subsequently, pathways are constructed on how the regulated stressor could cause harm to those specific protection goals, risk hypotheses are formulated and the data/information that is required to test those hypotheses is defined. Problem formulation thus provides context on how data are used in ERA which enhances reproducibility and maximizes the usefulness of ERA data for decision making. It can also serve as a communication tool to the wider public and help to minimize misinterpretations and confusions about the underlying data and (value) judgements that contributed to a regulatory decision.

8.03.5
Reliability of data from open data sources in the context of regulatory ERA
C. Moermond, RIVM / Centre for Safety of Substances and Products
Reliability is the inherent quality of a test report or publication relating to: 1) a clearly described experimental design to allow for the study to be repeated independently, 2) the way the experimental procedures were performed, and 3) the reporting of the results to provide evidence of the reproducibility and accuracy of the findings. Often, reliability is scored from non-reliable to reliable, either using a linear scale or specific reliability scores. In this presentation, different reliability scoring systems will be described, with special focus on the CRED criteria and the SCIRAP webtool that incorporates these criteria. Regulatory use of published articles or other open data sources for risk assessment is sometimes limited. Some assessors tend to focus only on GLP and OECD criteria, thereby excluding most data in the public domain. Using a systematic evaluation system, also non-GLP studies may be found to be reliable enough to use for regulatory risk assessment. In different regulatory frameworks such as REACH, the Water Framework Directive and the authorisation of pharmaceuticals, a non-GLP study may be acceptable as the most critical study on which the conclusions are based. Transparency is the key: both the publication and the study evaluation result should contain all information necessary for others to repeat the study or the evaluation.

8.03.6
The weight-of-evidence approach within the context of regulatory ecotoxicological risk assessment and the open science movement
K.R. Solomon, University of Guelph / School of Environmental Sciences
Weight of evidence (WoE) is a process whereby studies are evaluated for quality and relevance to a specific question(s) related to a regulatory or management decision. The open science movement is promoting the concept that all scientific studies be fully open. The WoE process makes use of a rubric of criteria that are used to characterize the quality and results of a study in a consistent way. This rubric must include key criteria, for example, in toxicity studies quality criteria, such as those in the CRED process, should be considered for every study. For other studies, such as environmental sampling, analysis of residues, and recording and analysis of data, other criteria of quality are needed. These criteria are scored numerically to allow the quality of each study to be ranked in a consistent way. For WoE assessment, relevance to adverse effects or exceedance benchmarks is also scored. Here different criteria such as statistical significance, concentration- or dose-response, effects only seen at unrealistic concentrations, etc. are used. These are then brought together in a WoE framework which will be described. In my experience, studies conducted under good laboratory practice (GLP) are completely reported, contain all the raw data, are easier to assess, and are of high quality. This is not always the case for studies in the open literature. Published studies should make better use of electronic SI to provide descriptions and all the data as is done for GLP studies. SI is virtually limitless and should be mandated in all papers published in journals and access fully supported by publishers. All in all, open science will greatly aid in determining quality and relevance of data from reports and published studies and will improve the conduct of regulatory ecotoxicological risk assessment.

8.03.7
New EU Transparency Regulation
A. Tuijtelaars, European Commission
In June 2019, the European Parliament and the Council adopted Regulation (EU) 2019/1381 on the transparency and sustainability of the EU risk assessment in the food chain1 based on a European Commission's proposal tabled in April 2018. Being a targeted amendment of the General Food Law Regulation and eight other sectoral Union acts, this new Regulation aims at increasing the transparency of the EU risk assessment in the food chain, at strengthening the reliability, objectivity and independence of the studies used by the European Food Safety Authority (EFSA), and at revisiting the governance of EFSA in order to ensure its long-term sustainability. It is a direct response of the EU Institutions to a successful European Citizens' Initiative and builds upon the findings of the Fitness check of the General Food Law Regulation, a comprehensive evaluation, completed in January 2018. The new "Transparency Regulation" will become applicable on 27 March 2021. As of that date, amongst others, all scientific data, studies and other information, supporting applications for authorisations/approvals in the area of the food chain - including supplementary information - will be made public, with the exception of duly justified confidential information, proactively and early on in the risk assessment process. This public disclosure will also be followed by a public consultation on the submitted scientific data to be carried out by EFSA, before it delivers its scientific output. The presentation will introduce the main features of the new Transparency Regulation and focus on how it will strengthen the transparency of the EU risk assessment in the food chain. Regulation (EU) 2019/1381 of the European Parliament and of the Council of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain and amending Regulations (EC) No 178/2002, (EC) No 1829/2003, (EC) No 1831/2003, (EC) No 2065/2003, (EC) No 1935/2004, (EC) No 1331/2008, (EC) No 1107/2009, (EU) 2015/2283 and Directive 2001/18/EC, https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32019R1381

8.03.8
The application of Open Science and FAIR principles to improve transparency in scientific advice used for policy decisions
J. Richardson, European Food Safety Authority / Evidence Management
For scientific risk assessments used in governmental policy it is essential that; the methodology and process are fully documented to provide clarity on the conclusions reported; supporting materials are publicly available; the analysis can be reproduced or repeated with alternative values (sensitivity analysis). EFSA is continuously improving its risk assessments by applying Open Science principles. Before starting a risk assessment, a protocol may be published for public consultation. To fill data gaps, Expert Knowledge Elicitation techniques or citizen science/crowdsourcing have been used. To increase the amount of published data available, the FAIR principles document a procedure for publishing survey and monitoring data. This includes identification of sensitive information, specification of metadata to support reuse and the highlights the importance of citation. An open evidence community for food and feed safety has been established where data, models, protocols and other supporting materials can be shared. In parallel there is an open analytics platform (R4EU) with an easy to use interface where users can run models. These initiatives seek to increase the opportunity for researchers, risk assessors, regulators, industry, methodologists, data scientists and citizens to engage with and contribute to environmental risk assessment. The New Transparency Regulation demonstrates that Open Science principles are also being taken up by legislators.

8.03.9
Use of Open data in decision making
C. Ajao, ECHA-European Chemicals Agency
REACH, the European Regulation on Registration, Evaluation, Authorisation and Restrication of Chemicals, is just one of the European legislation that encourages the use of public data. ECHA's Member State Committee (MSC) is responsible under REACH for resolving divergent views among Member States and ECHA as regards the information Registrants and Downstream Users are required to generate to show that their substance can be used safely without harm to man or the environment. Such information is requested following a dossier evaluation by ECHA or a substance evaluation carried out by an evaluating Member State. Furthermore, MSC can identify substances of very high concern (SVHC) for instance on the basis of CMR or PBT/vPvB-properties. These decision making processes require decision makers to assess the data available and use it in a weight of evidence to decide whether the dossier is fulling all the information requirements, or substance requires more information to demonstrate safe use or

can conclude that the substance needs further regulation. Oppenness and transparency of the decision making process are key to enable trust amongst the interested parties as well as the public. This presentation aims to provide insight on the regulatory processes in ECHA that use open data; the procedures in place for transparent decision making and the sharing of information through the publicly available chemicals database.

8.03.10
Are all data equal or are some data more equal than others?
A. Gladbach, Bayer AG, Crop Science Division / Research & Development, Disease Management
The environmental risk assessment (ERA) conducted in the registration process for crop protection products is based on data supplied by applicants, including relevant information from scientific literature. But why are not all data considered equally relevant when assessing the potential risk of substances? We aim to provide insight on the following topics from an applicants' perspective: 1. What are criteria for using data in a regulatory context? Good laboratory practice and clearly defined study guidelines aim to ensure consistency and comparability across tested substances. Some elements of these requirements would foster the consideration of more open scientific literature in ERA. 2. What can applicants contribute to Open Science? Since 2017, Bayer is not only providing access to reports of studies conducted for registration but accompanies them with educational material. In 2020 we will shed more light on the quality assurance system and most importantly will take transparency to the next level. 3. What are an applicants' expectations from Open Science? We strive for a dialogue on scientific data, their interpretation, reliability and relevance, potential conflicts of interest to ultimately re-install trust in the regulatory system.

Your Science or My Science? How Is the Way We Frame our Research Impacted by Values? A Multi-perspective Look at the Impact Organic UV Filters in Sunscreen Products Have on Coral Bleeching

8.04.1
The role of value judgements in scientific research
G. Oberg, University of British Columbia / IRES
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8.04.2
The concentration of UV filters around coral reefs and in reef organisms
C.L. Mitchelmore, University of MarylandCenter for Environmental Science / Chesapeake Biological Laboratory
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8.04.3
Environmental risk assessment of organic UV filters in sunscreen products and the disconnect between quality and quantity of science
M. Leonard, lOREAL SA / RECHERCHE ENVIRONNEMENTALE
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8.04.4
Development of a Global Framework to Assess the Environmental Risk of UV Filters to Coral
I. Davies, Personal Care Products Council / Science
Concerns over the exposure of coral to anthropogenic contaminants is an emerging research area. This interest has been largely driven by the potential toxicological effects from exposure to UV filters reported in a handful of recent studies. Moreover, monitoring data suggest these compounds are present in coastal coral environments. UV filters are suspected of entering the marine environment through recreational bathing (sunscreen wash-off) and wastewater effluent releases. As the science develops, it will be important to use these data to conduct environment risk assessments (ERAs) to identify whether risk management measures are necessary. A significant challenge to conducting a coral ERA is that the majority of ecotoxicological data and ERA current practice is geared toward temperate environments. Significantly less attention has been given to diverse tropical and subtropical ecosystems. In response, we have developed an ERA framework specifically for coral using principles from REACH and U.S. EPA endangered species assessments. We acknowledge that there is a lack of coral toxicological data currently available and to bridge this gap we propose leveraging existing ecotoxicological data within a tiered approach. As the science develops and the sensitivity of coral species becomes better understood, the framework can be refined to protect the diversity of the coral taxon.

8.04.5
A regulators perspective on the regulation of UV filters in the environment
C. Ajao, ECHA-European Chemicals Agency
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8.04.6
The impacts of UV filters on corals compared to other stressors
N. Imbert, Green Cross France et Territoires
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Poster Abstracts

Advances in Invertebrate Endocrine Toxicology (P)

1.01P.1

Pyriproxyfen and cadmium: what effects on the growth hormonal pathway in the amphipod Gammarus pulex ?

J. Jaegers, E. Gismondi, LEAE - University of Liege / Laboratory of Animal Ecology et Ecotoxicology

Due to the expected climate changes, it is predicted that disease-carrying mosquitoes will expand their geographical range, resulting in increased use of larvicides (insect growth regulators or IGRs) to face their proliferation. Among IGRs, the pyriproxyfen (PXF) is widely used and has been shown to prevent larvae from developing into adults and thus rendering them unable to reproduce. However, because of a similar endocrine system between crustaceans and insects, PXF could also impact aquatic crustaceans. In addition, when spreading in the environment, PXF is found in a mixture with other pollutants such as metallic trace elements, which could alter its action. Consequently, the present work was devoted to analyse the PXF effects on the growth hormonal pathway of the freshwater amphipod *Gammarus pulex*, as well as the combined binary effects with cadmium (Cd). After 7 days of exposure, concentration of the methylfarnesoate hormone (MF) was assessed, as well as the relative transcriptional expression of the farnesoic acid O-methyltransferase (FAMeT) (enzyme limiting the MF production), the methoprene-tolerant receptor (Met), known to bind MF to activate some transcription factors, such as broad-complex (BrC). Results revealed that exposure to PXF decreased the MF concentration, although an increase of FAMeT expression was measured. Besides, Met and BrC expressions were also increased suggesting the activation of the hormonal pathway. Nonetheless, the presence of Cd seemed to suppress these effects and even to achieve to opposite effects. Indeed, Cd-PXF binary exposure caused the diminution of MF concentration as well as under-expressions of FAMeT, Met and BrC. This work confirmed that PXF single exposure could impact non-target organisms such as amphipods through changes in hormonal pathway of methylfarnesoate. Nevertheless, when PXF was mixed to Cd, opposite results were obtained, suggesting different consequences on the long-term of the amphipod growth.

Advantages of Using Laboratory Model and Field Collected Invertebrates in Ecotoxicology: New Insights for Environmental Risk Assessment (P)

1.02P.1

Effects of acetaminophen on the Neotropical bivalve Anodontites trapesialis after acute exposures

M. Cabral, W. de Paula Santos, Universidade Estadual de Londrina / Departamento de Ciências Fisiológicas; C. Vidal, Chemistry Institute - University of Campinas / Analytical Chemistry; C.C. Montagner, Chemistry Institute - University of Campinas / Química Analitica; C. Martinez, Universidade Estadual de Londrina / Ciencias Fisiologicas

Acetaminophen (ACE) is an analgesic used worldwide to treat mild and moderate pain. Several studies have pointed to the presence of this contaminant in freshwater of different countries, but its effects on aquatic organisms remain unclear, especially in invertebrates. In this context, the aim of this study was to evaluate the effects of acetaminophen on the Neotropical freshwater bivalve *Anodontites trapesialis* by measuring hemolymph ions concentrations, lipoperoxidation (LPO) and the activities of glutathione S-transferase (GST), catalase (CAT) and acetylcholinesterase (AChE). Individuals of *A. trapesialis* were collected in a local fish farm (Parana, Brazil) and kept for ten days in tanks containing 400 L of dechlorinated and aerated water, with renovation every 24 h, photoperiod 12 h/12 h. Animals were fed with *Raphidocelis subcapitata* algae (~10,000 cells mL^{-1}) every two days. Then, bivalves were exposed individually in beakers (1.5 L) and four groups (n = 8 per group) were formed: dechlorinated water (CTR); and dechlorinated water with ACE at 3 µg L^{-1} (ACE 3), 30 µg L^{-1} (ACE 30) and 300 µg L^{-1} (ACE 300). After 24 and 96 h of exposure, hemolymph was collected for the determination of Na^+, K^+, Cl^-, Ca^{2+} and Mg^{2+} concentrations. The animals were then anesthetized to remove gills, mantle, and digestive gland for the determination of LPO and GST. CAT activity was determined only in gills, and AChE activity was measured in muscle (foot). There was no significant difference in ionic concentrations, glutathione S-transferase and catalase activity at any time and tissue tested among the different groups. After 96 h of exposure to ACE 300, animals showed a significant increase in LPO in the digestive gland, as well as an increase in AChE activity in muscle when compared to CTR. These results indicate that ACE promotes occasional changes in *A. trapesialis*. However, complementary analyzes will be performed in order to clarify the antioxidant defenses, possible oxidative damage in proteins and acid nucleic and osmoregulatory disorders.

1.02P.2

Responses of sympatric dreissenid species to contamination: comparison of zebra mussel and quagga mussel exposed to cadmium, and consequences for ecotoxicological studies.

E. David, Université de Reims Champagne Ardenne / UFR Sciences Exactes et Naturelles; M. Auffret, Institut Universitaire Européen de la Mer (IUEM) Université de Bretagne Occidentale (UBO) / Laboratoire des sciences de l'environnement marin (LEMAR); M. Bonnard, Université de Reims Champagne Ardenne; F. Bultelle, Université Le Havre / UMR-I 02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO); O. Dedourge-Geffard, University of Reims Champagne-Ardenne / SEBIO UMR-I 02; L. Delahaut, Université de Reims Champagne Ardenne / UMR-I 02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO); S. Devin, Universite de Lorraine / LIEC, CNRS UMR 7360; M. Lebreton, Université de Reims Champagne Ardenne / SEBIO UMR-I 02 INERIS-URCA-ULH (Environmental Stress and Aquatic Biomonitoring); F. Louis, Université de Reims Champagne Ardenne; B. Rocher, Université Le Havre / UMR-I 02 INERIS-URCA-ULH Environmental Stress and Aquatic Biomonitoring (SEBIO),; A. Tanguy, UPMC / Adaptation et Diversité en Milieu Marin, équipe ABICE, Sorbonne Universités, UPMC, CNRS – Roscoff, France; S. Pain-Devin, Universite de Lorraine / LIEC, CNRS UMR 7360

Biomonitoring programs rely on sentinel species whose specific features (basic levels, seasonal variability of parameters) must be precisely determined in order to efficiently interpret results. In freshwater ecosystems, zebra mussel Dreissena polymorpha is a commonly used model, for which numerous data are available. In this species, various set of biomarkers are developed to be used as tools for freshwater quality assessment. Yet, some zebra mussel populations have been recently reported to decline and another invasive close species of freshwater mussel, the quagga mussel Dreissena rostriformis bugensis became in turn invasive in western Europe. Most studies that seek to explain the replacement of zebra mussel by quagga mussel pointed ecophysiological differences linked to various tolerance to environmental parameters. To date, very few studies compared responses and sensitivity of both species to chemicals through biomarkers evaluation, most of them actually focusing on xenobiotics accumulation. In this context, a comparative exotoxicological approach has been driven. Individuals from both species were exposed to cadmium in laboratory conditions, during seven days at two concentrations (10 and 100 µg.L-1). Molecular and biochemical biomarkers associated to energy metabolism, antioxydant or detoxification mecanisms and genotoxicity were followed. On the whole, in zebra mussel, a slight increase of DNA damage, metabolic stimulation and defense activation were evidenced. However, quagga mussels showed higher levels of metal concentration in their tissue at the end of the experiment, but very few variations in biomarkers were observed. A proteomic analysis also revealed a more marked impact of cadmium on quagga than zebra mussels. These results show that different regulation pathways are involved in both species exposed to metal stress, zebra and quagga mussels presenting different levels of tolerance and adaptation to their environment. Same comparative approach has been managed on in situ dreissenid populations, and the whole project help to improve knowledge about species from Dreissena genus for further wide range environmental biomonitoring studies.

1.02P.3

Behavioural and biochemical responses of the mussel Mytilus galloprovincialis exposed to the red macroalga Asparagopsis armata exudate

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Lately, the continuous proliferation of invasive seaweeds has grown a great concern considering their impacts on native communities. *Asparagopsis armata* Harvey (Bonnemaisoniales, Rhodophyta) is an example of a red macroalga from the Pacific Ocean, which is classified as an invasive species in Europe, being established in few regions of the Portuguese coast. It is known to produce a chemical defense against consumers and epiphytic bacteria, through the exudation of halogenated metabolites, which act as surface-mediators and might have toxic effects to native rocky littoral communities. This study is a contribution to the evaluation of the potential impact of *A. armata* in the Portuguese coastal ecosystem, using its exudate as the study object. The mussel *Mytilus galloprovincialis* is one of the native species that was exposed to increasing concentrations of exudate to assess lethal and sub-lethal effects, evaluated as biochemical and behavioural responses. It exhibited a 96h-LC50 of 3.7 %, and based on this endpoint the following tests' concentrations were determined, i.e., from 0.25 up to 2 %. Exposure to sub-lethal concentrations caused no oxidative damage in the digestive glands of mussels, since levels of lipid peroxidation and protein carbonyl content were not affected. Nevertheless, there was a significant increase in the electron transport system activity and levels of total glutathione content in muscle, suggesting an increment of non-enzymatic antioxidant capacity and consequent increased energy to cope with potential pro-oxidant compounds. In another 96h exposure test, mussel's efficiency in the production of byssal threads and their post-exposure clearance rates were assessed. The number of functional byssuses tended to decrease along the gradient of exudate concentrations, besides there was a significant reduction in their *byssal attachment strength*. To measure the clearance rates, after the 96h exposure to *A. armata* exudate, known concentrations of the microalga *Isochrysis galbana* were used to estimate the rate at which mussels could remove algal cells from water. Preliminary results indicated that clearance rates declined with increasing exudate concentrations. Summarizing, the results suggest that the presence of *A. armata* might compromise the *M. galloprovincialis* integrity in the invaded coastal areas.

1.02P.4

Toxicity screening of sediments collected in three estuaries of the Bay of Biscay at different seasons using (Paracentrotus lividus) sea urchin embryos

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Estuarine sediments have undergone important inputs of different toxic substances over years and even today due to industrial exploitation and urban wastes release. Since polluted sediments could pose a risk for the environment and human health, information about their toxicity should be obtained in a rapid and accurate way to perform a proper management. For that, in addition to conventional chemical analyses, bioassays performed with field collected invertebrates such as sea urchin (*Paracentrotus lividus)* can be used. In fact, the sea urchin embryo development test is a standard toxicity bioassay broadly used for the screening of water and sediments. In addition, developmental abnormalities can be measured in exposed larvae since the sensitivity of skeletogenesis to specific contaminants has been demonstrated. Presently, a toxicity screening of sediments collected at different seasons in the Bay of Biscay was carried out with the aid of the sea urchin embryo test and the quantification of malformations frequency in embryos. *P. lividus* (adults) were collected in a reference place (Armintza 43º26'01.1"N 2º53'56.1"W; Bay of Biscay) in April 2019-2020. Tested sediments were sampled in the Nerbioi-Ibaizabal (4 sites Udondo, Kadagua, Benedicta, Zorroza), La Gironde (Plassac) and Charente (Rochefort) estuaries in October 2018 and April, July and October 2019. Sediment elutriates were prepared in seawater (SW) following DIN38414-S4, and pH, salinity, metals and PAHs were quantified. A range of concentrations (0-50 mg/L) of model ($CuCl_2$) and novel (AgNPs) pollutants dissolved in SW were also tested. Sea urchin embryos were exposed following ICES 2012. Exposure to Cu and AgNPs caused significant decrease in embryo size and occurrence of abnormalities from 0.005 mg/L onwards (EC_{50} 0.03 mg Cu/L and 0.09 mg AgNPs/L). Embryos exposed to elutriates from Udondo and Benedicta showed the lowest capacity to grow and evident malformations, more noticeably in sediments collected in April. The chemical analysis reinforced these results since high concentrations of Zn and Cd were recorded in those elutriates. According to the toxicity of elutriates a toxicity gradient was established: Udondo>Benedicta >Zorrotza> Kadagua=Plassac=Rochefort. Results prove the accuracy of sea urchin embryo test and embryo malformation quantification for a rapid assessment of polluted sediments. Acknowledgements: Basque Gov. (IT1302-19, IT1213-19), U. Basque Country and MINECO (seaDIMENTOX CTM2017-87766-R)

1.02P.5

The impact of algal food on the physiology and resistance to metal toxicity in Daphnia magna

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Trophic interactions play a major role for growth and performance of aquatic species in freshwater ecosystems. Among the aquatic organisms algae and daphniids have gained significant attention in relevance to ecology and ecotoxicology. Algae provide daphniids with their primary food source and, therefore, have an impact on their physiology, ageing and growth. In this study, the impact of algal food in relation to the lifespan, growth, reproduction, and responses to metal toxicity of daphniids was explored. Daphniids were fed with an algal suspension of Chlamydomonas rheinhartii, which was either prepared fresh (immediately from algal cultures) or derived from a frozen batch (of the same algal species). Comparative molecular insight over the impacts of food type to the metabolic level was evaluated with sensitive holistic hyphenated mass spectrometry approaches and molecular markers of oxidative stress, shed light to the impact of food also associated with changes in the physiology of daphniids. Daphniids fed with frozen algal food proved to live longer, have a slower growth rate and produced less offspring as compared to daphniids fed with fresh algal food. Keywords: Daphnia magna, food impact, physiology, metabolomics

1.02P.6

Daphnia as a model for understanding the sensitivity of aquatic organisms to chronic low-level ionizing radiation exposure

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Ionizing radiation is ubiquitous in the environment and potentially posing hazards to living organisms. While the acute effects of high-dose ionizing radiation have been extensively studied, knowledge on low-dose and chronic effects of ionizing radiation is scarce. In addition, determining how organisms activate adaptive responses to mitigate low-level radiation stress is essential for understanding the radiosensitivity of different species, and key to the development of universal models, such as Adverse Outcome Pathways (AOPs), for predicting the hazard and risk of ionizing radiation across taxa. The present study was conducted to investigate low-level gamma radiation-induced adverse and adaptive biological responses in aquatic organisms using the water flea *Daphnia magna* as a model testing species. Neonatal *D. magna* were exposed to seven dose-rates (0-100 mGy/h) of cobalt-60 gamma radiation at NMBU's FIGARO gamma irradiation facility for eight days, and samples were collected for transcriptomics and non-targeted metabolomics. In addition, the mitochondrial reactive oxygen species (ROS) formation, mitochondrial membrane potential (MMP), apoptosis, DNA damage and growth were measured. The transcriptomic analysis identified differentially expressed genes mainly enriched in pathways such as glutathione metabolism, carbohydrate metabolism, glycosphingolipid biosynthesis, protein digestion and PI3K-Akt signaling pathway. Results from the metabolomic analysis identified changes in metabolites involved in fatty acid metabolism, sugar metabolism and glutathione metabolism. At higher levels of biological organization, increased mitochondrial ROS, MMP, apoptosis and DNA damage in combination with decreased growth were observed. These results collectively suggest that multiple types of adaptive responses were activated to maintain homeostasis at radiation dose-rates between 0.4-40 mGy/h, whereas toxicity pathways were induced and contributed to reduced growth at the highest radiation dose-rate (100 mGy/h). A previously proposed AOP network for ionizing radiation-mediated adverse effects on aquatic organisms was refined based on the new experimental evidence generated. The present study has provided in-depth mechanistic insights into the adaptive strategies of *D. magna* in response to ionizing radiation exposure and the knowledge obtained may facilitate future investigation of radiosensitivity in various organisms such as aquatic insects and fish. *Acknowledgement* - This project was supported by the Research Council of Norway through its Centre of Excellence funding scheme, project number 223268/F50 and the NIVA Computational Toxicology Program (NCTP, www.niva.no/nctp).

1.02P.7

Response of neonates, juvenile and adults of Daphnia magna exposed to 3 non-steroidal anti-inflammatory drugs

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The presence of drugs in wastewater is a problem that has been intensifying since 20 years, because in countries like the U.S., Canada, Switzerland and France have been detected in ppb concentrations. Because these products are designed to be active in ppm or ppb concentrations, can cause deleterious effects to aquatic organisms. In this work the toxicity of 3 products was evaluated: Acetylsalicylic Acid, Diclofenac and Paracetamol in neonates, juveniles and adults of Daphnia magna. Static bioassays with a duration of 48 hours were performed, where six concentrations of the drugs (0.01, 0.1, 1, 10, 100 and 200 mg/L) were tested to determine the LC50. Subsequently, a 144-hours bioassay was performed where the organisms were exposed to sublethal concentrations (CL10 and CL1) to evaluate the degree of lipoperoxidation (Tbars) in the cladoceros tissues at 24, 48 and 144 hours. The LC50 values obtained in the bioassays with the drugs varied from 0.02 mg/L to 150.5 mg/L. Juvenile of D. magna (LC50 of 0.02mg/L at 52.4 mg/L) were more sensitive to drugs than neonates and adults. The most toxic compound for D. magna was Paracetamol (LC50: neonates: 67.8 ± 9.4 mg/L, juveniles : 0.02 ± 0.014 mg/L, adults: 71.8 ± 12.5 mg/L) and the least toxic drug was Diclofenac. The organisms exposed to sublethal concentrations (CL10 and CL1), presented degrees of lipoperoxidation that varied from 0.041 ± 0.012 to 0.29 ± 0.01 nM Tbars/mg (control group 0.032 ± 0.009 nMTbars/ mg), Paracetamol caused the greatest oxidative effect at 24 hours, and Acetylsalicylic acid at 48 hrs and 144 hrs. The drugs Acetylsalicylic acid, Diclofenac and Paracetamol were toxic to neonates, juveniles and adults of Daphnia magna, these drugs caused lipoperoxidation in cladoceros tissues in sublethal concentrations and adults exposed to drugs did not reproduce after being exposed to these compounds.

1.02P.8

Effects of copper exposure on osmo-ionic regulation in the freshwater crab Aegla castro

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Aeglids are unique freshwater decapods, entirely restricted to inland waters and widely distributed in southern South America. These anomuran crabs play a crucial role in maintaining trophic chains in the headwater of small streams. However, these habitats are being contaminated by metals, such as Cu, from industrial discharges and agricultural runoff. Nevertheless, no study addressed metal exposure effects on aeglids. Therefore, the purpose of this study was to investigate the effects of acute Cu exposure on osmo-ionic regulation of *Aegla castro*. Animals collected from a reference stream in Southern Brazil were acclimated during 5 days under controlled conditions of temperature, pH, dissolved oxygen, and turbidity. After that, males in intermolt (n = 16) were individually exposed for 24 h only to dechlorinated tap water under control conditions (n=8) or to water containing 12.4 ± 0.3 µg L^{-1} of dissolved Cu (n=8). This Cu concentration is under the limits for dissolved Cu in freshwaters according to Brazilian guidelines. Hemolymph samples were withdrawn from the base of the chelipods and were used for the determination of Na^+, K^+, Ca^{2+}, and Mg^{2+} concentrations. Osmoregulatory responses were assessed in the posterior gills by means of Na^+/K^+ ATPase, Ca^{2+} ATPase, H^+ ATPase, and carbonic anhydrase activities. Crabs exposed to Cu presented no alterations in Na^+ and K^+ hemolymph concentrations. In contrast, we observed an increased concentration of Ca^{2+} and Mg^{2+}. With respect to the enzymes we observed no alterations in Na^+/K^+ ATPase, Ca^{2+} ATPase, and H^+ ATPase activities. However, a decreased activity in carbonic anhydrase was observed in animals exposed to Cu. Although copper is an essential micronutrient being a component of the respiratory pigment of crustaceans, at high concentrations this metal can be potentially toxic. In this study, increased Ca^{2+} and Mg^{2+} levels may indicate that animals activated exoskeleton Ca^{2+} and Mg^{2+} resorption mechanisms, and may be interpreted as a stimulus to start a new molting cycle triggered by Cu. The decreased activity observed only in carbonic anhydrase may lead to an acid-base imbalance. It is probable that the exposure time was not long enough to promote effects on the other branchial enzymes. Metal exposure studies concerning aeglids are essential to understand how these animals respond to environmental contamination as well as their potential as alternative biological models in ecotoxicology.

1.02P.9

Ibuprofen: a new risk for Chironomus riparius populations

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In recent years, the consumption of drugs has increased, acting as emerging pollutants. Ibuprofen is one of the most used active pharmaceutical ingredients worldwide, being the third most sold in Spain (SNS,2008). In spite of that, 90% can be removed in wastewater treatment plants (WWTP) (Onesios et al.,2009). However, due to its high use and chemical properties (ubiquity and lipophilicity), it is present in several environmental compartments such as sediment, surface, and, even, drinking water (Santos et al., 2010; Tejon et al.,2010), with ranges from 0.01 to 85 µg/L (Aguire-Martinez et al., 2016; Blaise et al., 2006). Classified as a non-steroidal anti-inflammatory drug (NSAID), the water ecosystems are the main receptors of ibuprofen having the potential to bioaccumulate in aquatic organisms. Previous studies, mainly focused on physiological endpoints, observed effects on bacteria, algae, crustaceans, and fish (Ortiz de García et al., 2014). However, poor information is available about gene expression, with only a few works in fish (Gravel and Vijayan, 2007) and Daphnia (Wang et al. 2016). C. riparius is an invertebrate with high relevance and essential role in the food chain. Besides, it is a model organism in standardized toxicology tests validated by OCDE (218, 233, and 235). This study aimed to evaluate the effects of ibuprofen at environmentally relevant concentrations (0.01, 1, and 100 µg/L) on the fourth instar C. riparius larvae exposed for 24 and 96 hours. Transcriptional activity of genes related to detoxification mechanisms, stress response, and immune system was analyzed by Real-time PCR. Into the other hand, enzymatic activities Glutathione-S-transferase (GST), Phenoloxidase (PO), and Achetilcolinesterase (AchE) were also evaluated. Ibuprofen altered the expression of genes related to stress response (hsp24 and hsp27) and immune system [Prophenoloxidase (Proph) and Defensin (Def)]. Besides, increased GST and PO activity was observed. The results confirmed a strong effect of ibuprofen on the immune system and alterations on the stress response, two essentials processes in the response of the organism to aggressions. To sum up, ibuprofen shows toxicity in C. riparius even despite the low concentrations used. This work has been funded by Ministerio de Economía y competitividad CICYT (SPAIN), CTM RTI2018-094598-B-I00. A.B.M.G has pre-doctoral contract from UNED.

1.02P.10

Cloeon dipterum - developing robust ecotoxicological test systems for mayflies

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Cloeon dipterum is the most common mayfly species in ponds and ditches and also frequently part of the insect community in aquatic outdoor mesocosm studies. During the last years it moved more and more in the focus of ecotoxicology and there has been an increased interest in developing robust test systems to obtain reliable data on lethal and sublethal effects of chemicals on this species. Among acute toxicity studies, chronic long-term exposures as well as sublethal endpoints like the duration of the larval development or the inhibition feeding should be assessed. Furthermore, efforts should be made in reducing the control mortality and in finding an optimal relation between standardised test conditions and limiting stress to the test organisms. Against this background we are actually working on the development of test protocols for a) an acute toxicity test over 96h regarding mortality and immobility, b) a long-term toxicity test with monitoring of the larval deveopment and c) a feeding inhibition test. We studied the development of *C. dipterum* from small larvae to the emergence of subimagines in a long-term test system including the determination the aquatic development stage as descibed by Cianciara (1979) in regular intervals. The larvae were fed with natural peripyton cultivated in the laboratory on unglazed tiles. The periphyton quantity and the algae composition is determined by fluorescence measurements. This method is also used to monitor the grazing activity in terms of a feeding rate according to Maltby et al. 2000 of the Cloeon larvae in the feeding experiments. To characterise the different development stages and to investigate the differences of size between individuals of the summer and winter generation different size measures were determined. We compared these measures for a) fast growing larvae in the laboratory at 18°C, b) fast growing larvae in the field during summer and c) slow growing larvae in the field from autumn until late spring with a dipause during winter. We found that the larvae of *Cloeon diperum* seem to be well suitable test organisms in different laboratory test systems. In addition to acute effects, it is possible to investigate the development time and it might be possible to measure further sublethal endpoints e.g. feeding inhibition.

1.02P.11

In vivo and in vitro bioassays with Hediste diversicolor polychaete to assess the toxicity of polluted sediments

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Estuarine sediments represent a reservoir for chemicals introduced in coastal environments by human activities, acting as a sink and source of contaminants that can pose a risk for the environment. Hence, a proper assessment of the potential adverse effects exerted by contaminated sediments is mandatory in order to deliver suitable environmental management policies. A reliable assessment of the hazards resulting from pollutants in sediments can be achieved, after integrating chemical analysis and ecotoxicological studies. Among organisms inhabiting sediment, the polychaete Hediste diversicolor could be considered as a sentinel organism, commonly used in biomonitoring and bioassays. Moreover, in vitro approaches with their immune cells (coelomocytes) have been recently described as very useful and sensitive tools to determine pollution effects in ragworms. Nevertheless, the use of biomarkers in H. diversicolor is still scarce and the integration of responses measured at different biological complexity levels is poorly developed. The aim of this work was to assess the toxicity of polluted sediments measuring biomarkers after in vivo and in vitro exposure of H. diversicolor and their coelomocytes. For the in vivo studies, ragworms were maintained for 7 days in reference sediments (control), artificially polluted sediments ($CuCl_2$, 0-300 mg/kg) and sediments sampled in different points of the Nerbioi-Ibaizabal estuary. After exposure, survival, weight loss and metal accumulation and distribution in tissues were recorded. Coelomocyte number and viability and catalase activity and metallothionein induction were also measured. Regarding in vitro, coelomocytes were exposed to $CuCl_2$ (0-500 mg/L) and to elutriates coming from sampled sediments (following DIN38414-S4). After 2 and 24h, coelomocyte viability (Calcein-AM) was determined. In vivo exposure to Cu caused mortality, weight loss and impairments in measured biomarkers at 300 mg Cu/kg. Similar responses were recorded after exposure to sampled sediments, being the effects measured at different levels of biological complexity correlated with the pollutant levels in sediments. In vitro exposure established the LC50 at 1.7 mg Cu/L. The in vivo and in vitro bioassays performed with H. diversicolor and the integration of biomarkers measured provided useful information on sediment toxicity and thus, future use and management. Acknowledgements: Basque Gov. (IT1302-19), U. Basque Country and MINECO (seaDIMENTOX CTM2017-87766-R)

1.02P.12
Development of a test system with Crangonyx pseudogracilis in artificial aquatic mesocosms and laboratory tests compared to Gammarus spp.
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Today a wide range of chemicals is used and potentially cause stress in the environment and ecosystems. Therefore, different methods and models have been developed to determine the effects of substances and to gather knowledge, concerning the behavior of substances in the environment. Mesocosms are artificial aquatic freshwater-systems which are used to access the effects of chemicals on the ecosystem level under realistic conditions. The predictive power of such model ecosystems benefits from the high diversity that is present in the communities assessed. Crustaceans like Crangonyx pseudogracilis and Gammarus spp. are sensitive towards several chemical substances (e. g. plant protection products), hence relevant as test organisms for the assessment of chemicals. Until now, the establishment of a crustacean representative has not been successful with native Gammarus spp. in static outdoor aquatic systems. The natural habitats of Gammarus spp. are freshwater streams that provide constantly high oxygen levels and relatively constant temperatures in contrast to static pond systems. The aim was to establish a crustacean species which can cope with the conditions in a static aquatic pond system. The invasive species C. pseudogracilis is considered a promising candidate as it tolerates oxygen levels below 1% and higher temperatures. Therefore, specimens of the invasive C. pseudogracilis were captured from a moat in Karlsruhe. The study uses different methods, including free-ranging amphipods, or specimens exposed in cages within the outdoor mesocosms (bioassay-design), just as their performance and mortality/immobility were compared. The test substance was a formulation to control arthropods, specifically a mitochondrial complex X electron transport inhibitor known to be very toxic to crustaceans. The nominal concentrations tested were 10-3, 10-2, 10-1, 1 and 10 mg a. s. L-1. The results show a slightly higher sensitivity of C. pseudogracilis compared to Gammarus spp. An additional laboratory test carried out in vessels with nominal concentrations of 0.81, 2.59, 8.3, 26.56 and 85 mg a. s. L-1 showed an essential higher sensitivity of C. pseudogracilis in comparison with Gammarus spp.

Alternative Approaches to Animal Testing for Aquatic Ecotoxicity Assessments and Environmental Risk Assessments (P)

1.03P.1
Filling Data Gaps with Predictive Models for Chemically Related Products: A Case Study with Alcohol Ethoxylates
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Predictive toxicity models are quantitative and robust alternatives to animal toxicity testing. One approach to predicting toxicity that has gained scientific acceptance is the use of Interspecies Correlation Estimation (ICE) models. ICE models describe mathematical relationships between a pair of species that can be used to predict chemical toxicity from one surrogate test species to a species of unknown sensitivity. These models can be used to augment taxa diversity in Species Sensitivity Distributions (SSDs) from which hazardous concentrations (typically HC5) are derived. In this work, ICE models were developed for alcohol ethoxylates (AEs). AEs are nonionic surfactants comprised of molecules with varying alkyl chain lengths and degrees of ethoxylation, and are used in detergents, wetting agents, emulsifiers, degreasers and emollients. Aquatic toxicity data primarily from acute tests for 32 Shell AE products (Klimisch score 1 and 2) were used in these analyses. A total of 12 AE-ICE models, mostly for daphnia-fish and fish-fish pairs, were statistically significant (slope>1) and met established criteria for model reliability. AE-ICE models estimated toxicity with reasonable accuracy: 1) in 78 of 85 comparisons, model predictions were within a 3-fold difference of observed values not used in model development; and 2) model predictions were closer to the observed values than predictions for the same species pairs from related models (i.e., broad narcosis or specific nonpolar ICE models). The development of SSDs for AEs has been hindered by limitations in the available empirical data. However, toxicity predictions from the newly developed AE-ICE models could fill data gaps, helping address concerns about potential untested species sensitivity. Preliminary assessments indicated that HC5s from SSDs enhanced with AE-ICE predictions cover a relatively narrow range of values (0.13 to 2.88 mg/L). Within the same alkyl chain length, toxicity generally decreases with alcohol ethoxylation, while within the same alcohol ethoxylation, toxicity generally increases with alkyl chain length. Despite the lack of chronic data, for select products chronic HC5s from SSDs enhanced with AE-ICE predictions are within the same order of magnitude as acute HC5s. These and related predictive models are viable alternatives to standard toxicity testing with vertebrates, and could be used as additional weight of evidence in hazard estimation and chemical assessment evaluations.

1.03P.2
Evaluating the Xenopus Eleutheroembryonic Thyroid Assay (XETA) for relevance and sensitivity using thyroid-active reference substances
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The new Endocrine Disruptor (ED) identification criteria (Commission Regulation (EU) 2018/605) are applicable to pesticides since November 10th, 2018. The implementation of the ED criteria relies on the ECHA-EFSA guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (EFSA Journal 2018;16(6):5311). This guidance considers that the endocrine activity is sufficiently covered in environmental species when an Amphibian Metamorphosis Assay (OECD TG 231) and a Fish Short-Term Reproduction Assay (OECD TG 229) are available. However these tests require the use of a large number of animals and they are not highly specific regarding endocrine mechanisms (i.e. they can also respond to non-ED). Fish and amphibian embryo assays have been recently developed which give more direct access to endocrine mechanisms. Embryo assays are considered as "non-animal" tests within the EU because they use embryos at a development stage where independent feeding has not yet started while both the UK Animals (Scientific Procedures) Act, 1986 and the Directive 2010/63/EU on the protection of laboratory animals only apply to independently feeding larval forms. The embryo assays used for investigating endocrine mechanisms therefore appear as an ethical alternative to tests conducted with older larval stages, juveniles or adults. So far, the XETA (Xenopus Eleutheroembryonic Thyroid Assay) is the only embryo assay for which an OECD TG was published (TG 248). However, as it was adopted only recently, the current feedback from European regulators is that there is little experience with the XETA. EFSA also highlighted that this assay might have limitations with regard to the detection of (i) inhibition of thyroid hormone synthesis and (ii) interference with the transport of thyroid hormone. Therefore, there is still a need to consolidate the understanding of this assay in terms of interpretation of the thyroid-related mechanisms that can be affected. The European Crop Protection Association (ECPA) therefore launched a research project in collaboration with Laboratoire WatchFrog (France) to consolidate the level of knowledge and confidence in interpreting the results of the XETA. Within this project, twelve thyroid-active reference substances for which the mode of action is known (e.g., inhibition of thyroxyperoxidase, deionidase, sodium-iodide symporter) will be tested. The presentation will report on the first results of this project.

1.03P.3
Quantifying Conservatism in ecoTTC and CTD: Case Study of Chemicals with Regulatory Water Quality Values
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Ecological Thresholds for Toxicological Concern TTC (eco-TTC) utilise the wealth of ecotoxicological information in computing Predicted No-Observed Effect Concentrations (PNECs) on diverse chemical substances in the form of probability distributions. These enable toxicity prediction for untested chemicals based on grouping the chemicals according to their mode of action or structural features. The ecotoxicological database EnviroTox (https://envirotoxdatabase.org/), was recently developed to compile curated ecotoxicological data for more than 3900 chemicals across 1563 aquatic species. This ecotoxicity data is associated with physical chemistry data and curated taxonomic information for the organisms tested. Several mode of action schemes are also included to facilitate development of a best approach for grouping compounds. The database is publicly available online and is integrated with an online tool allowing calculation of the PNEC for the chemical (based on depth and breadth of data) and to build probability distributions. Those distributions allow to derive two types of environmental threshold concentrations: ecoTTC values (i.e. fifth percentile of a PNEC distribution) and chemical toxicity distribution (CTD)

values (e.g. fifth percentile of a Chemical Toxicity Data distribution, without the application of an assessment factor). This case study quantitatively assesses the level of conservatism in extrapolation approaches used in ecoTTC and CTD generation with the EnviroTox tool in comparison to available regulatory water quality values for aquatic life as benchmark values. EcoTTC and CTD values were generated for neurotoxicants (carbamates and pyrethroids) and benzyl compounds acting *via* a narcotic mode of action (MOA). The ecoTTC and CTD values were compared to available regulatory criteria and screening benchmark values for aquatic toxicity for these same groups of chemicals. Toxicity data were extracted from the ETOX (Information System Ecotoxicology and Environmental Quality Targets) database that contains information on various national and international environmental quality guidelines, targets, standards, criteria, and limit values. The preliminary results show that depending on the MOA of the chemicals, the ecoTTC or the CTD value could be a possible trigger value.

1.03P.4

Assessment of the risk for inhibition of hepatic clearance of pharmaceuticals in fish

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Fish are one of the most impacted species by pharmaceutical emissions in the water environment. Although the environmental risk assessment (ERA) guideline provided by the European Medicines Agency (EMA) addresses the bioconcentration risks associated with the uptake and elimination (hepatic clearance) of active pharmaceutical ingredients in fish, the mechanistic background of bioconcentration is not routinely determined as part of the ERA. In addition to being biotransformed by the hepatic enzymes, primarily the cytochrome P450 (CYP) system, pharmaceuticals may inhibit the hepatic clearance of other pharmaceuticals reversibly or irreversibly. In most severe situation, irreversible (time-dependent) inhibition of enzyme activity may result in complete loss of hepatic clearance capacity, and thus increased levels of pharmaceuticals (elevated bioconcentration risk), likewise in fish as in man. In human, drug-drug interactions caused by reversible/irreversible CYP enzyme inhibition are thoroughly characterized so that unfavorable (toxic) drug cocktails can be avoided, but there is very little information on the inhibitory effects of pharmaceuticals in fish although fish are constantly exposed to diverse chemical cocktails in vivo. In this study, we screened a set of marketed pharmaceuticals that are known to inhibit human CYP system, for their ability to inhibit CYP activity in fish microsomes (rainbow trout, Oncorhynchus mykiss). The model CYP substrates were selected based on OECD standards and scientific literature. Generally, the half maximal inhibitory concentrations (IC50) were much higher than measured environmental concentrations reported in the literature. However, on the basis of the IC50 shift (indicator of irreversible enzyme inhibition with threshold of >1.5) there were indications of the fact that irreversible inhibition of human CYP system may correlate with that of rainbow trout. These findings suggest that, besides hepatic clearance, the pharmaceutical's tendency for enzyme inhibition should be taken into account when predicting the in vivo bioconcentration risk based on the in vitro data.

1.03P.5

Eco-BER Case Study: Investigating the applicability of bioactivity data to inform quantitative hazard assessments for ecological species using bioactivity-to-exposure ratios

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Quantitative consideration of *in vitro* bioactivity data in ecological risk assessments is often limited. This is due, in part, to uncertainty with conducting and interpreting quantitative *in vitro*-to-*in vivo* extrapolations (Q-IVIVE) for species of ecological interest, such as fish. In the context of the APCRA[1] initiative, the present case study aims at developing a workflow for estimating an *in vitro* bioactivity threshold using Q-IVIVE and investigating its application as a quantitative line of evidence in chemical prioritization or ecological risk assessment. A set of chemicals with both *in vitro* bioactivity data and traditional *in vivo* chronic fish ecotoxicity data was identified and relevant data was extracted for each of those chemicals (chronic fish sub-lethal endpoints, bioactivity AC_{50} values, toxicokinetic and physico-chemical data). The Armitage et al. *in vitro* partitioning model was used to estimate true intracellular exposure concentrations for each chemical-assay pair. Several fish reverse toxicokinetic (R-TK) models are now being assessed and compared and the best performing model will be used to estimate *in situ* exposure equivalents from the bioactivity data. As a next step, the *in vitro* bioactivity threshold will be established and compared with traditional effect values to determine if bioactivity data can provide a conservative estimate of hazard potential. Besides advancing the consideration of *in vitro* bioactivity data as a quantitative line of evidence for the ecological risk assessment of chemicals, this case study may establish a method to conservatively translate *in vitro* bioactivity data into predicted *in situ* exposure equivalents for species of ecological interest using *in silico* tools. In addition, in an ecological bioactivity-to-exposure ratio (eco-BER) approach, the magnitude of the ratio may provide an indication of the level of concern with a particular chemical or set of chemicals, which may be valuable for chemical prioritization. [1] Accelerating the Pace of Chemical Risk Assessment (APCRA) is an international governmental collaborative initiative.

1.03P.6

Evaluation of the micronucleus frequency in gills of mullets Mugil cepalus as biomarker of environmental conditions

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The mullets (*Mugil cephalus*) is considered as cosmopolitan specie because it lives in the Atlantic and Pacific coastal areas in tropical and subtropical regions. Due to its wide distribution and because it is present in estuarine systems, could be used as an indicator species of environmental conditions. In this work an evaluation of the micronucleus frequency in gills of *Mugil cephalus* was carried out, to evaluate the use of this species in environmental biomonitoring studies. The mullets were collected in the following coastal systems: Alvarado Lagoon, Tamiahua lagoon, Sisal and in the Superior and Inferior lagoon. The gills samples were taken from 10 to 20 organisms. The tissues were fixed with a mixture of Alcohol-Acetic acid, (3:1). Preparations were made for each organism and stained with Giemsa for analysis. 1000 cells of each preparation were counted (in total up to 4000 cells per organism). Subsequently, a correlation analysis was carried out with the levels of metal and PAHs of the sampling sites. Significant differences were observed in the micronucleus frequencies of the mullets collected at different sampling sites. The system where the highest frequency of micronuclei were obtained was in the Tamiahua lagoon (1.36%) and the lowest micronucleus frequency was observed in the samples collected in Sisal Yuc. (0.06%). The results of the micronucleus evaluation agree with the contamination levels of the sampling sites. *Mugil cephalus* is a species that could be proposed as an species for biomonitoring studies.

1.03P.7

The superoxide dismutase (SOD) activity: optimization of a biomarker of exposure implicated in the oxidative stress response of organisms.

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The exposure of an organism to chemicals can be indirectly evaluated by the induction of detoxification mechanisms. For example, the superoxide anion is the most naturally produced reactive oxygen species (ROS). It is involved in biological metabolisms such as the mitochondrial respiratory chain or the innate immunity. Superoxide anions are supported by antioxidant defensive mechanisms. When these defenses are imbalanced due to the exposure of organisms to ROS inducer chemicals, the rate of superoxide is affected; the cells may be irreversibly damaged inducing pathologies such as lipid peroxidation or DNA damages. A key enzyme is involved within the antioxidant defenses: the superoxide dismutase (SOD). It metabolizes superoxide anions to hydrogen peroxide, a less reactive compound. The measurement of the SOD activity allows thus estimating the level of oxidative stress in exposed organisms. It is therefore necessary to develop robust and simple assays to estimate SOD activity. Several protocols are available in the literature but the level of SOD activity detected may not be low enough for ecotoxicological purposes. In this study, our goal was to optimize these protocols to decrease the SOD detection threshold, i.e. the sensitivity of the assay, in order to increase the detection power of the test. Three different compounds were tested and compared in the study: INT (Iodonitrotetrazolium), WST-1 (Water Soluble Tetrazolium-1) and DHE (Dihydroethidium) using a combination of statistical analyses commonly used to improve industrial processes. The first two are used with absorbance detectors and the last one with fluorescence detectors, which is potentially more sensitive than adsorbance. We observed that the solubility of tetrazolium's product yielded to a linear regression that allows detecting enzyme's concentration from concentrations as low as 0,005U/mL. We therefore dramatically decrease the detection limit that was 0.4U/mL. Furthermore, the results were replicable on a same micro-plate. A range of different SOD concentrations were realized after optimization to compare the several methods to identify the most sensitive assay. As a result, INT and WST-1 assay are more suitable than DHE but the DHE protocol need further optimizations. In the future, further assays should be performed in order to standardize these methods for a tracking tool.

1.03P.8

Non-lethal blood sampling from Rainbow trout in the laboratory and in situ

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The use of humane methods in animal research is an internationally recognized priority, but few Environmental Effects Monitoring programs use nonlethal methods with fish, and the ones that do are normally limited to behavior, morphology and reproduction. Non-lethal blood sampling methods support the three R's of humane experimental technique (Replacement, Reduction, and Refinement). Small tissue samples can be sampled from fish to measure exposure to contaminants; however, for understanding biological effects, tissue samples are often limited to only one type of physiological response. One of the advantages of blood, serum, and plasma is that these fluids contains protein, metabolites, and signaling information from all tissues and organs within the entire organism, which facilitates a systems-level understanding of whole organism health. Much to our surprise, we can find no published protocol or studies that outline procedures for effective and low-impact non-lethal blood sampling in fish. Thus, the goal of the present study was to determine the impacts and survival of larger bodied fish after sampling small volumes of blood. In our approach, we housed 80 rainbow trout (*Oncorhynchus mykiss*) purchased from a local hatchery in our flow-through aquatic facility (Ontario Tech U, Oshawa, ON). We then anaesthetized the fish using MS-222 and sampled 1 µl of blood per gram of mass from each fish. We tested four different post blood-sampling treatments on the puncture wound: (1) pressure only, (2) pressure and application of a liquid bandage, (3) pressure and a swab of betadine, or (4) pressure and a swab of the fish's own skin mucous. After blood sampling, fish were examined weekly for 5 weeks. Overall, we observed 90% survival among all treatments; the most effective approach was the pressure-only (100% survival), while the post-treatment with the largest impact on fish survival was the use of betadine (75% survival). Based upon these results, we repeated the blood sampling with no-post treatment using 20 rainbow trout (freshly purchased, not previously tested upon) *in situ* using suspended cages at a nearby freshwater creek, and monitored fish behavior and survival for 3 weeks post sampling. In this presentation, we will present the detailed results of these combined studies and describe what was determined to be the safest non-lethal blood sampling protocol.

1.03P.10

Evaluation of a flow-through multiwell plate for application in the FET test using zebrafish and fathead minnow

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The evaluation of chemicals in a regulatory context relies on the assessment of their (eco)toxicological hazard. In addition to other criteria, the assessment is based on the evaluation of the toxicity in whole-organism bioassays. In this context, the Fish Embryo Toxicity (FET) test has emerged as an important bioassay for the evaluation of the teratogenic potential of chemicals. The test is based on the prolonged incubation of fish embryos in polystyrene multi-well plates. However, single dosing exposure experiments using hydrophobic and charged substances were shown to be problematic as concentrations rapidly decline using this standard well plate type. This was also observed when static renewal of the exposure medium occurred at frequent intervals. Hence, it has been proposed to use miniaturized flow-through systems for exposure control in the FET test. The constant renewal of the exposure medium improves the stability of the concentrations of the target compound, and the potential for automation can improve the applicability of whole-organism bioassays in high-throughput study designs. This study evaluated a BioWellPlate (BWP) designed for the incubation of organisms under flow-through conditions in a multiwell plate format. We compared the impact of different exposure regimes within the FET test using zebrafish (*Danio rerio*) and fathead minnow (*Pimephales promelas*) embryos. Static, static renewal and flow-through exposure with carbendazim and fluoxetine were compared. Additionally, larval locomotor responses and apoptosis were assessed at the end of each test. The influence of the exposure regimes on substance concentrations was assessed using chemical analysis. In this study, we present a system that has the potential to be used in the FET test as an addition for the testing of lipophillic or charged compounds. This approach for controlled compound concentration exposure using the BWP not only addresses the limitations of the substance concentration depletion as seen in other studies, it can be automated and applied continuously in-field e.g for environmental monitoring studies.

1.03P.11

Non-targeted metabolites profiling based approach on adverse effects of diclofenac to zebrafish embryo

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Using zebrafish embryo or larvae as a toxicological model has been increasingly interested due to its advantages such as cost effective and easy to manage on the target chemicals. In addition, within Europe, zebrafish embryo until the stage of 5 days post-fertilization (dpf) are not classified as animal, and thus this toxicological model could be accepted as an animal alternative testing (OECD TG236). In this study, diclofenac (DCF), one of NSAIDs (Non-Steroidal Anti-Inflammatory Drugs), was selected as a target chemical due to its known adverse effects on environmental biota as well as biotransformation into other active metabolites, and various concentrations of DCF were exposed to zebrafish embryos from 0 to 5 dpf to evaluate Maximum Tolerated Concentration (MTC). As a result, MTC of DCF to zebrafish embryos was derived as 10.0 µM for both 5 day exposure with up to 5 dpf and 1 day exposure with 4 and 5 dpf. From this MTC value, various concentrations of DCF, 0.2, 1.0, 5.0, and 10.0 µM, were exposed to 100 embryos/L for further experiment. This DCF exposed embryos were then prepared by liquid-liquid extraction (LLE) with cold acetonitrile for the analysis with UPLC coupled with Thermo TSQ Quantum Orbtrap MS (Thermo Scientific, Waltham, MA, USA). Full scan MS data were acquired in both positive and negative ion modes, and all MS data were analyzed by using XCMS online (https://xcmsonline.scripps.edu). Through MS profiling, two significant MS data with m/z=312.0195 and 334.0014 were achieved and these peaks were proton and sodium adduct for hydroxylated DCF based on mass defects (Δm). As increasing DCF concentrations to zebrafish embryos, these peaks were exponentially increased, and thus hydroxylated DCF would be exogenous active metabolite related to toxic effects of DCF to zebrafish embryos. In addition, two endogenous metabolisms, 'L-dopa degradation' and 'glutathione biosynthesis', were delivered from MS profiling based on the potential metabolites of '3-methoxy-4-hydroxyphenylpyruvate' and 'γ-L-glutamyl-L-cystein' with m/z=233.0418 and 214.0422, respectively. In future, based on these results, targeted-metabolomics will be performed to understand the toxicological interactions between DCF and zebrafish embryos.

1.03P.12

Characterization of vitellogenesis in in the long-term cultured 3D spheroids of zebrafish liver cell line

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In vitro platforms based on fish cell lines can be considered as an alternative to animal tests for assessing the adverse effects of chemicals in aquatic animal species. In view of bioethics and 3Rs (replacement, reduction, and refinement) policy, the precise and reliable *in vitro* tests are required as the increased presence of endocrine disruptor chemicals in the ecosystem. However, the conventional monolayer fish cell cultures have numerous limitations on endogenous and exogenous metabolic functions to support such demands. For the *in vitro* platforms, the vitellogenin (Vtg), a precursor of the egg yolk proteins, can be utilized as a marker for reproductive toxicity. In our study, we apply a zebrafish liver (ZFL) cell line to a three-dimensional (3D) spheroid culture system which is based on an ultra-low attachment plate. The cell viability and synthesis of Vtg is described. Our results show that the culture conditions of 5,000 and 10,000 ZFL cells are viable for 28 days within 10% of death ratio, and synthesize vitellogenin, unlike conventional 2D cell cultures. Moreover, the Vtg levels in 3D spheroids were increased with the function of time and 3D spheroid treated with 17β-estradiol (E2) appears higher vitellogenin synthesis levels than the non-treated 3D spheroid. This result suggests that 3D ZFL spheroids have improved hepatotypic functions compared to 2D cell cultures and can be used as an *in vitro* model system for chronic assessment of the reproductive toxicity.

1.03P.14

Do Daphnia magna and Ceriodaphnia dubia Acute and Chronic Tests Show Equitoxic Results?

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Environmental risk assessments are typically based on toxicity data derived from only a handful of standard model organisms. Some standard regulatory assays limit the species in scope, others like the OECD 203 acute fish assay can be considered valid if one of seven recommended species are tested. While it is important to consider the relative sensitiv-ity of other species, it is equally important to understand the sensitivity variation that is already contained within the standard regulatory assays. The OECD 202 Acute *Daphnia* Immobilization Toxicity Test requires the use of *Daphnia magna* or another "suitable *Daphnia* species (e.g., *D. pulex*)." The daphnid species, *Ceriodaphnia dubia,* is not con-sidered a standard test species for chemical registration in Europe despite the availability of ISO, USEPA, ASTM, Environment Canada standard acute and chronic test methods and its wide use and acceptance in other countries. Previous comparative work by Versteeg et al. (1997) suggests that *D. magna, D. pulex, and C. dubia* are acutely equisensitive. Here, we employ a big data approach to critically evaluate the comparative sensitivity between *D. magna* and *D. pulex* and contrast that to the similar species sensitivity of *D. magna* and *C. dubia*. These big data approaches will be contrasted against the smaller, more curated web-ICE regressions. A total of 207 chemicals that had both *D. magna* and *D. pulex* acute toxic-ity data were identified (5,443 studies). This orthogonal regression has a slope of 0.881 and an intercept of 0.484. As both *D. magna* and *D. pulex* are accepted OECD 202 standard test species, this inherent biological difference in sensitivity is accepted under the regulatory guidelines. A total of 193 chemicals that had both *D. magna* and *C. dubia* toxicity data were identified (5,465 studies). The orthogonal regression for the acute toxicity studies has a slope of 0.919 and an intercept of 0.599. The relative species sensitivity differences between *D. magna* and *D. pulex* and *D. magna* and *C. dubia* are of the same magnitude and have nearly identi-cal slopes. *D. magna* and *C. dubia* chronic toxicity (83 chemicals, 1062 entries), also showed similar sensitivity with a slope of 0.841. Interspecies modeling demonstrates similar sensitivity for *Daphnids,* and *C. dubia,* and the interchangeability of these taxa is recommended for chemical testing. *This presentation does not necessarily reflect the views or policies of the US Environmental Protection Agency.*

1.03P.15
Environmental classification of Essential Oils: Pros. and Cons. of each standard method and some alternatives.
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As a result of their extraction methods, Essential Oils (EO), used as raw materials in finished products, are covered by the REACH regulation in Europe and subject to the classification and labeling requirements of the CLP Regulation.Following this Regulation, the approach for classification of aquatic environmental hazards of EOs, and mixtures in general, is tiered, and is dependent upon the type of information available for the EO itself and for its components. Elements of the tiered approach include three possibilities: classification based on ecotoxicological experimental studies available on the EO itself, using the Water Accommodated Fraction (WAF) method; classification based on bridging principles; or the use of “summation of classified components” and/or an “additivity formula”.Due to the lack of thermodynamic considerations, the “additivity formula” overestimates the experimental results from a WAF test and therefore can overestimate the classification of the EO. In addition, the “summation of classified components” may be biased by the use of harmonized classification under revision (e.g. recent case of limonene) or from direct transfers of environmental classifications established under pre-existing European rules.WAF tests are recommended to determine the aquatic toxicity of mixtures under the CLP Regulation although there is no requirement to generate new data for classification purposes alone. Furthermore, WAF tests are costly and time consuming and it may not be wise to perform studies on fish as the popularity of vertebrate studies is decreasing steadily.Bridging principles on similar mixtures reduce the cost of new experimental studies, but a scientifically justified read-across must be provided in accordance with the “Read-Across Assessment Framework (RAAF)”. This state of the art method is highly time consuming and sometimes difficult to justify based on the complexity of mixtures thus is not widely used.Another alternative to experimental studies is the iSafeRat® in silico calculation method, developed by KREATiS, which has already been applied in ca. 40 REACH EOs dossiers and is recommended in the IFRA Guidance. This alternative method takes into account all the constituents at their expected concentrations in water using a thermodynamic based calculation and provides both a high precision global toxicity value for the WAF loading rate and a mechanistic understanding of the results based on each constituent.

1.03P.16
Petri Nets as a Unifying Tool to Link Physiologically Based Toxicokinetic Models to Adverse Outcome Pathways
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Physiologically based toxicokinetic (PBTK) Models and Adverse Outcome Pathways (AOPs) are common tools used in predictive and computational toxicology. PBTK models are used to predict internal tissue concentrations of a contaminant in an organism from various exposures. AOPs describe a pathway leading from a molecular initiating event to an adverse outcome. We present Petri Nets (PN) as a way to quantitatively link PBTKs to AOPs, providing a complete exposure to outcome model and linking effect data across multiple levels of biological organization. The present study demonstrates this conceptual approach by 1) expanding an existing AOP for selective serotonin reuptake inhibitor (SSRI) antidepressant drug effects on feeding in fish models, 2) evaluating the causal relationships between events along the AOP, and, 3) providing quantitative links to unify data from the AOP continuum through application of a PN for fish. The waterborne exposure of the SSRIs fluoxetine, venlafaxine, and sertraline, and the resulting inhibition of time to capture prey in hybrid striped bass (Morone saxatilis x. M. chrysops) were used as an example. Using the model, we were able to predict dose response curves for observed appetite inhibition versus exposure concentration. Using Monte Carlo sensitivity analyses, we determine which parameters should be revised through further experimentation and measurement. Finally, we describe novel ways that the model can be used to predict time-course, probabilistic, magnitude of response endpoints. Acknowledgements: Financing - RCN 268294 “Cumulative hazard and risk assessment of complex mixtures and multiple stressors (MixRisk)”, and NCTP: NIVA’s Computational Toxicology Program, NCTP (www.niva.no/nctp).

Epigenetic and Evolutionary Effects of Environmental Stressors on Environmental and Human Health (P)

1.04P.1
Resurrected Daphnia as a model organism to assess evolution of natural populations to environmental stressors
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Long-term exposure to environmental stressors such as temperature changes and chemical pollution can lead to genetic adaptations in exposed populations of organisms. Evolutionary toxicology and resurrection ecology offer powerful tools for the investigation of changes in sensitivities and adaptive trajectories of populations exposed to contaminants and environmental stressors over decades to centuries. Dormant resting eggs produced by Daphnia species as a result of unfavourable environmental conditions are archived in sediments and can be dated and hatched to produce clonal lineages (i.e., same genotypes) of historical populations. Our research examines how genotypes of clonal lineages of Daphnia species from single populations, separated through generations of evolution, differ in their response to exposure of environmental stressors. Specifically, 20 resurrected D. magna lineages collected from the field (Lake Ring, Denmark), spanning approx. 100 years and previously sequenced, were exposed in 48-h immobilization tests (OECD 202) to phenanthrene, a common polycyclic aromatic hydrocarbon (PAH) ubiquitous in aquatic environments. The EC50s observed among the clonal lineages ranged from 6 to >30 µM, indicating a wide response in sensitivity of the daphnia. In addition to the acute studies, 21-d daphnia tests (OECD 211) were conducted to investigate the effects of aqueous phenanthrene exposure in combination with changes in temperature on the sensitivity and fitness, as well as genomic analysis of the clonal lineages of D. magna. The genomic analysis, including metabolomic profiling and transcriptomics, of tolerant and non-tolerant Daphnia populations will aid in elucidating the micro-evolutionary adaptations of genes in response to changing environments, providing further insight into the mechanisms of stress tolerance and adaptation. Overall, the toxicological and genomic data obtained from exposure of daphnia populations to environmental stressors provide unprecedented opportunities to gain insight into long-term and potentially future evolutionary responses of a keystone sp., providing feedback for risk assessment and future management of lake systems (e.g., collapse of grazers/impacts on upper trophic levels).

1.04P.2
Multigenerational exposures of Daphnia magna to pristine and aged silver nanoparticles: epigenetic changes and phenotypical ageing related effects
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Engineered nanoparticles (NPs) undergo physical, chemical, and biological transformation after environmental releases, resulting in different properties of the ‘aged’ variants compared to the engineered ‘pristine’ forms. While many laboratory studies have investigated the ecotoxicological effects of silver nanoparticles (Ag NPs), the majority focus on ‘pristine’ engineered Ag NPs in simple exposure media, rather than investigating realistic environmental exposure scenarios with transformed NPs. Here, we systematically evaluated the effects of both ‘pristine’ and ‘aged’ Ag NPs (with different surface coatings) on *Daphnia magna* over four generations, comparing the impact of continuous exposure versus a parallel parental only exposure for three generations, the latter considered as a recovery approach. Biological endpoints including survival, growth and reproduction and genetic effects associated with Ag NP exposure were investigated. The study shows that pristine NPs in standard culture media had the most toxic consequences. Life history traits revealed that parental exposure to ‘pristine’ Ag NPs had an inhibitory effect on reproduction, induced the expression

of key antioxidant stress related genes and caused a decline in survival. The pristine Ag NPs also induced morphological changes including tail losses and lipid accumulation in the heart, abdomen and abdominal claw which are associated with ageing phenotypes. These effects were further displayed in two generations removed post maternal exposure (F2 and F3) showing epigenetic traits. However, exposure to identical Ag NPs (at the same concentrations) aged for 6 months in environmentally realistic water containing natural organic matter showed considerably reduced toxicological effects in the continuously exposed generations and reduced effects to the recovery generations.

1.04P.3

Applying DNA Methylation as a Biomarker to Assess Latent Effects of Acute Exposure of Benzene in Simulated Chemical Accident Using Zebrafish Model

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Chemical accidents through which high concentration of chemicals were released by fire or explosion cause latent effect on aquatic environment. Benzene is one of the chemicals often detected near the oil-spill accidents. DNA methylation regulating gene-expression without alteration in DNA sequences has recently risen as a biomarker. We exposed male zebrafish (F0), in a short-term (i.e., 3 minutes), to high concentration of benzene (i.e., 0, 10, 100, 600 mg/L). DNA methylation was measured before and after transfer to clean water for recovery. Exposed and transferred male zebrafish were mated with un-exposed female zebrafish 2 times after 7 and 14days. Global DNA methylation was observed in testis and liver of F0 male zebrafish and in offspring (F1) embryos at 5dpf (day post-fertilization). Gene-specific DNA methylation related with reproductivity was analyzed by pyrosequencing and expression of the genes were measured using qPCR. Down-regulated global DNA methylation was observed both in testis of F0 and in F1 embryos. In particular, the expression of star gene was significantly up regulated. In conclusion, our data suggest that DNA methylation has a potentiality as a biomarker to identify the latent effect of high concentration of chemicals in a simulated chemical accident.

1.04P.4

Zebrafish differential sensitivity to environmental stressors during early development and implications for subsequent exposures

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Aquatic systems are affected by a wide range of stressors including chemical pollutants and alterations in abiotic factors. Some stressors have been shown to affect the epigenome, leading to persistent effects long after exposure, however little work has been done to determine whether susceptibility to epigenetic alteration varies during development. To address this, zebrafish embryos were used as their epigenetic reprogramming periods have been studied in detail, and we hypothesise that embryos may be particularly sensitive when exposures occur during this period. We also hypothesise that exposure during this period causes alterations in susceptibility later in development. To test these hypotheses, we exposed embryos to toxic metals (copper, silver and methylmercury), and 5-azacytidine (a positive control), during and after reprogramming to determine impacts on embryo physiology and the epigenome. The timing of exposure caused significant differences in the toxicity of copper, methylmercury and 5-azacytidine after 48 hours, with 5-azacytidine almost ten times less toxic when the exposure period began after reprogramming. We are now investigating whether the rate of chemical uptake into embryos varies with the developmental stage at which exposures are initiated, to determine if the differences observed are due to altered chemical uptake. To investigate if non-lethal exposure during early development affected larval response to re-exposure, embryos were exposed for 24 hours beginning either at 0 or 4hpf, before a depuration period until 72hpf. At this point, embryos were re-exposed for 24 hours to a concentration causing 50% mortality in the control populations and responses were measured. Embryos exposed to copper for 24h during early development had delayed hatching at 72hpf, but no developmental delay. However, upon re-exposure, hatching was further delayed and a significant proportion of embryos remained unhatched at 96hpf. These groups also showed significantly lower mortality at 96hpf during re-exposure compared to naïve embryos exposed in parallel. In contrast, silver and methylmercury did not cause delayed hatching, and a 24h pre-exposure did not cause significant differences in mortality when embryos were re-exposed. Identifying periods of increased sensitivity during development and the associated mechanisms is important when defining environmentally safe concentrations and to describe the impact early exposure may have on later development.

1.04P.5

Assessing Eco-genotoxicity for Prioritizing Chemicals Using Version 2.0 of the Ecological Risk Classification Approach

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In 2016 ECCC developed the Ecological Risk Classification (ERC) approach used for classifying (prioritizing) 640 organic chemicals for further ecological risk assessment under the third phase of the Chemicals Management Plan (CMP) in Canada. ECCC has recently developed version 2.0 of the ERC (ERC2) for re-prioritizing ~12200 organics on the Canadian Domestic Substances List (DSL) which were not ‘categorized’ as a concern using PBT metrics in 2006. ERC2 is a rule-based weight of evidence approach for classifying the risk of organic chemicals to ecological receptors. ERC2 uses data consensus within and among in silico, in chemico, in vitro, in vivo and in situ data to classify hazard and exposure and risk, classification confidence and classification severity. Classification of hazard, exposure and risk is conducted according to rules based on potency indices (hazard) and probability (exposure). Confidence is scored according to data consensus and severity scored according to number of rules triggered. ERC2 also uses fundamental chemical interactions with biological tissues to help target “effects of concern” (e.g., developmental and reproductive toxicants). ERC2 thus uses a high amount of data that would traditionally be in the human health domain and consequently promotes the notion of “one health” assuming comparable cross species susceptibility. To profile eco-genotoxicity, reactive toxicity from covalent interactions with proteins and nucleic acids is targeted. Several descriptors (in silico, in chemico, in vitro, in vivo) are thus used to explain plausible mechanism(s) consistent with adverse outcomes. A comparison of ERC2 hazard classifications for covalent interactions with results from an in vitro “multiflow” DNA damage assay was performed for 10 chemicals as a proof of concept study only. For non-human organisms, ECCC is mainly interested in genotoxic responses related to developmental and reproductive effects and thus mainly mutagenic responses. Exposures to epigenetic chemicals with wide environmental distribution and long residence time in the environment is a main focus of ERC2 because transmissible population level impacts may continue even after emissions are stopped and “safe levels” are reached (e.g., based on chronic median lethal PNEC). Results of the ERC2-in vitro DNA damage proof of concept study will be discussed as a case study demonstrating the use of eco-gentoxicity for eco-prioritization of organic chemicals in Canada.

1.04P.6

Identification of Differentially Methylated Regions in zebrafish eleutheroembryos exposed to TBT

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Environmental exposures to chemicals can affect the epigenetic status of ecotoxicologically relevant species of fish, plants and invertebrates. The prevalent hypothesis is to use the "epigenetic foot-print” as a tool to evaluate the exposure of a given organism to toxicants, with the ultimate goal of determine if epigenetic signatures characterized after early developmental exposures are maintained through time once the pollutant exposure ceased, and if those can cause adverse effects on the exposed animals. Indeed, several publications have demonstrated that EDCs can act as epigenomic disruptors. However, the mechanism of action remains unclear. For that reason, further studies are needed to better understand the involvement of upstream mechanisms, such as epigenetics, and elucidate how EDCs act on epigenetic endpoints capable to regulate gene expression. Zebrafish has been recognized to be a good model species to study environmental epigenetics.In the present study we have exposed zebrafish eleutheroembryos from 2 to 5 days post fertilization to 3, 30 and 100 nM of TBT. Extraction of RNA and DNA was performed simultaneously from pools of 8 eleutheroembryos. RNA sequencing revealed that transcriptomic dysregulation of TBT suggested a general, rather unspecific toxicity pattern, since most of the affected pathways were related to cell viability and development. This was in concordance with morphometric results previously published that showed that the affected traits were more related to developmental delays rather than to a specific mode of action of TBT. However, steroid biosynthesis pathways that lead the synthesis of cholesterol and vitamin D_3appeared as the modules that become affected at the lowest TBT concentrations. Furthermore, five replicate samples per condition were sequenced for control, 3 and 30 nM of TBT using whole genome bisulfite sequencing (WGBS). Results from this analysis will be integrated with transcriptomic data obtained. The ultimate goal of this study is to identify potential epigenetic biomarkers of exposure to EDCs and to better understand the modes of action of EDCs using holistic approaches.

1.04P.9

Observed individual plasticity in a generational study of Daphnia magna

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The term that defines the ability of a single genotype to generate a variety of phenotypes based on environmental cues is phenotypic plasticity. Increased tolerance to environmental challenges facilitated by phenotypic plasticity over longer time can furthermore be described as acclimation. The extend of phenotypic plastic responses thus describe the range of trajectories that an organism has the ability to take. For instance, developmental switches can respond to environmental signals, allowing a single genotype to take multiple developmental routes and give rise to many alternative phenotypes. In laboratory studies, this becomes especially important when comparing generational lineages over longer time scales as there will be a likelihood for different developmental trajectories in replicates resulting in individual phenotypes leading to higher variance. Phenotypic plasticity in organismal responses to toxic compounds, is thus an essential feature and possible source of variation when studying toxicity across generations. In a 3-generation study of the highly plastic model organism *Daphnia magna* we studied toxic effects beyond exposure of the azole fungicide prochloraz. The study included one treatment group where the first generation (F0) was exposed to prochloraz and then we followed the subsequent two generations (F1+F2) through individual lineages. These lineages were compared to lineages from a non-exposed control group. Effects were assessed at different levels of biological organization ranging from transcriptomics, enzyme activity and key phenotypic effects. When comparing the global gene expression of the grand-maternally exposed organisms (F2) to the control animals, principal component analysis showed that individual grandmaternally-exposed clonal lineages were gradually more divergent form the controls (i.e. inter-lineage plasticity). Furthermore, this correlated with lineage-specific offspring length (F3). The pattern showed that the more similar gene expression in F2 was to the control animals, the smaller the neonates. This first of all demonstrates that a gradual plastic adjustment is taking place. It also provides some insight into potential trade-offs in energetic costs. Finally, we see that this is not synchronized between the replicate lineages. These results therefore underline the importance of considering plasticity as a source of variation and exemplifies the importance of high statistical power in multigenerational studies.

1.04P.10

The effects of waterborne chromate (Cr6+) on protein abundance patterns in Lake trout (Salvelinus namaycush) and White sucker (Catostomus commersonii)

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In anticipation of the development of chromium mining in the Ring of Fire Region (Northern Ontario, Canada), we assessed the effects of Cr^{6+} on the plasma proteome of Lake trout and White sucker – two species' which are native to that region. Juvenile Lake trout were exposed in the laboratory to waterborne Cr^{6+} (0, 0.2 ppb and 3 ppm). Exposures lasted for 21-days in flow-through conditions, with the exception of the highest (3 ppm) treatment because we had observed mortalities after 7-days in our pilot study. Plasma proteins were analyzed using a reverse-phase liquid-chromatography tandem quadrupole time-of-flight mass spectrometry system with data-dependent full scan acquisition, and peptide spectral files were subsequently sequenced and matched to proteins using bioinformatics software. Results from liver transcriptome analyses from these same fish indicate there was significant differential gene expression in both the 0.2 ppb and 3 ppm treatments. There were also significant changes in the concentration of some plasma metabolites. Plasma protein abundance will be compared to these results to detect where there is an agreement between the datasets and we will discuss how changes in gene expression caused by exposure to Cr^{6+} could result in higher-level biological effects, and be used for monitoring fish health in Ring of Fire mining activities.

Fish Model Species in Human and Environmental Toxicology (P)

1.05P.1

Oestrogenic effects in wild fish in English Rivers: comparing contemporary and historical impacts

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A wide range of natural and anthropogenic chemicals interfere with the normal functioning of endocrine systems of both humans and wildlife. Exposure to such endocrine disrupting chemicals (EDCs) can cause disruptions in development, alter sexual differentiation and function, and impact adversely on reproduction. Some of the most persuasive evidence for adverse effects of EDCs in wildlife globally comes from the identification of widespread feminisation of males in fish populations linked with exposure to oestrogenic substances. Some of the pioneering studies on fish were carried out in England where widespread feminisation of wild roach (*Rutilus rutilus*) in rivers was shown to be associated with exposure to wastewater treatment works (WwTW) effluents. The historical surveys for these effects on roach in English rivers were undertaken in 1995-1998, and 2002-2003 and the feminising effects seen included a high incidence of intersex and in males' high levels of the egg-yolk protein precursor vitellogenin (VTG). Feminisation severity (frequency of oocytes in an otherwise male testis) correlated with WwTW effluent concentration in the river, as well as the size and age of the fish.There have been no studies that have systematically compared current intersex levels to those reported previously. To remedy this, in 2017, we conducted a new survey specifically revisiting ten sites with feminisation data from the previous surveys, including 'low', 'medium' and 'highly' impacted populations. As with previous assessments, intersex frequency and severity, VTG and feminised sperm ducts were investigated. In the 2017 survey, the genetic sex of the sampled fish was also determined to confirm whether intersex fish were feminised males or masculinised females, and to determine the possibility of fully sex-reversed fish.The 2017 survey indicates that feminisation in wild male roach is still a widespread phenomenon; the frequency of intersex was not significantly changed compared with the historical surveys. The genetic sex-probe confirmed the hypothesis that the majority of intersex fish are genetic (feminised) males. The 2017 survey also shows some reduction in the feminised responses. None of the male fish had feminised ducts, compared with historical analyses where 94% of sites had males with feminised ducts. Male plasma VTG concentrations were also significantly reduced, although they remained elevated above the natural male baseline levels.

1.05P.3

Vitellogenin Analysis and the use of Aprotinin

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Endocrine activity of the test substance may be indicated by measuring the glycoprotein, vitellogenin (VTG), which is a simple and sensitive biomarker for endocrine active chemicals. VTG, a pre-cursor of yolk proteins, is normally produced by the liver of female fish in response to circulating endogenous oestrogen. By measuring VTG it is possible to detect various oestrogenic modes of action of chemicals, for example, increased levels in male fish indicating estrogenic effects and decreased levels in female fish indicating anti-estrogenic effects. Samples can be taken from blood, either from the caudal vein/artery or heart, liver or whole body which are then processed and VTG concentration determined by Enzyme-Linked Immunosorbent Assay (ELISA). The OECD 229 guideline recommends that when blood is sampled from the caudal vein/artery, the separated plasma should be stored with aprotinin (a protease inhibitor) prior to VTG analysis. However, some evidence is available in the literature^[1] which indicates that the addition of protease inhibitor may reduce the amount of VTG in the sample. An experiment was therefore conducted whereby blood samples from the caudal vein/artery were taken from adult male and female fathead minnow (*Pimephales promelas*). The plasma from each sample was separated and divided into two samples one of which was stored with aprotinin and one without. The samples were stored deep frozen prior to VTG analysis. This poster will discuss the experiment conducted and if the storage of blood plasma samples with or without aprotinin affects the VTG concentration in the sample. [1] Brodeur JC, Woodburn KB, Zhang F, Bartels MJ, Klecka GM. Plasma sampling and freezing procedures influence vitellogenin measurements by enzyme-linked immunoassay in the fathead minnow (Pimephales promelas). Environmental Toxicology and Chemistry, 2006 February 25(2):337-48

1.05P.4

Effects of Sub-Lethal Atrazine Concentrations on Embryogenesis, Larval Survival and Growth of African Catfish, Clarias gariepinus (Burchell, 1822)

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Freshly fertilized eggs of *Clarias gariepinus* were exposed to environmentally significant concentrations of atrazine in a static renewal bioassay for 72hours. This study was aimed at determining the effects of sub-lethal atrazine concentrations on the seven broad stages of embryogenesis: zygote, cleavage, morulation, blastulation, gastrulation, segmentation and hatching; growth and survival of *C. gariepinus* larvae. The embryonic development was observed, photographed and documented live with the aid of a light microscope equipped with a digital camera connected to a computer. At the end of the embryonic exposure to atrazine concentrations of 0 (control), 4, 8, 12, and 16μgL^{-1} in water, the first mitotic cleavage in the control group occurred at 40minutes post-fertilization (pf). First mitotic cleavage, however, occurred at 60minutes pf in the 4 and 8μgL^{-1} concentrations and 70minutes pf in the 12 and 16μgL^{-1} concentrations. First larvae emerged at 22 hours post-fertilization (hpf) in both control and treatment groups at a controlled temperature of 27.0 ± 0.5°C. However, there was a significant difference ($p < 0.05$) in the hatching rate as well as percentage deformities between control and treatment groups. In the control group, 73.3% of the eggs hatched at 22hpf while only 3.3% of the eggs hatched in the group treated with 16μgL^{-1} atrazine. Larval deformities such as scoliosis, kyphosis, lordosis, detached yolk, and c-shaped body curvature were observed among treatment groups but were more severe with increasing concentrations of atrazine. The most common deformity observed was kyphosis with 60% of the emerged larvae exhibiting the deformity in the groups treated with 12 and 16μgL^{-1} atrazine and 26.6% in the 4μgL^{-1} treatment group. No deformity was observed in the control group. Larvae morphometry showed significantly reduced body length, head length, mean body weight and yolk diameter in the 16μgL^{-1} compared to the control. These results showed that atrazine delayed embryogenesis and hatching with possible physiological and morphological implications.

1.05P.5

Endocrine disruption in zebrafish following exposure to a combination of bisphenol A and ketoconazole

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Since bisphenols occur as mixture in water environment, adverse effects on aquatic organism associated with chemical interactions are also of concern. In the present study, the effects of the combined exposure to bisphenol A and ketoconazole on reproduction were investigated using zebrafish (*Danio rerio*). Egg production, gonadosomatic index, sex hormone levels, and transcriptions of genes related to vitellogenesis and steroidogenesis were observed in adult zebrafish exposed to bisphenol A alone or mixed with ketoconazole for 21 d. In male zebrafish exposed to bisphenol A alone, a significant decrease in egg production and gonad weight, an increase in 17β-estradiol (E2) to testosterone (T) ratio, and an upregulation of *vtg*, *erα*, and *cyp19a* genes were observed. The presence of ketoconazole potentiated the bisphenol A-induced estrogenic responses in the male and anti-estrogenic responses in the female fish. The increase of the E2/T ratio and the downregulation of *cyp17* and *17βhsd* were more pronounced following the combined exposure to bisphenol A and ketoconazole in male zebrafish. In female fish, the stimulation of the bisphenol A-mediated anti-estrogenic responses was observed in accordance with the mixed exposure. The present study indicated that bisphenol A exposure alone alters vitellogenesis and steroidogenesis, and combination with azole compound could increase the endocrine disrupting effect of bisphenol A. Acknowledgement - This study was supported by the National Research Foundation of Korea (Project NRF-2019R1A2C1002712).

1.05P.8

Phthalates - a plastic-related chemicals disrupt host immunity via effects on microbiome-gut axis

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To improve physical characteristics of plastics such as flexibility and durability, producers enrich plastics with phthalates, specifically di-2-(ethylhexyl) phthalate (DEHP). DEHP is considered to be a high production volume chemical, and it is frequently found in the environment. This project aimed to determine the key mechanisms related to how phthalates initiate adverse health outcomes. Our research focused on the microbiome-gut axis as it is a key regulator of health. A two-month dietary exposure was conducted, and male and female zebrafish were fed DEHP at 3 ppm. We used an integrated approach to study the effect of phthalates on the microbiome-gut axis that included both transcriptomics and metagenomics. We identified dysregulation of biological processes in both the host microbiome and gastrointestinal tract, that revealed mechanisms into how phthalate affect a wide array of physiological functions including metabolism, gut integrity and homeostasis, and immune function. We identified associations to phthalate-induced metabolic dysfunction via dysregulation of receptors that are important in lipid metabolism and energy homeostasis. Our results describe for the first time the involvement of adaptive immunity (i.e. helper T cells; Th) in adverse processes including phthalate-induced dysregulation of cell-cell communication, as well as inhibition of cell surface transporters that lead to changes in gut integrity. To understand the effects of phthalates on biological processes and the intestinal immune system, and to reveal interactions between the host gastrointestinal tissue and the microbiome, we utilized several computational approaches and predictive functional models using microbial metagenomics and intestinal transcriptomic data. Our novel approach determined the effect of phthalates on host microbial composition and diversity, and described the impact on microbial production of bioactive molecules (metabolites). The newly identified metabolites have the potency to stimulate intestinal adaptive immunity, which are considered to be key regulators and initiators of several disease states including chronic inflammatory diseases. These data suggest that the microbiome-gut axis is a target for metabolic disruptors and emphasizes the value of multi-omics approaches to study microbiome-host interactions. This research was supported by Marie Skłodowska-Curie actions no.707241.

1.05P.9

The organochlorine pesticide dieldrin affects microbial communities related to heme and selenium biosynthesis in the gastrointestinal system of zebrafish (Danio rerio)

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The gastrointestinal (GI) system is the first line of defense for dietary exposures to environmental contaminants. Long-term ingestion of chemicals can lead to GI pathology and an altered microbiome. This is important as gut dysbiosis is significantly associated with animal and human disease. The organochlorine pesticide dieldrin is a legacy environmental contaminant, accumulating in tissues of organisms. The objectives of this study were to determine how environmentally relevant levels of dieldrin affected gut morphology and its microbiome in zebrafish. Adult male zebrafish, 5 months of age, were fed a measured amount of pelleted feed of either control or dieldrin (measured at 16 ng/g and 163.5 ng/L dry weight). Zebrafish in the three experimental groups did not show any difference in weight gain over 4 months. Body burden levels of zebrafish at the end of the exposure were 10.8 ng/g and 18.32 ng/g wet weight (10x and 20x above background levels). Dietary exposure to dieldrin did not induce any notable gut pathology. The gut content was extracted from males in each group and 16S rRNA sequencing conducted, followed by QIIME pipeline. Interestingly, dieldrin fed to fish at 16 ng/g lowered the abundance of Firmicutes, bacteria involved in energy resorption, and one that is common to many disease states (e.g. diabetes, obesity). Detailed evaluation on class level revealed a decrease in Clostridia, Betaproteobacteria and Verrucomicrobiae species following dieldrin treatment. To understand the adverse outcomes of the microbial shift, we employed computational approaches and predictive functional models to study potential interactions with the host. Our results suggest that dieldrin may affect heme biosynthesis and the levels of porphyrins in host microbiome. Further, we determined that the lower dose of dieldrin affected several selenium related metabolites and selenium metabolism. This study demonstrates that low dose exposure to dieldrin can dysregulate the host microbiome and shift metabolite production that may promote adverse affects in the host.

1.05P.10

Investigation of the influence of pH on the toxicity, uptake and biotransformation of Ibuprofen in zebrafish (Danio rerio) embryos

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Recently, there is a great interest from the scientific community and regulatory authorities in xenobiotic substances (such as pharmaceuticals), since most of them are detected in aquatic ecosystems. Although slight variation in the pH can cause considerable changes in the uptake and the toxicity, until now, the pH-effect has not rigorously been considered in risk assessment. In the light of their potential risk to the aquatic environment and organisms, immediate action is required. Ibuprofen belongs to one of the most important and widely used groups of pharmaceuticals worldwide (nonsteroidal anti-inflammatory drugs (NSAIDs)). It has been detected in numerous environmental water samples, including river waters and waste water treatment plant effluent. Therefore, it is necessary to assess its potentially toxic effects on the aquatic organisms. Zebrafish (*Danio rerio*) has been considered as a well characterized experimental model organism in the fields of molecular genetics and developmental biology. Moreover, it has been characterized as a powerful and promising model organism, since it is widely used in ecotoxicology research studies, as well as for environmental risk assessment.The aim of this study was to assess the influence of different pH values of the test medium on the toxicity of Ibuprofen in zebrafish embryos. Furthermore, the internal concentrations at different pH values as well as the biotransformation processes of ibuprofen in zebrafish were evaluated. Meanwhile the effect of pH values on the occurrence of biotransformation products of ibuprofen were estimated. Also, it was examined if biotransformation data could be used in a complementary way to the internal concentration of the parent compound, for a holistic interpretation of toxicity at different pHs. The overall scope of this study was to estimate the influence of pH to the uptake, potential bioaccumulation, biotransformation and toxicity of ibuprofen in fish embryos. Moreover, the zebrafish embryo toxicity test was used to calculate the LC50 of Ibuprofen at different pH values. Exposure experiments were conducted at three different pH values (5, 6 and 8), to assess potential pH-dependent differences in an environmental relevant pH range. For the analysis of the exposure water samples and the zebrafish extracts, RPLC and HILIC methods were used, in both positive and negative electrospray ionization mode, to cover the widest possible range of polarities, using LC-ESI-QTOF-MS.

1.05P.11

Toxic effects of Mebendazole on zebrafish Danio rerio

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Drug pollution is a problem that is increasing due to its continuous contribution to aquatic systems, so it is important to conduct studies on how these compounds affect aquatic organisms since they are the main targets of these substances. Mebendazole is a nonprescription drug with genotoxic effects and affects glucose synthesis. Because its sequels in fish are not fully known, the objective of this study was to evaluate the toxic effects of Mebendazole on the zebrafish *Danio rerio*. Acute bioassays lasting 96 hours were performed where the fish were exposed to six concentrations of the drug in duplicate (0.5, 2.5, 5, 25, 50 and 250 mg/L), to determine the LC50. Subsequently, a bioassay was carried out with a sublethal concentration (LC1) with a duration of 168 hours, in which the degree of lipoperoxidation (tbars) in gill, the activity of the AchE enzyme in muscle, the concentration of sugars in liver and the micronucleus frequency in peripheral blood were evaluated, The results obtained showed that Mebendazole is toxic to zebrafish, the LC50 calculated at 24, 48 and 72 hours were: 11,482 ± 4.3 mg/L, 9.47 ± 6.2 mg/L and 9.47 ± 6.2 respectively. In the sublethal bioassays it was observed that the degree of lipoperoxidation was significant difference between the exposed organisms and the control group. AchE enzyme activity was 10% lower in fish exposed to the drug. A decrease of up to 70% in sugar levels was detected in organisms exposed to the drug compared to the control group. The frequency of micronuclei in the exposed organisms increased up to 52%. The results show that Mebendazole in sublethal concentrations (< 1 mg/L) has neurotoxic, genotoxic and hypoglycemic effects in zebrafish.

1.05P.12

Effect of cytostatic substances in zebrafish embryos

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Cancer is one of the top causes of death in humans. The currently available treatments depend on the type of cancer and may include cytostatic substances. Methotrexate (MTX) is a cytostatic drug and it has been used for cancer treatment, ectopic pregnancy, and autoimmune disorders. Their presence in water, even though in low concentrations, are of particular interest due to the risk of consumption by drinking water and its potential effects. However, knowledge of its impact on aquatic organisms is still limited. Therefore, this study aimed to evaluate the effects of MTX (0.1 up to 18000 $\mu g.L^{-1}$) on zebrafish (*Danio rerio*) focusing on different biological endpoints (e.g. heartbeat rate, swimming behaviour and biochemical endpoints associated with neurotransmission, antioxidant defences and energy metabolism). Overall, zebrafish embryos demonstrated a high sensitivity to MTX. The obtained results have the same pattern both in heart beat rate and swimming behaviour (e.g. promoting an increase at 0.1-1000 $\mu g.L^{-1}$, however it decreased in the highest tested concentration (18000 $\mu g.L^{-1}$)). Our findings demonstrate that MTX uses similar pathways in humans and zebrafish, once MTX induced disruption in the heart beat and behaviour.

1.05P.13

MS/MS proteomic-based approach in liver of Sheepshead minnow (Cyprinodon variegatus) exposed to perfluorooctanesulfonic acid (PFOS)

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Perfluorooctane sulfonate (PFOS) is a synthetic compound with a hydrophobic chain of 8 carbons with all H atoms replaced by F, bond to a polar sulfonate group. This molecular structure gives this chemical the property to repel both water and oils, and thus be used for protecting the surface of textiles, leather, paper and metals. Despite cessation of PFOS precursor production in 2002, PFOS is frequently detected in organisms from lakes and rivers worldwide. The synthesis of proteins related with stress response, energy metabolism, lipid metabolism, DNA metabolism and thyroid metabolism is known to be altered by PFOS exposure. However, there is a lack of experiments carried out on marine species. Here, we designed an experiment to test the effects of PFOS in the marine fish Sheepshead minnow (*Cyprinodon variegatus*), a model for proteomic and ecotoxicological studies. Two treatments were tested: low (0.5µg/L) and high (5µg/L) PFOS concentrations. After seven-day exposure fish were euthanized and liver samples were taken. We used a labeling mass spectrometry-based proteomic approach MS/MS and the software PEACKS for identification and quantification of peptides and proteins of that tissue. In parallel, the expression of genes CYP1A, PPARD and ER1 was analyzed using qPCR technique in the same organ. Proteomic approach allowed identification of 2553 proteins, 628 of them showing significant changes on their expression levels in the high treatment. These proteins are involved in several processes and pathways like lipid metabolism or general stress response, among others. Our qPCR results highlight a non doses-response behavior, and for this case, we observed that the 18S gene works better as housekeeping gene than the RPL7.

1.05P.14

Exposure to tetramethylbisphenol F (TMBPF) induces developmental effects and disrupts thyroid endocrine systems in zebrafish larvae

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Tetramethylbisphenol F (TMBPF) is widely used as coating epoxies as an alternative compound of bisphenol A (BPA). BPA and its analogues are well-known as endocrine disruptors, however potential adverse effects of TMBPF have not yet been elucidated. In the present study, the developmental effects and changes in expression of the genes related to the hypothalamus-pituitary-thyroid (HPT) axis were investigated in zebrafish embryo/larvae exposed to TMBPF. Zebrafish embryos exposed to the 5 mg/L TMBPF showed an increase in embryo coagulation, leading to death after hatching. Phenotypic observation revealed that exposure to TMBPF caused pericardial edema and bent spines. Exposure to 0.5 mg/L TMBPF resulted in significantly decreased body weight and up-regulated genes related to the HPT axis. Although the effective concentration for endocrine disruption was greater than that of BPA or bisphenol F, the actions of TMBPF on the HPT axis was similar to the effects of BPA exposure. Acknowledgement: This study was supported by National Research Foundation of Korea (NRF; Project no. 2019R1A2C1002712).

1.05P.15

Effects of trabectedin on the development, biochemical markers and DNA damage of Danio rerio embryos

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Although there is a significant increase on consumption and consequent release of anticancer agents (AAs) into the environment, they are one of the least studied groups of pharmaceuticals concerning environmental impacts. AAs are classified according to their MoA and are biologically active compounds that interact with a target molecule and though encountered at low concentrations in the environment (ng/L to Ÿg/L), may promote toxicity through different mechanisms or affect organisms with the same target molecule. Although, most AAs are well described in terms of human health effects, new mechanisms of action have been claimed, as well as new properties, e.g. alterations of host s microbial activity and community structure. The aim of the current study is to assess the effects of trabectedin, a cytotoxic AA indicated to treat patients with advanced soft tissue sarcoma, to a non-target aquatic species, the zebrafish Danio rerio, using an integrative approach through diverse and sensitive endpoints that are disregarded by pharmaceutical environmental risk assessment. Early-life and adult stages of the model aquatic species Danio rerio were exposed to trabectedin at different concentrations (0.1 µg/L from 50 µg/L) according to international standards to assess lethal and sublethal endpoints (alterations on developmental, behavioral and biochemical as well as DNA damage). Preliminary results indicated high toxicity of trabectedin to D. rerio larvae where, after 96 h of exposure, the LC50 was 66.9 µg/L while the EC50, based on malformations (e.g. pericardial edema, tail deformities), was 48.9 µg/L. At a biochemical level, the exposure of D. rerio to 50 µg/L of trabectedin increased catalase activity, decreased cholinesterases, glutathione-S-transferase, and lactate dehydrogenase activity, while glutathione reductase activity was not affected. DNA damage to the D. rerio larvae with no developmental malformations was also registered at low concentrations of trabectedin. These effects at such low concentrations hint the high probability of severe damage when sensitive and related to AAs' MoA endpoints will be used and then provide robust information for use in regulatory risk assessment.

1.05P.16

Functional benchmark dose (BMD) approach in the study of the dose-response transcriptomic effects exerted by tributyltin (TBT) in zebrafish eleutheroembryos

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Exposure to tributyltin (TBT), used as antifouling for decades, has been related to imposex in mollusks and to obesogenicity and masculinization in fish, although the molecular mechanisms underlying these effects are not fully characterized yet. This study presents a dose-response transcriptomic analysis in zebrafish eleutheroembryos exposed to 3-100 nM of TBT from 2 to 5 days post fertilization. Benchmark dose analysis stablished a transcriptomic PoD (point of departure) of 16.5 nM, similar to the one found at the metabolomic level (PoD = 20.5 nM). Both of them were about one order of magnitude lower than the one found at the morphometric level (PoD = 121.0) and the estimated median lethal concentration (LC50, 166.9 nM). In the performed analysis per pathway, steroid metabolism appeared as the physiological function most affected at low concentrations of TBT (< 50% PoD), whereas transcripts related to general stress, developmental delay, cell cycle and DNA damage became affected only at TBT concentrations two to five-fold above the PoD. Cholesterol and vitamin D3 biosynthesis pathway appeared among the most TBT-sensitive disrupted ones, which could explain the endocrine-disruption effects of TBT. Lipid-related pathways only were affected at high concentrations. These results suggest a relatively narrow TBT concentration margin between sublethal, endocrine-disrupting effects found and its general toxicity, compared with the usual margin of other typical endocrine disruptors. Indeed, the application of the benchmark dose approach at the functional level, rather than specific differentially expressed genes observations, facilitates the elucidation of the toxic mode of action of TBT and the corresponding molecular initiation event (MIE).

1.05P.18

Ecological risk assessment of bisphenol S and bisphenol SIP on freshwater organisms

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Considering its various application and endocrine disruption, bisphenol A (BPA) alternatives such as bisphenol S (BPS) and bisphenol SIP (BPSIP) have been used to replace BPA in consumer products. However, research on the chronic toxicity and ecological risk of BPA alternatives is extremely limited. In this study, chronic toxicity of BPS and BPSIP were investigated using Daphnia magna (21 d), Moina macrocopa (7 d), and Danio rerio (21 d). The predicted no-effect concentration (PNEC) of BPA, BPS, and BPSIP were derived by the assessment factor (AF) method and the species sensitivity distribution (SSD) method. Finally, an ecological risk assessment was evaluated by estimating hazard quotient (HQ) based on the measured environmental concentrations (MECs) in surface waters and the derived PNECs. The chronic NOEC of BPS in D. magna , M. macrocopa, and D. rerio was generally lower than that of BPSIP. PNEC values derived using the AF approach was one order of magnitude less than the PNECSSD. The HQ of BPA and BPS exceeded 1, suggesting that potential ecological effects at highly contaminated sites cannot be ruled out. Potential long-term consequences and ecological risk of BPA alternatives in the water environment deserve further investigation. Acknowledgement: This study was supported by National Research Foundation of Korea (NRF; Project no. 2019R1A2C1002712).

1.05P.19

Ecotoxicological screening of water extracts from River Aconcagua (Chile) using Danio rerio embryos - Teratogenic and behavioural effects of environmental chemical mixtures

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Chemicals are omnipresent in the aquatic environment as mixtures and their effects are affecting the ecological balance of aquatic ecosystems. The toxicity of chemical mixtures is particularly problematic because scientific evidence shows that the toxicity of a mixture exceeds the toxicity of each individual compound. The fish embryo test (FET) using *Danio rerio* represent a good surrogate for acute toxicity fish testing and for the characterization of mode of actions. The River Aconcagua is a catchment with increasing competition for water between agriculture, urban development, hydroelectricity and mining. A large-scale copper mine, is located in the headwaters. Downstream, the central valley supports intensive agriculture (including avocado and grapes), while several urban areas are located throughout the catchment. In the present study, we applied a modified *in vivo* assay with *Danio rerio* to test teratogenic and behavioural effects of water samples from River Aconcagua. *Danio rerio* embryos were exposed to several dilutions of water extracts collected in three different locations using an on-site large volume solid phase extraction device. Testing was conducted by following the OECD 236 for 96h with few modifications. We registered abnormal malformations and impairments in the behaviour at two different time points (24 and 96h post fertilization). Furthermore, the effect on the spontaneous tail coiling (STC) and swimming activity in a dark/light transition test were recorded and the activity of the acetylcholisterase (AChE) and the total proteins after 4 days were measured. Results showed adverse effect of water extracts to fish species with a calculated LC_{50} of 12, 16 and 19. Strong teratogenic effects and malformations (i.e. yolk, bended spine) were observed at concentration near the LC_{50}. While hyperactivity at 24h was observed only in one location at low concentration. All samples showed a general decrease of the STC at concentrations closer to the calculated LC_{10}. A strong decrease in the swimming activity (40%) at 96 hpf was observed only in one location. No effect was registered for the AChE and total proteins. Our study showed potential problems in the behaviour of *Danio rerio* embryos at environmental relevant concentrations, which may influence the fitness of native fish species. Further analyses need to be conducted to elucidate and prioritize specific causative chemicals (i.e. neuroactive compounds) responsible of the observed effect.

1.05P.20

Environmental effects assessment using in situ fish embryo testing

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Recent research has generated ample evidence that exposure to low concentrations of organic pollutants cause deleterious effects on early life stages of marine fish. Effects observed in developing larvae after short term exposure during embryogenesis are pericardial oedema, jaw and craniofacial deformations, yolk sac oedema, lack of pigmentation, small eyes and spinal curvatures. In controlled laboratory studies, these effects are even observed after exposure to very low exposure concentrations. Importantly however, the potential for these effects to occur in the native populations following exposure to complex chemical load from anthropogenic and industrial effluents remains unknown. Marine organisms, like blue mussels, have been widely used for environmental monitoring, however, they are less sensitive than fish embryos to toxicant exposure. Fish eggs have very rarely been applied for the purpose of environmental monitoring. Early life stages of the lumpsucker (*Cyclopterus lumpus*) have properties that are highly favourable for field-applications. Their hard chorion makes them robust to mechanical stress, and their high lipid content facilitates accumulation of pollutants from surrounding water. Lumpsucker is widely distributed in the Northern Atlantic Ocean, and spawning lumpsuckers are available almost year around, so they are flexible in terms of timing for field-application. As they develop over a relatively long period of time (300 day-degrees), this enables the ability to keep them placed in the field for several weeks. Shortly after fertilization, lumpsucker eggs are sticky and can be moulded into monolayers of eggs. Such monolayers are very stable over time and have shown >95% hatching success in controlled laboratory experiments. In total, lumpsucker eggs have a huge potential to be used as a model fish egg for field studies. This project has developed technologies and strategies to enable the use of fish ELS for the purpose of environmental monitoring of anthropogenic stressors in the marine environment. A prototype container for housing fish eggs in the sea over periods of several weeks have been designed and successfully implemented. After collection of exposed embryos, a battery of techniques to study effects of exposure, including highly sensitive, high-resolution analytical techniques to measure bioaccumulating contaminants, and several toxicological endpoints have been applied.

1.05P.21

O. melastigma as a marine alternative to the freshwater model species D. rerio for fish embryo toxicity

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In the field of freshwater ecotoxicology, several laboratory model fish species such as the zebrafish have been established, while the marine research basically lacks standardized models. *O. melastigma* (marine medaka) is emerging as a potential alternative laboratory model species for the brackish and marine environment, as it is easy to breed in laboratory conditions, highly tolerant to external factors including temperature and salinity, and has a short generation time. Also with respect to early life stage (ELS) testing and omic technologies several advantages including continuous spawning throughout the year, a transparent chorion as well the assembly and annotation of the entire genome promote the usage of this species as a marine counterpart to the zebrafish. Against this background, the current presentation describes adaptions of classical ecotoxicological methods standardized for zebrafish ELS testing using *O. melastigma*. Besides acute fish embryotoxicity (FET), biomarker protocols (EROD, AChE, CAT) were successfully established and validated using positive control substances (benzo[a]pyrene, paraoxon-methyl). Basal biomarker responses as well as optimum measurement time windows during early development were elaborated. A high sensitivity towards exposure to positive control substances was observed in late pre-hatching developmental stages (6 -8 days post fertilization). Furthermore, first results on the application of the marine model in the field of oil spill research were compared to effects observed in zebrafish embryos from experiments generated in the same laboratory using comparable experimental setups and identical crude oil samples. The acute toxicity of low energy water-accommodated fraction from a naphthenic crude oil was lower for marine medaka than for zebrafish embryos. However, phenotypical endpoints with cardiotoxicity as the main effect were conserved across both marine species. The results demonstrate the importance of including marine species for a reliable risk assessment. Additional endpoints for ELS testing including swimming behaviour will be established. Overall, current results indicate that *O. melastigma* can be a useful marine alternative to zebrafish. While already established in several Asian regions, the marine medaka can be promoted as a model species also in Europe.

1.05P.22

Biochemical and behavioural effects of SiO2 nanoparticles on Danio rerio embryos

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As the interest and progress in the field of nanotechnology deepens, new nanomaterials are being launched as an integral part of commercial products or innovative industrial methods. SiO2 nanoparticles are one example of nanomaterials that have been employed in numerous applications over the years, ranging from food products to adsorbents of organic pollutants and metal ions in aqueous media. As a result of their use in a vast number of applications, their release in large amounts may be expected (e.g. through industrial effluents). Furthermore, their use in environmental applications may pose as a risk if the potential toxicity of SiO2 nanoparticles is not understood in depth. Thus, it is of paramount importance to evaluate the effects of these type of nanomaterials in aquatic organisms and to assess the potential risks to the aquatic ecosystems. This study aimed to evaluate the size and surface charge-related effects of SiO2 nanoparticles on Danio rerio embryos focusing on biochemical and behaviour endpoints.

1.05P.23

Ecotoxicological effects of a "smart" engineered nanomaterial in zebrafish

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Layered Double Hydroxides (LDH) are anionic nanoclays, composed by positively-charged layers with divalent or trivalent metal cations (e.g. Zn^{2+} or Al^{3+}) and stabilized by anions (e.g. nitrates) and water molecules in the inter-layers. LDH have a remarkable capacity to control the release of their anions in the presence of specific triggers (e.g. pH changes), high stability, specific surface area, layered structure and ability to swell in water. LDH have been regarded as â_smart” engineered nanomaterials and lately received great interest in multiple areas, such as medicine (e.g. scaffold), pharmaceutical (e.g. drug delivery) and industry (e.g. anti-corrosive and anti-fouling coatings), among others. Previous studies showed that these nanomaterials may cause low toxicity to humans, mammals or marine organisms. However, ecotoxicological effects on freshwater organisms are scarcely studied, which is critical for regulatory purposes. The zebrafish *Danio rerio* is a well-established freshwater model organism that is widely-used in toxicological and ecotoxicological assessment of a wide range of substances, e.g. pharmaceuticals, nanomaterials. The present study aimed to assess the short- and long-term effects of Zn-Al LDH in *Danio rerio.Danio rerio* eggs were exposed to Zn-Al LDH to assess developmental, behavioral, biochemical and molecular (at DNA level) alterations. Eggs were exposed to Zn-Al LDH according to the OECD 236 FET protocol, where mortality and malformations were documented daily during 96h. For the behavioral assay, embryos were exposed to Zn-Al LDH sub-lethal concentrations for 120h and locomotory activity of larvae tracked using the Zebrabox (Viewpoint, France). Following the same principle, eggs were exposed to sublethal Zn-Al LDH concentrations and neurotoxicity and oxidative-stress related biomarkers, as well as DNA damage (comet assay), were assessed at the end of 96h. Exposure concentrations ranged from 0.01 to 1200 mg/L.Results after 96h showed that the no observed effect concentration (in terms of mortality) was 415.2 mg/L, while the LC_{50} was 559.9 mg/L. LDH caused malformations in *D. rerio* embryos, such as pericardial edema, yolk sac absorption and tail deformities with an EC_{50}value of 172.4 mg/L after 96h of exposure. In terms of behavior, LDH alters locomotor activity even at levels that cause no developmental effects (143.7 mg/L). The results obtained in this study suggest that Zn-Al LDH is of low toxicity towards zebrafish embryos.

1.05P.24

Genotoxic and biochemical effects of the insecticides imidacloprid and λ-cyhalothrin, alone and mixed, in Danio rerio embryos

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Currently, Brazil is the largest consumer of pesticides in the world, among them the pyrethroid λ-cyhalothrin (λ-CL) and the neonicotinoid insecticide imidacloprid (IMI). These insecticides have been used in agricultural areas, where their mixtures are constantly applied to improve efficacy and reduce cost. However, the use of this mixture may result in combined effects on various organisms, as pesticides may reach aquatic ecosystems and promote impacts at different levels of biological organization, from the molecular level to the population and community structure. Fish represent a very important group in toxicity studies because they are in direct contact with contaminants present in water. A widely used model is the *Danio rerio* embryo, due to its rapid development and easy maintenance, being an ideal fish model for this study. Thus, the goal of this work was to evaluate the effects of λ-CL (0.15 μg L^{-1}), IMI (300 μg L^{-1}), and the mixture (λ-CL + IMI), on genotoxic (DNA damage) and biochemical (biotransformation enzyme, oxidative stress parameters and acetylcholinesterase activity) biomarkers in embryos of *D. rerio*. Embryos were exposed to the different treatments or only to water (CTR) during 96 h in 90 x 15 mm petri dishes with 50 embryos per petri and eight replicates per experimental group. After exposure, the embryos were homogenized in K phosphate buffer (0.1M and pH 7.5). Cells of embryos were used for the comet assay, for the determination of lipid peroxidation (LPO), catalase activity (CAT), glutathione S-transferase activity (GST) and acetylcholinesterase activity (AChE). Scores of DNA damage significantly higher were observed in IMI group (153.38 ± 7.96 n = 8) in relation to CTR group (122.25 ± 3.18 n = 8). In relation to biochemical biomarkers (GST, LPO, CAT and AChE) no significant variations were observed among the treatments tested. These results show that imidacloprid insecticide may be more harmful than λ-cyhalothrin because it was able to promote genotoxic damage in *D. rerio* embryos.

1.05P.25

Alternative methods to assess the effects of a realistic Persistent Organic Pollutant mixture (POP Mix) following a chronic exposure in zebrafish larvae (Danio rerio).

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There is a growing concern related to the so-called Persistent Organic Pollutants (POPs). These compounds originate from different sources, some of them being synthesized with specific purposes and others just randomly appearing as by-products (e.g. incomplete combustion). The research into POPs has shown that they have several adverse effects. Cancer, metabolic disorders, effects in the nervous system have been described amongst many other adverse health effects after POP exposure. The POPs have been generally studied as single/individual compounds, and data is scarce related to mixtures that are not uncommon in the environment and even within humans. Thus, a realistic-environmentally significant Persistent Organic Pollutant mixture (POP Mix) as found in the serum of a Scandinavian population was tested in zebrafish larvae (*Danio rerio*). The zebrafish has been commonly used in the toxicology field, mainly for toxicity tests where the endpoint is lethality. In this study, a set of different approaches was used to assess the effects of a POP mix exposure on zebrafish larvae after 96 hours post-fertilization. In our scope, we mainly focused in endpoints such as development of the lateral line system, malformations, etc. Our results suggest that there are no major effects on the development of the lateral line at any of the different concentrations tested. However, some other morphological traits were undergoing deficiencies during development, such as edemas (pericardial or yolk sac). Thus, we have proven that the POP mix has some deleterious effects at realistic concentrations and further analysis is required.

1.05P.26

Zebrafish embryos for interspecies prediction of toxicity of alkoxy/alkyl alcohols and valproic acid by application of the adverse outcome pathway concept

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A way to innovate human health and environmental hazard and risk assessment of chemicals, in line with the 3R principles, is to develop methods that compare toxicological effects among and across vertebrate species. For this purpose the Adverse Outcome Pathway (AOP) concept is applied, assuming that the same compound will invoke the same or a similar molecular initiating events regardless of species involved. Although pathways, including apical adverse outcomes (AOs), may diverge in a species-dependent way further downstream, early key events (KEs) may still predict these AOs correctly. The zebrafish embryo (ZFE) can be assumed to represent these KEs in early development-related AOPs in vertebrates, because these developmental processes are well conserved. To explore the potential of ZFE as a predictive model for mammalian developmental toxicity we have investigated the toxicokinetics in ZFE for a range of alcohols and their respective transformation products, carboxylic acids, and measured early key events and apical endpoints in AOPs, as defined for rodent models. Developmental toxicity of alkoxy acids was identified in ZFE comparable to mammalian species, and alkoxy ethanols were significantly less potent to induce developmental effects than their corresponding metabolites. Similarly, effects in ZFE also appeared indicative for chronic toxicity in adult fish. Toxicokinetic analysis indicated a low metabolic capacity of ZFE compared to adult fish and mammalian species. Marker gene expression analysis in ZFE after exposure to a set of 12 test compounds confirmed regulation of the molecular pathways similar to rodents. Marker gene expression analysis in the ZFE thus appears to be a useful tool to confirm early key events along a defined AOP, to contribute prediction of developmental effects in other species.

1.05P.27

Thyroid hormone disruption of two UV filters (EHMC and BP-3) in embryo-larval and adult zebrafish

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Increased use of UV filters including 2-ethylhexyl 4-methoxycinnamate (EHMC) and 2-hydroxy-4-methoxybenzophenone (BP-3) leads to their widespread occurrences in the aquatic environment worldwide. While there is laboratory evidence that indicating EHMC and BP-3 may alter sex hormonal activities, thyroid hormone disruption potential of these chemicals in aquatic organisms is not well characterized. In this study, we investigated thyroid disrupting effects of EHMC and BP-3 using the embryo-larval and adult zebrafish. The zebrafish embryos were exposed to series of concentrations of EHMC (1-30 μM) or BP-3 (0.14-1.40 μM) for 6 days. Embryolarval survival, hatchability, malformation rate, and transcriptional changes of thyroid regulating genes were observed. In addition, adult male zebrafish were exposed to 1-30 μM of EHMC or 0.14-4.40 μM BP-3 for 21 days, and transcriptional changes of hypothalamic-pituitary-thyroid (HPT) axis and thyroid hormone metabolism (liver) genes were quantified. Exposure to EHMC or BP-3 significantly altered thyroid hormone regulation of both zebrafish larvae and adults. Following exposure, embryolarval survival, hatchability and malformation rate were not influenced, except for decreased hatchability at 1.40 μM of BP-3. Following exposure to EHMC, significant down-regulations of *tshb* (≥10 μM) and *sult1 st5* (≥10 μM), and *ttr* (≥3 μM) genes were observed in zebrafish larvae. Following exposure to BP-3, significant up-regulation of *tg*, *ugt1ab* and *dio1* genes at 1.40 μM, and increased up-regulation of *tshb*, *tpo*, *nis*, *pax8*, *dio2* and *ttr* genes were observed in zebrafish larvae. In adult male zebrafish, T3 hormone level was decreased along with significant down-regulation of *tra* and *trb* genes in brain, *tshr*, *tg*, *nis*, *tpo*, *dio1*, *dio2* genes in thyroid, and *dio1* and *dio2* genes in liver after exposure to EHMC. Exposure to BP-3 also significantly lowered T3 levels of adult male zebrafish, however, different pattern of gene regulation were observed. These changes included dose-dependent up-regulation of *tshb*, *trh*, *nkx2.1*, *tra* genes in brain, *tshr*, *tg*, *nis*, *tpo*, *dio1*, *dio2* genes in thyroid, which indicates negative feedback mechanisms. Our observations clearly demonstrate that exposure to two commonly used sunscreen chemicals could disrupt normal thyroid function toward decreaed thyroid hormones but through different modes of action. Further studies are needed to confirm different modes of thyroid disruption by these frequently used sunscreens and related ecotoxicological consequences.

1.05P.28

Determination of mixture neurotoxicity using the hyper- and hypoactivity behavior of zebrafish embryo in the spontaneous tail coiling test

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Recently, there has been an increase in the number of people suffering from neurological diseases and incidents of nervous system-related diseases are increasingly associated with exposure to pesticides and pharmaceuticals. These chemicals interfere with the functioning of the nervous system and very little disruptions can lead to long term neurological diseases such as Parkinson and autism. Generally, chemicals occur in the environment as mixtures and it is therefore more realistic to assess the toxicity of chemical mixtures rather than single chemicals. To detect mixture neurotoxicity, it is important to be able to differentiate neuroactive mode of action for single chemicals within a mixture of other chemicals. Even more important is to investigate the additive, synergistic and antagonistic effects of neuroactive chemical mixtures within a pool of other substances. Zebrafish embryo is a suitable tool to test mixture effects. While only few studies have studied behavioral effects of mixtures, most studies on zebrafish embryo were based on lethal and sublethal endpoints. Since behavioral endpoints might be more sensitive to detect neuroactive substances and also more relevant for ecological effects, then it is probably more reasonable to encourage the use of behavioral tests for screening neuroactive chemical mixtures. In this study, we employed the use of the spontaneous tail coiling (STC) of the zebrafish embryo to detect the effect of both single and mixed neuroactive substances. We addressed the questions: 1.) Can the STC test predict the mode of action of neuroactive substances i.e. acetylcholinesterase inhibition by chlorpyrifos or activation of Gamma aminobutyric acid receptor by abamectin. 2.) What is the resulting effect of a mixture of neuroactive substances with similar and dissimilar mode of action? First results showed that chlorpyrifos and abamectin caused hyperactivity ($EC50$ = 2.57μM) and hypoactivity ($EC50$ = 0.54μM) of the STC respectively and this was assumed to be indicative of their mode of action in zebrafish embryo. The results for similar and dissimilar mixtures with varying mixture approaches predicted by concentration addition and independent action models will be presented.

1.05P.29

Quantitative histological validation of a semi-quantitative grading system of fish gonadal maturation usable for reproductive toxicology - Space for improvement?

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In fish reproductive toxicology it is often needed to evaluate the maturation degree of the gonads and how toxicants may disturb it. Yet, there is no universal way of doing it. Here, we use adult comet-tailed goldfish (*Carassius auratus*) to evaluate the strength of an OECD recommended (but not widely adopted) semi-quantitative gonadal histology-based maturation system [1], by validating this convenient method quantitatively. Injections with a luteinizing hormone-releasing hormone analogue, combined with a dopamine antagonist pimozide, in a saline solution that was injected (i.m.), was used to induce maturation in some fish. Procedures were put in place under anaesthesia. Animals were sacrificed with an overdose of anaesthetic and gonads were fixed in Bouin fluid (24 hours). The pieces were processed, first dehydrated in alcohol then cleared with xylene, and finally infiltrated with paraffin. For histological screening and quantitative computer-assisted stereology and image analysis, serial 4 µm thick sections were made. We began by analysing the ovaries and then extending the approach to the testes. We have confirmed that the ovarian maturation progression can be monitored and categorised as a whole using the OECD grading system. We could rate ovaries from stage 0 (undeveloped) to stage 4 (late development / hydrated). However, as the application of the criteria is based on the perceived relative abundance of the types of follicles, there is an ambivalence in assigning grades to certain gonads. This unquantified ambiguity may lead to biases when comparing fish groups. The semi-quantitative grading biases can be enhanced by stereology. The latter can estimate the relative volume compartments of the oocyte groups and even their average size-related variance, which should create better boundaries (cut-offs) between grades. The relative sperm volume, germ epithelium and lumen for the testis correspond to what is proposed in the OECD grading system. Overall, our quantitative approach suggests that every ovary and testis stage has a unique average stereological signature. Our study mostly supports the use and corroborates the stages defined in the OECD grading system, despite minor caveats, and offers new facts that can be used to adapt the systems and make it more precise and of universal use. Future work could focus on refinements and automation. [1] Johnson R et al. Guidance Document 123 (OECD) (2009), 1-114.
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1.05P.30

Energy-budget modelling for early-life stages of Atlantic cod and haddock

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The early life stages of organisms are of considerable interest in ecotoxicology as they constitute a vital aspect of population dynamics and often display considerable sensitivity towards toxicants. Furthermore, embryo and early-life stage toxicity tests for vertebrates (i.e., fish and amphibians) are increasingly being suggested as alternatives for testing with the later (and legally-protected) life stages. To interpret the patterns of effects observed in toxicity tests, and to extrapolate such results to field conditions, requires mechanistic models. Such models should consider both the toxicokinetic (TK) and toxicodynamic aspects (TD) of toxicity. Substantial research efforts are currently concentrating on the molecular level. However, molecular-level approaches need to be combined with energy-budget models to allow for a causal link between exposure and the whole-organism life-history traits (as represented by AoPs). This combination is necessary as life-history traits are not independent, but connected through the energy budget (e.g., structural growth and development are causally linked to the available yolk and to respiration rates), and feedbacks at this level cannot be explained from the molecular level up. In this contribution, we report on our progress in applying the simple DEBkiss model to yolk-feeding stages of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). The model was previously calibrated on literature data for cod, and we now compare the calibrated model to extensive data on growth, development and respiration gathered in the DiTail project (https://ditail.no/en/). Experiments were performed in presence of stressors (mine tailings and copper). However, the stress levels were apparently too low to produce sufficient effects on the biometry to allow modelling, and therefore this presentation will focus on the control responses. In a later stage of the project, we will use our model to interpret toxicity data and to investigate the links between molecular-level data and the energy budget.

1.05P.31
MEOGRT - A Practical Experience

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The Medaka Extended One Generation Reproduction Test (MEOGRT)[1] is a standard OECD Test Guideline (No. 240) using multiple generations of fish to generate data relevant to ecological hazard and risk assessment of chemicals. The test evaluates population relevant parameters, such as survival, gross development, growth and reproduction but also includes endpoints which may provide evidence of endocrine disrupting properties of a chemical such as measuring vitellogenin (Vtg), phenotypic secondary sex characteristics (SSC) and histopathology. The MEOGRT guideline has been designed for use with the Japanese medaka (*Oryzias latipes*) and was issued in 2015. The small size, relatively short life-cycle, easily identifiable phenotypic secondary sex characteristics and genetic sex determination makes *O. latipes* a suitable species for laboratory testing. However, the test is highly complex with several, highly stringent control validity criteria. Although multigenerational validation tests were conducted by several laboratories prior to the guideline being issued, the finalised guideline was not subject to ring-testing. Previous publications[2] suggest that not all validity criteria were achieved in the initial validation tests. The test requires the use of nearly 2000 fish and can cost over half a million euros therefore the risk of having to repeat studies due to failure to achieve the control validity criteria not only has financial implications but also animal welfare issues. This presentation compares experimental control data from two MEOGRT studies and looks at practical modifications to achieve control validity criteria and implement improved animal welfare. [1] OECD Guideline for the Testing of Chemicals Medaka Extended One Generation Reproduction Test (MEOGRT) OECD240 Adopted: 28 July 2015 [2] Salinas ER, Weltje L. 2018. In: Abstract book, SETAC Europe 28th Annual Meeting; 2018 May 13-17; Rome, Italy, Pensacola (FL); SETAC. 495 p.

1.05P.32
Ecotoxicological assessment of fish reproduction: an automated methodology for egg counting and viability evaluation - case study using zebrafish eggs

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Fish are excellent experimental models for toxicological research, and fish have become the major vertebrate models in aquatic ecotoxicology. Research in fish ecotoxicology has been conducted mainly under acute exposures, highlighting effects on survival and growth, whereas the effects on reproduction have been scarcely studied. Reproduction tests, such as OECD guideline N229, highlight the effects of chemicals on egg production, fecundity and viability. However, this requires the daily counting of eggs and further assessment of their viability, which constitutes a laborious and exhaustive work, that is obviously subject to human error. The aim of this work is to present an automated methodology to evaluate fish reproduction, providing total eggs and discriminated egg counting; meaning counting and differentiation of i) unfertilized or underdeveloped egg, ii) fertilized "good" eggs, and iii) coagulated eggs. Total eggs and counting differentiation were achieved using the electronic device D-counter (WO2016067202A1). The discrimination is achieved based on the light absorbance level detected in every single event. An event is considered as such when the passing of a particle through the serialization component match the criterion defined for the particle under study – the zebrafish egg. Every single egg is treated as single event. Results obtained in the automated counting and discrimination registered different patterns based on the light absorbance registered for every single egg/event, allowing to discern three egg viability stages. The automated methodology can be applied in ecotoxicology assessment of fish reproduction providing egg counting and viability evaluation by discriminating and counting separately fertile and not fertile eggs. The methodology could also be applied in other fish reproduction studies such as in aquaculture (eg. maternities).

Human and Environmental Lipid Disruptors (P)

1.06P.1
Effects of crude oil on lipid composition in calanoid copepods terminating diapause

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Calanoid copepods are important zooplankton species in the North Atlantic and Arctic Oceans, primarily due to their role in transferring nutritious lipids from phytoplankton to higher trophic levels. Their presence in surface waters is seasonal, as most of the juvenile stage C5 enter a dormant state (diapause) before the final molt into adulthood. Diapause lasts for several months, and termination of this state may depend on endogenous changes in lipid content or composition. In a recent study, we showed that expression of genes related to lipid metabolism was affected in calanoid copepods exposed to the water-soluble fraction of a crude oil while terminating diapause. Specifically, petrogenic oil exposure resulted in temporary pause in lipid catabolism in the copepods, an effect which was not observed in the control copepods. In the present study, we aim to address whether the composition of lipids is affected by petrogenic oil exposure during diapause termination. We collected copepods in diapause in a Norwegian fjord and subsequently exposed a subset of these to the water-soluble fraction of a North Sea crude oil for five days, followed by a recovery period of two weeks. Copepods were sampled and analyzed for lipid content and composition at four time points during the exposure experiment. We analyzed lipid classes and fatty acid composition using GC-MS in WSF-exposed and control copepods. Our main hypothesis is that we will observe differences in total lipid content that reflect our previous observations, i.e. that the lipid content in exposed copepods will be higher than in control copepods sampled at the same time. We will also assess whether the oil exposure will affect the fatty acid composition in the copepods.

1.06P.2
The use of zebrafish liver cells (ZFL) to characterize and predict the effect of lipid disrupters

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There is increasing awareness that exposure to endocrine disrupters interfere with lipid homeostasis in vertebrates, including fish. Many of these compounds exert their action by binding to nuclear receptors, such as peroxisome proliferator-activated receptors (PPARs) and retinoid X receptors (RXRs). This work investigates the use of zebrafish liver cells (ZFL cells) as an alternative in-vitro method for the detection of lipid disrupters in the aquatic environment by assessing changes in the cell's lipidome after exposure to the model compounds, tributyltin chloride (TBT), and *all*-trans retinoic acid (ATRA). TBT is a ligand of RXR and PPARɣ, while ATRA is an endogenous and physiological ligand of RARs. ZFL cells were exposed to TBT (50-200 nM) and ATRA (1 µM) for 24h, and lipid extracts analyzed by FIA-ESI(+/-) Orbitrap. This methodology allowed the identification and quantification of about 120 different lipid species and the detection of a concentration-dependent accumulation of triglycerides (TG) and diglycerides (DG) in ZFL cells exposed to TBT. At the highest concentration tested (200 nM TBT), a decrease of plasmenyl/plasmanyl phosphatidylcholines was observed and associated to oxidative stress. In contrast, exposure to ATRA led to an accumulation of TGs with no alteration of DGs. According to the observed lipidic fingerprints, both TBT and ATRA up-regulated the expression of markers of lipid synthesis (*fasn, scd, elovl6*), while ATRA down-regulated *dgat1a*. Retinoic metabolism related genes (*cyp26b1*, *lrata*) were highly up-regulated by both TBT and ATRA. Overall, ZFL cells showed comparable sensitivity and responses to those previously reported in mammalian models. These results support the use of ZFL cells in combination with FIA-ESI(+/-) Orbitrap analysis as a robust approach for the screening of lipid and metabolic disrupters in fish.

1.06P.3
Three-dimensional (3D) brown trout primary hepatocyte model for assessing 5α-dihydrotestosterone effects on lipidic target genes
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The use of *in vitro* 3D fish models in ecotoxicology is limited, despite the recognized advantages of these systems regarding the maintenance of *in vivo* specific tissue architecture/functions. Sex steroid hormones, particularly estrogens, are known to display pivotal regulatory roles in lipid metabolism, with consequences for hepatic energy homeostasis. The androgenic effects in fish lipidic pathways are comparatively less studied. Thus, the aims of this study were to: 1) establish a novel 3D primary hepatocyte spheroid model from juvenile brown trout (*Salmo trutta* f. *fario*); 2) apply the 3D system for testing the effects of a model non-aromatizable androgen, 5α-dihydrotestosterone (DHT), on the expression of a selection of lipidic targets (acyl-coenzyme A oxidase 1-3I – Acox1-3I; peroxisome proliferator-activated receptor gamma – PPARγ; acyl-CoA long chain synthetase 1 – Acsl1; apolipoprotein AI – ApoAI and fatty acid binding protein 1 – Fabp1). After primary hepatocyte isolation, the optimization of culture conditions was carried out by using a multiparametric characterization of the spheroids (cellular viability, biometry and morphology). Then, the spheroids were exposed to DHT (10 and 100 μM) between the 14^{th} and 18^{th} days in culture. Relative mRNA levels were assessed by performing quantitative real-time polymerase chain reaction (RT-qPCR). The optimal medium was selected as Dulbecco's modified eagle medium/nutrient mixture F-12 medium (DMEM/F-12) with 10% (v/v) fetal bovine serum (FBS), 15 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 10 mL/L of antibiotic/antimycotic solution at 18 ºC, without additional supply of O_2/CO_2, and at a constant agitation of ± 100 rpm. Morphological stabilization of the spheroids occurred from the 14–16^{th} days onwards, with a median equivalent diameter of 217 μm. The spheroids remained viable for 30 days in culture. A down-regulation was observed for Acox1-3I and PPARγ mRNA levels, following 100 μM of DHT exposure. On the contrary, Acsl1 and Fabp1 mRNA levels were up-regulated at the highest DHT dose, and the ApoAI levels were not altered. The present data reinforces the importance of the study of androgens' impacts in fish lipidic pathways, and to infer their physiological consequences. The developed 3D model is suitable for metabolic, biochemical and genetic studies, with potential for its application in ecotoxicological testing. Funding: Supported by FCT and ERDF (UID/Multi/04423/2019) and ICBAS.

Neuroendocrine and Neurochemical Disruption in Vertebrate and Invertebrate Models (P)

1.07P.1
Alteration of neuro-dopamine and steroid hormone homeostasis in wild Bank voles in relation to PFASs exposure at a Nordic skiing area
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Perfluoroalkyl substances (PFASs) are persistent anthropogenic chemicals in the environment that are applied in a wide range of consumer products, including ski products. Recently, we reported higher PFAS concentrations in Bank voles (*Myodes glareolus*) from a skiing area in Trondheim, Norway, compared to a reference area, suggesting that these PFASs are, most likely, derived from skiing products. The present study investigated the neuro-dopamine and cellular steroid hormone homeostasis of Bank voles from a Nordic skiing area, in relation to tissue and environmental concentrations of PFASs. We performed biochemical (dopamine metabolism), molecular (gene expression) and physiological (steroid hormone) analysis using standard techniques. We show that brain dopamine levels were significantly higher in Bank voles from the skiing area, compared to a reference area with no skiing activities and low PFAS concentrations. Dopamine metabolites (3,4-dihydroxyphenylacetic acid: DOPAC and homovanillic acid: HVA), were lower in voles from the skiing area, compared to the reference area. The ratios between dopamine and its metabolites is normally used as a measure of dopamine turnover. The DOPAC/dopamine ratios were significantly lower in voles from the skiing area, compared to the reference area, indicating lower dopamine turnover in the areas with high PFAS levels. PFASs could possibly alter the intra-neuronal metabolism of dopamine to DOPAC via monoamine oxidase (MAO), leading to lower MAO activity. It has been shown previously that the dopaminergic homeostasis can indirectly regulate cellular oestrogen (E2) and testosterone (T) biosynthesis. In line with this, we observed lower T levels (albeit not significant) in male voles from the skiing area. In contrast, there were no significant differences in hormone levels in female voles. In summary, the dopaminergic system is an intricate network with several connections to other systems and may thus represent an important target for PFASs with potential severe health consequences.

1.07P.2
Endocrine disruption of volatile PAH concentrations in urban and rural environments
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The levels of air pollution have been rising considerably hand in hand with increasing urbanisation and industrialisation. One notable group of common air pollutants are polycyclic aromatic hydrocarbons (PAHs). They are known to disrupt endocrine functioning. Peroxisome proliferator-activated receptor (PPAR) is the key pathway linking PAH exposure and endocrine signaling to the commensal microbiota. However, studies comparing PAH exposure and endocrine disrupting potential of PAHs among urban and rural areas are lacking. The aim of this study was to measure associations between volatile PAH concentrations in the ambient air and PPAR pathway in the endocrine system, and compare two distinct living environments; urban and agricultural areas. The participants were elderly people; thirty lived in agricultural areas and 32 in urban areas. Passive samplers were utilized to measure the ambient PAH concentrations as they simulate the process of bioconcentration in the fatty tissues. Endocrine signaling pathways were analysed from stool bacterial metagenome with Kyoto Encyclopaedia of Genes and Genomes. High phenanthrene level in ambient air was associated with decreased PPAR signaling pathway. The difference between the urban and rural concentrations were not notable, but the variation within the samples of the two areas ranged significantly. Both the highest and the lowest PAH concentrations were measured in the agricultural sites whereas the urban measurements varied only slightly from each other. Some agricultural measurements were markedly higher than the urban average, which was due to the use of heavy equipment (such as tractors, combine harvesters etc.) and grain dryers in active farms. The timing of this study had a major impact on the PAH concentrations as the measuring campaign occurred as the farmers were in the midst of drying their (grain) harvest. Thus, the PAH concentrations tend to highlight the use of grain dryers as well as other farming equipment and their impact on the local air quality. The results of this study demonstrate well how the use of fossil fuels cause considerable air pollution regardless of the location. Our study supports the hypothesis that air pollution disrupts endocrine signaling.

Not all Oil Spills Behave the Same: Unconventional Oils, Advanced Characterization and Ecotoxicity (P)

1.08P.1
Novel biomarkers in a marine copepod exposed to crude oil
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Biomarkers are detectable molecular, biochemical and tissue-level changes that indicate physiological effects after exposure to pollutants (Smit *et al.*, 2009). Previous studies have revealed diverging results regarding expression of chemical stress response genes in marine copepods (Lauritano *et al.*, 2012), and the usefulness of molecular biomarkers in relation to contaminant exposure in marine copepods is debatable. In copepods, expression of genes involved in detoxification responses, like CYP330A1, CYP1A2, GST, SOD, CAT, HSP70, HSP90 and ubiquitin, have repeatedly been investigated in response to petroleum oil exposure, primarily in experiments lasting 96 hours (reviewed by Tarrant *et al.*, 2019). Results have been ambiguous, and in essence, the reliability of these genes as biomarkers of oil exposure is uncertain. To assess exposure or effects of crude oil exposure at low trophic levels in marine ecosystems, it is essential to identify genetic biomarkers that are expressed in a stable way over time in marine copepods. We collected copepods in diapause in a Norwegian fjord and subsequently exposed a subset of these to the water-soluble fraction of a North Sea crude oil for five days. Then followed a recovery period of two weeks. Copepods were sampled and analysed for RNA sequencing at four time points during the exposure experiment. Our main findings were that previously studied genetic biomarkers in copepods did not show stable expression with time, except for one Gluthathione-S-transferase gene. We identified three potential novel biomarkers which had stable expression over time: a thioredoxin, a small ubiquitin modifier and a transcriptional repressor. These new biomarkers should be further investigated and their expression should be assessed in relation to more experimental conditions.

1.08P.2
Ecotoxicology for Sustainable Biofuels
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The increasing industrialization and growth of population have led to an increase

in the global energy and fuel demand, particularly in the transportation sector. Moreover, the use of fossil fuels, such as petroleum-derived diesel fuels, also poses a significant risk for the environment and the global climate. Therefore, research on developing alternative and renewable fuels is increasing rapidly. Biofuels represent promising alternatives to fossil fuels. Biofuels are considered to be more environmental friendly and more sustainable than fossil fuels. However, an important factor, the toxicity of these novel fuels, is often not considered. Toxicity investigation of biofuel candidates should be integrated in the fuel development process and can provide important information on potentially hazardous biofuels. With regard to the large amount of fuels required by the transportation sector, and the consequential contamination of the environment by fuels spills and leakage, the introduction of sustainable, non-toxic fuels provides an opportunity to avoid environmental contamination and harmful effects on human health. Therefore, we want to propose a framework for the integration of a "Green Toxicology" approach in the fuel-design process. It consists of *in silico* toxicity prediction tools, ecotoxiological bioassays and investigation of fuel mixture toxicity as well as the assessment of the toxicity of combustion products. A QSAR tool for prediction of aquatic toxicity has been applied for range-finding purposes and a first identification of potential toxic fuel molecules. A test battery including aquatic toxicity tests (waterflea, fish embryo) as well as genotoxicity test was applied. Potential biofuels investigated exemplary were 2-Methylfuran, 2-Methyltetrahydrofuran, 2-Butanone, 1-Octanol, Dimethoxymethane and Diethoxymethane. Results of the present research demonstrate that a "Green Toxicology" approach can provide important information for biofuel development. The identification of the potentially hazardous biofuel candidates 2-MF and 1-Oct suggests that further research should focus on the less toxic biofuel candidates 2-MTHF, MEK, DMM and DEM. Further investigations within the "Fuel Science Center" will focus on fuel mixture toxicity and the toxicity of combustion products. This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany´s Excellence Strategy – Exzellenzcluster 2186 „The Fuel Science Center".

1.08P.3

Influence of a chemical dispersant on oil toxicity: a multilevel approach in adult zebrafish*

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The application of chemical dispersants is a widely used response strategy to combat oil spill events in aquatic ecosystems. Their ability to break down oil into small droplets, thus allowing very fast dispersion and faster degradation, make them suitable tools in specific oil spill scenarios. However, an increased toxicity of chemically dispersed compared to initial oils towards aquatic wildlife has been reported. This work aimed to gain knowledge on the effects of the water accommodated fraction (WAF) of a naphthenic North Sea crude oil with and without the addition of a chemical dispersant (Finasol OSR 52) using zebrafish as model organism. For this, (1) 1:200 oil:water (WAF_{oil}) and (2) 1:200 (dispersant:oil, DOR= 1:10):water (WAF(oil+D)) low energy WAFs were prepared. Adult zebrafish were exposed for 3 and 21 days to 5% and 25% WAFoil and to 5% WAF(oil+D). 75% of the water volume was exchanged every three days. An unexposed control group was run in parallel. Fish mortality was recorded only in the group exposed to WAF(oil+D) from day 3 of exposure onwards. This treatment presented the highest levels of target PAHs in WAFs along the exposure as well as in the fish carcasses after 21 days. In general, no significant effects were detected in WAFoil-exposed fish across all biological endpoints, while the dispersed oil induced significant alterations compared to unexposed controls. At the gene transcription level significant up-regulation was detected in the liver for xenobiotic biotransformation- and oxidative stress-associated genes (*cyp1a*, *gstp1*, *sod1* and *gpx1c)*. At enzyme level, significantly increased activities were detected for EROD in the liver (3 and 21 d) and in the gills (3 d). Also, GST (3 and 21 d) and CAT (21 d) showed significantly increased activity in gills. A potential genotoxicity was indicated by the increased frequency of micronuclei in erythrocytes after 21 d of exposure. All together, these results demonstrated an increase of the PAH bioavailability and resulting bioactivity (activation of biotransformation metabolism and oxidative stress) of chemically dispersed crude oil on different biological organization levels of zebrafish. *Funded by EU H2020-BG-2005-2 project GRACE (grant agreement #679266), Spanish MINECO (NACE project CTM2016-81130-R) and MECD (FPU grant to A.E.), Basque Government (consolidated research groups IT810-13, IT1302-19, IT1213-19). Thanks to staff at Driftslaboratoriet Mongstad, Equinor (former Statoil) for supplying the oil.

1.08P.4

Toxicity to sea urchin embryos of three types of WAFs weathered under ice, alone and mixed with dispersant

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The Arctic Ocean, Northern Atlantic Ocean and Baltic Sea present particular environmental conditions (presence of ice cover, low water temperature and slow rate of water renewal), added to increasing maritime transport and exploration and extraction of gas and oil resources, increase the probability of oil spill accidents. The presence of ice can affect the weathering, spreading, natural attenuation or encapsulation of oil in the water, which could modify the potential toxicity of spill products. The toxicity of the water accommodated fraction (WAF) of oil and oil in the presence of dispersant have been widely studied but there is still a knowledge gap on potential toxic effects of WAF weathered under ice. WAF weathering of three types of oil (Naphthenic North Atlantic (NNA), Intermediate Fuel OIL 180 (IFO180) and Marine Diesel Oil (MGO) alone and mixed with Finasol OSR52 dispersant was conducted under ice inside an industrial refrigerator at -4 ± 2 ºC in complete darkness over 2 months. To compare toxicity, WAFs without weathering (LEWAF) were also produced at 10ºC for 40h and the toxic effects of under ice weathering were assessed by means of acute toxicity bioassays using larvae of sea urchin *Paracentrotus lividus*. After the exposure period, larvae length, unpaired development and larvae abnormalities were measured. Catalase activity and DNA strand breaks were analysed in larvae as biomarkers for oxidative stress and DNA damage. Chemical analysis of PAHs were measured as total aromatic hydrocarbons by UV-fluorescence. Weathering under ice increased WAF toxicity and the presence of dispersant slightly modified the toxicity of the oils. However, lighter oils (such as NNA) showed a higher increase of toxicity in under ice conditions than intermediate or diesel oils. Results suggest that oil spill toxicity depends on the type of spilled oil, the presence of ice or not and the application of dispersants. Acknowledgements- This work has been funded by the EU H2020-BG-2005-2 project GRACE (grant agreement number 679266), Spanish Ministry of Education, Culture and Sport (PhD fellowship L.dM FPU15/05517 grant) and the Basque Government (Consolidated Research Group GIC IT810-13). Thanks to Total Spain for kindly providing the dispersant.

1.08P.5

Different Mechanisms of Oil Hydrocarbon Biodegradation by Shallow and Deep Water Microbial Communities from the Caspian Sea

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Accidental oil spills can occur during oil & gas exploration and production activities and may adversely affect human and environmental health. The structured spill impact mitigation assessment (SIMA) process exists to facilitate selection of appropriate response option(s), while minimizing the ecological, socio-economic and cultural impact of an oil spill. Response methods to combat an oil spill include skimming, shoreline cleanup, application of dispersants, burning of floating petroleum, and bioremediation. Of these methods, bioremediation (biodegradation by microorganisms) is effective and has minimal adverse effects on the environment. However, knowledge about identity and metabolic capabilities of the microbial communities is necessary to predict petroleum biodegradation and inform response planning (i.e., SIMA). The Caspian Sea is an important global energy source. Our previous microcosm experiments revealed, surprisingly, that resident microbial communities from this basin degraded oil in anoxic conditions more efficiently than in oxic conditions, which is important information for response planning in the event of a spill. Half-lives, determined by first-order rate kinetics calculations, showed faster anaerobic than aerobic biotransformation, particularly of PAHs. Increased transformation of most oil compound groups was also shown for chemically dispersed oil when compared to physically prepared dispersions. A limitation of that previous study was that it only assessed taxonomic composition in deep waters and lacked metabolic characterization. The aim of this study was to characterize taxonomic and metabolic changes that shallow water (aerobic) and deep water (hypoxic/anoxic) microbial communities undergo during biodegradation of Caspian crude oil. We collected water samples in the field (25 m and 350 m) and transported them to the laboratory under controlled environmental conditions (on ice in amber bottles) for subsequent microcosm incubation experiments. Biodegradation experiments were conducted using appropriate environmental conditions (22°C, oxic for 25 m samples; 8°C, anoxic for 350 m samples) and characterized using a combination of standard microbiology methods, multi'omics community sequencing (16S rRNA, shotgun DNA, shotgun mRNA), and advanced analytical hydrocarbon quantitation, including biodegradation half-lives.

1.08P.6

Assessing Oil Spill Toxicity Accurately: A Novel In-Situ Approach

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An in-situ exposure and effects tracking system was developed for assessing oil spills. This NOAA funded project was demonstrated in oil seeps near Santa Barbara, CA. The assessment tool combines two demonstrated systems, the SEA Ring (Sediment Ecotoxicity Assessment Ring, SERDP ER-201130) and the Drifting Exposure (DrEx) Systems (SERDP ER-201432). A combination of the in-situ bioassay capabilities of the SEA Ring, and the tracking and sampling capabilities of the DrEx provided a robust approach allowing for real-time acute and chronic toxicity assessments of oil spills. The system is called the Drifting Exposure and Effects Assessment Ring (DEEAR) and comprised of a GPS float with iridium modem, the drifter drogue, the SEA Ring exposure system, and ancillary sensors and samplers. This multi-system unit pumps ambient water (and oil/dispersants if present) through organism exposure compartments for 1 to 14 day exposures. A polyethylene sampling device (PEDs) is attached to capture organic contaminants in concert with a UV fluorescent sensor to characterize oil exposures, verifying whether or not the system is within the spill. Sensor responses are logged continuously with potential to interface to the Iridium modem, providing real-time monitoring. The system is being tested with a variety of invertebrates and early life stage fish. Pilot testing was successful with exposures of Mysids and Topsmelt embryos and larvae. The DEEAR system was easily deployed and retrieved with two people and tracked oil slick exposures. This novel technology allows for more accurate and efficient determinations of oil spill and dispersant toxicity.

1.08P.7
Containing Oil Spills with Novel Acrylamide/Carboxylic Acid Copolymers
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Abstract Crude oil spills are a major threat to marine biota and the environment. When light crude oil spills on water, it forms a thin layer that is difficult to clean by any oil spill response method. In this study, a series of copolymers of acrylamide (AM) and long-chain carboxylic acids (CA), (C8 – Octanoic acid, C10-decanoic acid, C12-Lauric acid, C14-Myristc acid, C-16-Palmitic acid), with different AM:CA ratios were synthesized by free radical solution polymerization. The results show that adding 5 wt% (polymer/oil) of polymer to the oil spill decreases the contaminated area by more than 80% after a short time. After the polymer addition, the viscosity of the oil increased 3 times, which facilitated its removal by mechanical means. In this presentation, we will describe how to synthesize these copolymers and how to use them as *herders* to remediate oil spills on water. **Keywords:** ***herder, polyacrylamide,*** *oil spills*

Novel Developments in Endocrine Disruptor Testing with Vertebrates (P)

1.09P.1
Multi-Endocrine Disruptors Screening in Zebrafish
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In vitro tools are inexpensive and scalable for high-throughput platforms, however, they pose low relevance to vertebrate species. On the other hand, work 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* model. 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). Endocrine disruptors (EDCs) are chemicals that by interfering with the endocrine system can have an adverse effect at developmental, neurological, immune and reproductive level. Thyroid Disrupting (TD) compounds specifically alter the function of the thyroid gland through the interference with the synthesis, transport and/or binding of the thyroid hormones. The negative impact of 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. The zebrafish is currently used as a model for the evaluation of acute and developmental toxicity and for the screening and testing of potential thyroid (TD) and endocrine disrupters (EDCs), as described in the OECD Guidelines. The two major endpoints used to evaluate EDCs, vitellogenin concentration and change in sex ratio, have several limitations. With the purpose of expanding the number of tests available to identify estrogenic and androgenic substance, we evaluate gene expression of 4 biomarkers in 5 dpf zebrafish larvae after exposure, from 48 hpf, to 10 compounds reported as EDCs. Expression assay over known markers of thyroid pathway was also developed to evaluate 9 environmentally relevant TD substances. This screening methodology showed to be a sensitive and cost-effective assay to screen and evaluate potential EDCs chemicals.

1.09P.2
Interaction of thyroid hormones and eye development in fish: An adverse outcome pathway approach
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Chemical compounds are meanwhile being used at ever increasing amounts in different fields of modern life, including industries, agriculture, medicine and cosmetics. Among these numerous substances, endocrine disruptors (EDs) affect the hormonal system of humans and wildlife, mainly aquatic organisms. Some EDs are known to affect the thyroid hormone (TH) system, which is involved in the regulation of many important physiological and developmental processes, one being the development of eyes in vertebrates. A chemical-induced impairment of the visual system can have severe consequences for the survival rate in the wild, especially for developing fish, which are thought to be very sensitive to thyroid disrupting chemicals (TDCs). However, the direct interaction between THs and eye development in fish has not been fully understood yet. Especially with regard to the environmental consequences of impaired eye development, it is important to understand the full chain of events from molecular interaction with the TH system, over morphological and physiological changes up to the adverse outcome at the individual and population levels. To this end, we performed an extensive literature review of approximately 80 studies (until end of 2019) into the effects of modification of TH levels on eye development in different life stages of different fish species. The reviewed studies include a variety of different techniques for TH disruption, including TDCs, transgenic or mutant fish, microinjection, morpholino oligonucleotides, thyroid ablation etc. Endpoints range from gene and hormone level modifications to cellular changes in the eyes (e.g. cell size, cell layer structure and organization, amount of photoreceptors, pigmentation etc.), morphological changes (e.g. eye size or shape), but also physiological and behavioral changes (e.g. optokinetic response, light response, swimming activity, etc.). Based on these data, we developed an adverse outcome pathway (AOP) to describe the different processes involved in the interaction between THs and eye development in fish. This AOP will build the basis for further research on the consequences of thyroid disruption in fish, a research field with many knowledge gaps.This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825753 (ERGO). This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

1.09P.3
Behavioural responses of zebrafish (Danio rerio) embryos to direct or parental thyroid disruptor exposure
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Thyroid hormones (THs) serve multiple functions; among others, THs regulate neuromorphological and eye development in vertebrates. In the developing eye, they affect the spectral sensitivity of visual cones. Since the thyroid system has also been described to interact with the interrenal system, there may be a crosstalk between THs and glucocorticoids. These factors might affect the behaviour of organisms exposed to thyroid disrupting chemicals. To investigate this potential interaction, zebrafish embryos were exposed to sublethal concentrations of propylthiouracil (PTU), a TH synthesis inhibitor. Behavioural changes of the embryos were assessed using the light/dark transition (locomotion) test. The test consists of several cycles of alternating phases of 10 min dark and 10 min light, and the reaction of the embryos is recorded as distance moved. It addresses anxiety-like behaviour with, in the case of zebrafish embryos, the dark phases representing the more stressful condition.Various tests were conducted with different light intensities and wave lengths (red: 623 nm; green: 525 nm; blue: 470 nm) for 5 cycles of dark and light phases. Embryos exposed to 200 and 300 mg/L PTU reduced movement during the dark phases, except in tests of low intensity green light. However, extending the low intensity green light test to include more light/dark cycles, effects could be documented down to 80 mg/L PTU, when embryos *increased* the distance moved. In contrast, unexposed embryos whose parents had been exposed to 80 mg/L PTU for 4 - 18 days *decreased* movement during dark phases. Embryos exposed to 80 mg/L PTU, whose parents had also been exposed to the same PTU concentration, behaved similarly to control embryos (no parental or embryo exposure). These results suggest a hormetic (biphasic) response of zebrafish embryos to PTU exposure with a positive (stimulating) effect at lower PTU concentrations (interpreted as anxiety-like behaviour) and a negative (depressing) effect at higher PTU concentrations. Conversely, parental exposure to low PTU concentration decreased anxiety-like behaviour in embryos. The combination of parental and embryo exposure cancelled the effect of PTU on embryo behaviour. Funding: European

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1.09P.4

Transgenerational effects of a thyroid disrupting compound on the development of the swim bladder of zebrafish, Danio rerio, embryos

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Among other functions, thyroid hormones (THs) regulate morphological development in fish embryos. THs deposited in the egg by the mother are most likely functional, as the embryo expresses thyroid receptors, before it is able to produce THs by its own. Consequently, parental exposure to thyroid disrupting compounds, if influencing parental deposition of THs into eggs, might affect offspring development.In order to test this hypothesis, the effect of exposure to propylthiouracil (PTU), an anti-thyroid drug that inhibits thyroid peroxidase and reduces TH synthesis, was tested in zebrafish. Among other endpoints, swim bladder inflation, which is known to be directly regulated by THs, was analysed. Parental fish were exposed to PTU (0 - 78 mg/L) for 4 - 18 days. Likewise, eggs of the F1 generation were exposed to PTU concentrations of 0 - 78 mg/L for 5 days. The relative area of the swim bladder at 125 h post-fertilization was reduced, when parents were exposed to 78 mg/L of PTU; in contrast, no effect was seen in PTU-exposed embryos originating from unexposed parents. At molecular level, we checked for gene expression of thyroid- and swim bladder-related genes. We observed a compensatory overexpression of thyroid peroxidase (*tpo*). The expression of surfactant protein b (*sp-b*), hypothesised to mediate the observed effect, did not differ between exposure treatments, nor did thyroid receptor beta (*thrb*). Additionally, embryo exposures to 150 and 200 mg/L PTU were tested. In the absence of parental exposure, only the highest concentration of embryo exposure (200 mg/L) impaired swim bladder inflation. Consequently, swim bladder inflation can be concluded to be more sensitive to parental than to embryo exposure. To discriminate between possible underlying mechanisms of hormonal deposition or PTU transfer into the egg, PTU and TH concentration in eggs are currently being analysed.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") and from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825753 (ERGO).

1.09P.5

Reversibility of thyroid disrupting effects on eye and swimbladder development in zebrafish (Danio rerio) embryos

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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 disrupting chemicals, TDCs). Eye and swimbladder development of fish are known to be directly regulated by THs and can, therefore, be used as thyroid-related endpoints in endocrine disruptor testing with fish. We were interested to see whether TDC-induced malformations of eyes and swimbladder during early development would be reversible after depuration in clean water. To this end, zebrafish (*Danio rerio*) embryos were exposed to either propylthiouracil (PTU), a TH synthesis inhibitor, or tetrabromobisphenol A (TBBPA), which interacts with TH receptors, until 5 days post-fertilization (dpf). Subsequently, half of the embryos were further exposed to the TDCs until 8 dpf or kept in clean water to check for reversibility of effects. The continuous TDC exposure induced altered eye size and pigmentation together with changes in the cellular structure of the retina. By TDC treatment, swimbladder inflation was significantly delayed and impaired. Moreover, the pigmentation of the swimbladder epithelium was significantly reduced. Interestingly, the effects on both eyes and swimbladder were partly reversible after the short recovery period of 3 days. These results document that eye and swimbladder development in zebrafish embryos are very sensitive to TDC treatment; however, repair mechanisms apparently allow for rapid and effective recovery from early exposure to TDCs.This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 825753 (ERGO).

1.09P.6

Sensitivity of the common frog Rana temporaria to the anti-thyroid drug 6-propylthiouracil (PTU): effects and new potential endpoints

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Amphibians are very sensitive to different stressors and frog species are in danger of extinction worldwide. Due to their permeable skin, they are particularly sensitive to chemicals present in water. As thyroid hormones play a critical role in amphibian growth and metamorphosis, thyroid disrupting chemicals can have detrimental effects on their development. Moreover, the thyroid system is very well conserved amongst vertebrates and frogs thus constitute a good model species to study the effects of thyroid disrupting chemicals in humans. OECD assays designed to detect or identify thyroid disrupting chemicals, e.g. Amphibian Metamorphosis Assays (AMA), use Xenopus laevis, the African clawed frog, as a model species. However, as opposed to most frog species, X. laevis has a 100% aquatic life cycle. Besides, X. laevis is an African species, and assays using this species may consequently not be able to predict the sensitivity of European and partly terrestrial frogs to thyroid disrupting chemicals. In this context, this study aims at comparing the sensitivity of the common frog Rana temporaria with that of X. laevis, when exposed to 6-propylthiouracyl (PTU), a well-known anti-thyroid drug used to treat hyperthyroidism. Eggs were collected in spring 2019 in a pond in Odense, Denmark, and randomly allocated to tanks with different PTU concentrations: 0 mg/L (control), 1.25 mg/L, 4.43 mg/L, 13.82 mg/L and 48.13 mg/L in water. The eggs and later tadpoles were exposed to PTU throughout metamorphosis and were then sampled for morphological measurements, thyroid hormone concentrations and thyroid histopathology. Analyses of developmental stage, length and weight showed strong differences between control and treated groups. At the end of the exposure, while most of the controls reached the climax of the metamorphosis, treated individuals developed more slowly and with a concentration dependent effect. Besides, when comparing the body length and weight for Gosner stages 35 to 38, individuals treated with PTU concentrations from 4.43 mg/L and higher had higher length and weight than controls. Those results, suggesting that PTU stops the development but not the growth, are consistent with what was already shown in the literature regarding the effects of anti-thyroid drugs in amphibians. Thyroid histopathology, considered to be more sensitive than the biometric measurements, was also used to look for relevant endpoints, besides the ones already used in OECD assays.

1.09P.7

Chemical selection for the EU-NETVAL Thyroid Validation Study

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As a follow up on the European framework for Endocrine Disruptors (EC, 2018), the European Reference Laboratory for alternatives to animal testing (EURL ECVAM) is coordinating the validation of a battery of 17 thyroid-targeted test methods (OECD, 2014). The aim is to assess the potential of chemicals to disrupt the thyroid hormone (TH) axis, including various mode of action, namely interference with central regulation, TH synthesis, TH transport and distribution, TH metabolism, excretion, or cellular uptake, intracellular TH (de)activation via peripheral deiodinase type-1, or T3 nuclear receptor binding. Sixteen of the proposed in vitro methods are based on human or mammalian test systems. One method uses the zebrafish embryo, a non-protected life stage under current EU legislation, as an integrated in vivo model. Fourteen laboratories from the European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL), are participating in this two-part validation study: - Part 1, currently ongoing, is aimed at defining the methods and assessing their robustness and reliability. A few positive, negative control and reference chemicals (OECD, 2018) are being tested for each method, identified previously during test method research and development. - Part 2 will aim at assessing the

overall relevance of the test methods and will use a larger set of around 30 test items, which will be screened using all 17 methods. A particular challenge for the success of this validation study is selection of relevant test item chemicals, covering the range of expected response from the various modes of action. In support of the chemical selection for Part 2, an expert meeting was organised in November 2019. Prior to this meeting, experts were surveyed to propose potential validation set chemicals with known activity in at least one of the methods/mechanisms of action: 87 chemicals were initially suggested. During the meeting, these initial proposals were reduced to a short list of 40 chemicals that in the collective opinion best cover the assays and modes of action. In addition, it was agreed that negative control compounds would be included and several were considered. The shortlist will undergo further refinement over the coming months to yield 30 validation set chemicals. This presentation will describe the strategy taken for chemical selection and the outcome of the survey and the meeting. EC 2018. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. Towards a comprehensive European Union framework on endocrine disruptors. COM(2018) 734 final. OECD 2014: New scoping document on in vitro and ex vivo assays for the identification of modulators of thyroid hormone signalling. Series on testing and assessment n° 207, ENV/JM/MONO(2014)23. OECD, Paris OECD 2018: Guidance Document on Good In Vitro Method Practices (GIVIMP). Series on testing and assessment n° 286, ENV/JM/MONO(2018)19. OECD, Paris.

1.09P.8

Can androgens modulate classic estrogenic targets in brown trout primary hepatocytes?

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Endocrine disruption by estrogens is one of the major focuses of ecotoxicological studies. Estrogenic biomarkers, such as vitellogenin (Vtg) and the zona pellucida proteins (ZPs) are frequently used in the survey, screening and monitoring of estrogenic compounds. A few studies, however, have revealed the potential of androgens to mimic estrogenic signalling effects. The mechanisms behind those interferences have been relatively unexplored. The most suggested hypotheses are the aromatisation of androgens to estrogenic compounds and the direct binding of androgens to estrogen receptors (ERs). The purpose of this study was to evaluate the effects of two model androgens – testosterone (T) and 4,5α-dihydrotestosterone (DHT), which are aromatizable and non-aromatizable androgens, respectively, in a selection of well-known estrogenic targets, using primary hepatocytes from juvenile brown trout (*Salmo trutta* f. *fario*). The exposure conditions were: control (C), solvent control (SC) and six androgen concentrations (1, 10 and 100 nM and 1, 10 and 100 μM). The estrogenic targets were VtgA, ER alpha (ERα), ER beta (ERβ) and two zona pellucida genes, ZP2.5 and ZP3a.2. The expression of the selected targets was studied by quantitative real-time PCR (qRT-PCR) and immunohistochemistry (IHC). The VtgA and ERα mRNA levels were significantly up-regulated at 1, 10 and 100 μM of DHT and 10 and 100 μM of T. By contrast, after 10 and 100 μM of DHT and 100 μM of T, ERβ was significantly down-regulated. The mRNA levels of ZP2.5 significantly increased at 1, 10 and 100 μM of DHT and T, while the ZP3a.2 was up-regulated by 100 μM of DHT and 10 and 100 μM of T. Correlation analysis revealed medium to high positive correlations between VtgA and ERα mRNA levels, and both ZPs and ERα. The changes found by IHC mostly confirmed the molecular findings, especially in the case of Vtg and ZP proteins. These findings suggest that both androgens acted through direct binding to the ER's, most likely ERα. The present data illustrate the unfeasibility of Vtg and ZPs as exclusive biomarkers of estrogen exposure, and thus, caution must be taken when interpreting the induction outputs as specific estrogenic disruptions. Funding: Supported by FCT and ERDF (UID/Multi/04423/2019) and ICBAS.

1.09P.9

Retinoid pathway disruption: in vitro and in vivo bioactivity of retinoid compounds detected in the environment

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Cyanobacterial water blooms and their production of diverse bioactive compounds have been linked with adverse effects on exposed organisms and potential health risks. Newly detected compounds produced by cyanobacteria are retinoid-like compounds, but there is little information on their production, levels in the environment or potential adverse effects and risks associated with their occurrence. Detected compounds in the environment include all-*trans* retinoic acid (ATRA), 9/13-*cis* retinoic acid (RA), 4OH-RA, retinal (RAL) or 4keto-RAL as well as some novel metabolites, such as 5,6epoxy-RA or 4keto-ATRA. Some of these are known as strong teratogens and disruption of the retinoid signalling pathway can have fatal consequences as it regulates crucial processes during the early development of vertebrates such as formation of nervous system. Our studies employed four luciferase reporter cell lines for the characterization of the retinoid- (p19/A15 and GeneBlazer UAS-bla HEK 293T with retinoic acid receptor- RAR; and retinoid X receptor- RXR) and thyroid receptor (PZ-TR) disrupting potencies of the pure compounds. *In vitro* potencies were compared with their potency to cause teratogenicity *in vivo* using zebrafish (*Danio rerio*) embryo toxicity test (zFET). Additionally, to provide further information on adverse outcomes that might result from retinoid pathway disruption the exposed zebrafish embryos were subjected to label-free proteomic analysis and qPCR analysis of selected genes expression. The results document the ability and potency of the compounds produced by cyanobacteria and commonly detected in the environment to interfere with retinoid signalling and provide a unique comparison between the responses in individual specific cell-lines and apical effects *in vivo*. Effective *in vivo* concentrations for single compounds are in nM range, which is lower than the levels of individual retinoids detected in the environment. Relative potencies of the retinoids determined in the *in vitro* assays showed relatively good predictability towards their *in vivo* potency and with the additional information from the proteomics and gene expression analysis it is clear that these compounds are able to disrupt the retinoid signalling pathway and early development at very low concentrations. This work was supported by the RECETOX Research Infrastructure (LM2015051), Czech Science Foundation project No.18-15199S and CIISB Research Infrastructure (LM2015043) funded by MEYS CR.

1.09P.10

Cytotoxic Effect of Polychlorinated Biphenyl (PCB-153) on Human Placental Trophoblast Cells

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Polychlorinated biphenyls (PCBs) are environmental pollutants and known endocrine-disrupting chemicals. The potential human health risk of PCBs is considered a major public health. Despite their worldwide ban, PCBs can still be detected in the environment and general population due to chemical stability and lipophilic properties. The most prevalent measured in human tissues are PCB-28, 52, 101, 138, 153 and 180. A great deal of concern exists that low levels of PCBs transferred to the fetus across the placenta or to nursing infants via breast milk may induce long-lasting neurological damage. Epidemiological and animal studies demonstrated that the developing fetus is vulnerable to PCB exposure. In utero exposure to PCBs has been linked to increased incidence of fetal resorption and abortion, reduced birth weight, and fetal growth. Estrogens affect a variety of tissues, including reproductive tissues, bone, liver cardiovascular system and brain. Several studies have shown that human placenta bind estradiol, and estrogens influence various aspects of placental function and fetal development in human and primates. The aim of this study was to examine the toxic potency of PCB-153 in human placental trophoblast (BeWo) cell model, and to examine the effect of PCB-153 on estrogen-induced cell proliferation. BeWo cells were treated for different exposure times and various concentrations of PCB-153 in absence and presence of 17β-estradiol (E2). Cell proliferation and viability, and apoptotic cell death were assessed. Exposure to PCB-153 at a concentration range of 0.001 to 10μM increased cell proliferation and viability of BeWo cells whereas a significant reduction in cell proliferation and viability was observed in a concentration-dependent manner after exposure to more than 10μM of PCB-153. Estrogen at low concentration (0.01-10nM) significant induced cell proliferation in a time-dependent manner. Low concentrations (0.01-10μM) of PCB-153 reduced estrogen-induced cell proliferation. The estrogen antagonists, ICI 182,780, and tamoxifen, did not blocked these effects. By contrast, these antagonists inhibited estrogen-induced cell proliferation. This study demonstrated that exposure to PCB-153 can cause toxic effect which may not be directly mediated through an estrogen receptor. Also, it will provide relevant evidence on the action of PCB-153 as environmental risk factor. Supported by Title III.

1.09P.12

Deciphering the mode of action of thyroid active chemicals using the XETA (Xenopus Eleutheroembryonic Thyroid Assay).

D. Du Pasquier, Watchfrog S.A.

The ATHENA project belongs to to the EURION cluster aiming to develop new testing strategies for endocrine disrupters. In this project the consortium will develop new methods for incorporating into existing OECD test guidelines which could can capture the consequences of thyroid hormone disruption. The XETA (Xenopus Eleutheroembryonic Thyroid Assay) is an *in vivo* assay using xenopus embryos to detect thyroid active compounds. The test relies on transgenic embryos bearing a genetic construct where Green Fluorescent Protein expression is controlled by the thyroid pathway. Thyroid active molecules, hormones and endocrine disrupters induce increases or decreases of fluorescence, depending on their mode of action. The XETA was published as the OECD test guideline n°248 in spring 2019. By nature, the XETA provides a degree of information about the likely mode of action of thyroid active chemicals. Each chemical is tested either in the presence (T3 spiked mode) or absence of T3 (unspiked mode), interpreting the results obtained in the two modes helps to hypothesised on the mode of action. Thyroid receptor agonists induce an increase of fluorescence in the spiked and unspiked mode while thyroid receptor antagonists induce a decrease of fluorescence in T3 spiked mode. Substances modulating the clearance or metabolism of the hormones modify the fluorescence only in the presence of thyroid hormones, increases or decreases in fluorescence could be observed depending of the enzyme inhibited by the substance. In the ATHENA project, we are developing strategies using additional co-treatment to help to distinguish between the different mode of action of the substances modulating the fluorescence. By adding additional co-treatments such as thyroid receptor antagonist (i.e. the pharmacological inhibitor NH3), it will be possible to delineate whether the effect is mediated through the thyroid receptor and excluded potential false positive. Another example is that by comparing the results obtained with either a T_4 or a T_3 co-treatment, substances acting as deiodinase inhibitors can be identified. Depending of the deiodinase inhibited, the change in fluorescence in T_4 and T_3 could potentially be observed in both directions. We are validating this co-treatment strategy on a set of reference compounds to establish the addition of these co-treatments in the XETA test guideline.

Novel Tools and Bioassays for the 21st Century Environmental Toxicology (P)

1.10P.1

Multi-omics Analysis Reveals that Co-exposure to Phthalates and Metals Disturbs Urea Cycle and Choline Metabolism

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The aim of this study was to obtain mechanistic insight into how co-exposure to phthalates and metals causes neurodevelopmental perturbations based on in vitro assays. HepaRG cells were exposed to two mixtures of DEHP, DiNP, and BBzP phthalates, methylmercury and total mercury. The concentrations of the pollutants were multiplied by ten for the second mixture. The effective concentrations of the chemicals in vitro were estimated through extrapolation from human biomonitoring data through internal dosimetry modeling using the INTEGRA computational platform. Multi-omics analysis was performed on the treated cell models including transcriptomics, proteomics, and metabolomics. Integrated pathway-level analysis of transcriptomics and proteomics data revealed that co-exposure to phthalates and heavy metals lead to the perturbation of the urea cycle due to alterations in the expression levels of arginase-1 and -2, argininosuccinate synthase, carbamoyl-phosphate synthase, ornithine carbamoyltransferase, and argininosuccinate lyase. Co-mapping of proteomics and metabolomics data revealed that their common drivers are responsible for the homeostasis of metabolic pathways related to choline, phosphatidylcholine, phospholipases and triacylglycerol metabolism. The identification of the urea, phosphatidylcholine biosynthesis I and phospholipases metabolic pathways is of particular interest since these pathways have been also identified in human samples from the REPRO PL and PHIME cohorts using untargeted metabolomics analysis and have been associated with impaired psychomotor development in children at the age of three to six. Our work reveals that co-exposure to plasticizers and metals disturb biochemical processes related to mitochondrial respiration during critical developmental stages that are clinically linked to neurodevelopmental perturbations.

1.10P.2

Could we offer a comprehensive assessment of chemical mixtures without previous knowledge of the individual components?

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One of the challenging tasks in environmental toxicology is the assessment of complex mixtures of pollutants. Mixtures could produce different effects than their individual components. Therefore, it is difficult the understanding of the overall mechanisms of the mixture if the composition and ratio among the compounds have not been assessed in advance. The classical approaches limit the assessment of mixtures to specific biomarkers and pathways without a global molecular view inside cells or organisms. Here we propose the use of the bioactive thermal proteome profiling (bTPP) approach to assess mixtures with the aim to get an unbiased and comprehensive assessment of a network of mechanisms of action. Applying one single analytical technique, bTPP could identify in a proteome-wide manner all possible protein targets of the chemical mixture, supporting the 3R, 3M principles and providing the key knowledge required to develop Adverse Outcome Pathways and identify novel biomarkers for further analysis. Previously, the bTPP approach was used to identify the protein targets of compounds with therapeutic uses. In this approach, a soluble proteome is exposed to a compound of interest followed by a thermal shift assay. Using proteomics, the thermal stability of identified proteins is determined. Protein targets are those proteins that show a shift in their thermal stability. As proof of concept we performed the bTPP approach to assess the effects of 25 nM 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), 10 uM alpha-endosulfan and its combination on extracted proteins from hepatocytes. After an exposure to TCDD, 32 protein targets were identified out of 3155 proteins evaluated. Some of the proteins identified as targets are involved in pathways related to glucose metabolism corresponding to the results found in other studies. We also found that many other key enzymes involved in essential metabolic pathways were affected by TCDD. Experiments with alpha-endosulfan and the mixture are ongoing but based on TCDD findings, promising results are expected. Our study shows that bTPP approach offers a comprehensive assessment of chemical mixtures based on the unbiased identification of protein targets within the soluble proteome of any cell type. Those targets are the driver of the mechanisms of actions that have been affected causing an integrated cellular response that could be mapped. The uniqueness of this methodology is that neither previous knowledge of the chemical composition of the mixture, nor the ratio or stoichiometry is required. Using bTPP method to assess chemical mixtures, we could solve one of the challenges of environmental toxicology today understanding the effects of exposure to unknown chemical mixtures, facilitating early warning for possible risks for human and environmental health.

1.10P.3

Protein structural similarity for extrapolation of toxicity knowledge across species

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Bioinformatic approaches for understanding protein structural similarity are advancing rapidly. In fact, computational "virtual" screening for bioactive molecules using advanced molecular modeling approaches is common practice in the pharmaceutical industry, mitigating required time, costs, and risks for late-stage drug-candidate failure. Typically, these methods are used to understand which structures, from hundreds, can optimally bind to a given protein target to select candidates to move forward in drug development. In principle, these same virtual screening pipelines could be applied in support of chemical safety assessments and used to evaluate a chemical's interaction with hundreds to thousands of proteins representing both the diversity of toxicologically-relevant protein targets in the body as well as the variations in those proteins among species (e.g., vertebrates, invertebrates, plants, fungi, etc.). Here we describe a pipeline that can be used to 1) fill knowledge gaps in instances where chemical molecular targets are unknown by "virtually" identifying if and how chemical(s) bind to proteins across species and 2) provide quantitative information for chemical-protein interactions in species where limited or no chemical toxicity data exist. Harnessing the power of computing is the future for cross-species chemical safety screening which is why the US Environmental Protection Agency Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool was created and continues to evolve. SeqAPASS predicts chemical susceptibility across species based on protein-sequence similarity. The output from this tool provides an initial line of evidence for cross species extrapolation, however currently does not provide a numerical value for how well the chemical is expected to bind to the protein target in species predicted susceptible. Computational advances in drug-discovery are available to take this evaluation further to specifically examine protein structural interactions with chemicals using available x-ray crystallography and advanced bioinformatic methods to predict binding affinity. Such virtual methods are intended to significantly reduce the cost associated with current high-throughput in vitro screening methods and address the limitations in using model organisms which are primarily used as surrogates due to convenience or convention, rather than appropriateness. This presentation describes a computational pipeline to evaluate protein sequence and structural similarity in the context of biological pathways to gather lines of evidence for conservation of chemical targets for cross species extrapolation.

1.10P.4

A Weight-of-Evidence Approach for Androgen Receptor Conservation Across Vertebrate Species

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The US Environmental Protection Agency's endocrine disruptor screening program (EDSP) is tasked with assessing thousands of chemicals for their potential to adversely impact human health and the environment through perturbation of endocrine pathways. Traditionally, chemical screening is performed using a tiered toxicity testing strategy that includes whole-animal

studies. This approach, however, has proven challenging due to the extensive time, resources, and animals needed to evaluate a single chemical for endocrine activity. Therefore, the EDSP has been transitioning towards the use of in-vitro high-throughput screening (HTS) techniques to rapidly and efficiently prioritize chemicals for further testing. Despite their utility, the ability of these mammalian-based HTS assays to accurately reflect chemical interactions with non-mammalian targets remains uncertain. One goal of the EDSP is to evaluate biological pathway conservation across taxa to understand how broadly these HTS results can be extrapolated to non-mammalian species. Identification of chemicals that modulate the androgen receptor (AR) is of interest to the EDSP because many chemicals have androgenic activity that can disrupt the endocrine system by mimicking or antagonizing natural hormones. Therefore, the objective of this study is to gather weight of evidence for AR pathway conservation across species using a combination of in-silico structural comparisons and systematic review of available toxicity literature, both in vitro and in vivo, to determine whether current AR HTS assays are predictive of activity across vertebrate species. Results of in-silico structural comparisons suggest that the AR ligand binding domain, as well as four amino acid residues critical in AR-ligand binding (N706, Q711, R753, T878) are conserved across all vertebrate species with available sequence information. To date, systematic review of available in-vitro receptor binding and transcriptional activation data supports functional conservation of AR among mammals, fish, birds, amphibians, and reptiles. Overall, this work provides lines of evidence toward structural conservation of AR across vertebrate species and suggest that chemicals known to interact and bind to the AR ligand binding domain in mammalian assays should behave similarly in non-mammalian vertebrates.

1.10P.5

Application of in vivo and in vitro assays for studying the chemosensitizing potential of environmental chemicals in zebrafish embryos

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The uptake of chemicals from the water into zebrafish embryo tissue is controlled by an Abcb4 ortholog. Man-made chemicals can be abundant in aquatic environments and we were interested, to which extent such chemicals interfere with efflux transporter activity acting as chemosensitizers. We applied ATPase assays with recombinant zebrafish Abcb4 protein generated with the baculovirus system for obtaining indication for interaction of test compounds with the efflux transporter. Assays were run with concentration series of test compounds. From more than 30 environmental pollutants, comprising agro-chemicals, industrial chemicals, ingredients of personal care products and drugs, 20 caused either stimulation or inhibition of the basal Abcb4-ATPase activity and/or inhibition of the verapamil-stimulated ATPase activity. This indicates that interaction of environmental pollutants with zebrafish Abcb4 is prevalent. The finding that a majority of the compounds (almost 20) inhibited the verapamil-stimulated ATPase activity indicates that chemosensitizer action could be a common effect. For determining how potent these chemicals are as chemosenitizers *in vivo* we established a dye accumulation assay with live zebrafish embryos. Embryos at 72 hours post fertilization are exposed to chemicals along with rhodamine B, a fluorescent substrate of zebrafish Abcb4, and the dye accumulated in the embryo tissue is quantified using the VAST (Vertebrate Automated Screening Technology) platform, which allows taking bright field and fluorescent images of live embryos in an automated way. If a test compound interferes with the efflux transporter the fluorescence signal increases, as more dye accumulates in the embryo tissue. Cyclosporin A, for instance, a known inhibitor of zebrafish Abcb4, caused an up to 2- to 3-fold increase of the fluorescence signal in the embryo tissue in a concentration-dependent way.

1.10P.6

Tetraselmis chui as a marine green microalgae model specie for toxicity testing

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The OECD guideline 201 “Alga, growth inhibition test” and the ISO 10253:2016 Water quality — Marine algal growth inhibition test with *Skeletonema sp.* and *Phaeodactylum tricornutum* are routinely used in aquatic ecotoxicology to assess the toxicity of chemicals on phytoplanktonic species. The tests are performed in liquid test medium incubated under controlled conditions for 72 hours. *Tetraselmis chui* is a common species in European estuaries and other coastal areas and has been used as a representative species of marine primary producers in ecotoxicology in previous research. The specie is easy to culture and currently widely used as a feed in aquaculture but is also considered for biofuel production. This research aims to investigate the suitability of *Tetraselmis chui* as a marine green microalgae model specie for toxicity testing according to OECD guidelines. *Tetraselmis chuii* stock cultures were maintained in an illuminated incubator under a temperature of 20 ± 1 °C and a photoperiod of 12 h light: 12h dark. The culture medium was F/2 Guillard medium (Guillard, 1975), prepared with filtered (0.45 µm) and autoclaved seawater collected from the coast of Malta. To prevent aggregation and sedimentation of *T. chuii*, cultures were shaken twice a day. Test cultures to asses toxicity of 3,5-dichlorophenol were cultured in a constant illuminated incubator at 20 ± 1 °C and shaken twice a day. Growth was monitored by cell counts using a Neubauer Improved brightline chamber at 0, 24, 48 and 72h. Growth curves and toxicity tests were performed with the reference item 3,5-dichlorophenol.

1.10P.7

Newly developed Ames RAMOS test allows mutagenicity detection by online monitoring of respiration activity

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The Ames test is one of the most widely used mutagenicity tests. It is based on histidine auxotrophic Salmonella typhimurium strains, which can back-mutate to histidine prototrophy during growth on initially added histidine. The more mutagenic an added substance is, the more back-mutated cells (revertants) develop and can continue to grow after histidine is depleted. The original Ames test takes place on agar plates and is standardized by the OCED. In addition, the Ames fluctuation test was developed, which is standardized by the ISO. It is carried out in liquid culture in microtiter plates allowing a higher throughput than the original Ames test on agar plates. Nevertheless, both standardized test systems are laborious and require a determination of revertant growth after the test procedure. In this study, a novel online-monitoring high-throughput Ames test in microtiter plates, the Ames RAMOS test, is introduced. Herby, RAMOS stands for respiration activity monitoring system. The mutagenicity of a sample in the Ames RAMOS test is evaluated by online measurement of respiration activity (oxygen transfer rate, OTR). Hereby, the OTR correlates with the test strain growth. The faster the OTR increases, after consumption of the initially added histidine, the more mutants have developed. Thus, the more mutagenic a sample is considered. By using 48-well microtiter plates, the Ames RAMOS test can be performed at high-throughput. Due to the automatic online monitoring of the OTR, no additional determination of revertant growth after the test procedure is necessary and a sensitive monitoring of mutagenic effects is feasible. By optimizing the preculture strategy, the transfer of additional unreproducible histidine amounts into the test culture is eliminated, leading to a highly reproducible test system. By using 5 mg/L initial histidine instead of the standardized 1 mg/L initial histidine, a higher sensitivity of the test system is achieved, allowing a better detection of weak mutagenic substances. Dose response curves were generated for test strains TA 100 and TA 98 without and with the metabolic activation system S9. Hence, the newly developed Ames RAMOS test allows for a high-throughput mutagenicity evaluation. Due to the online monitoring of respiration activity, the workload is reduced compared to the original Ames test systems. An optimized preculture strategy as well as an increased amount of initially added histidine lead to a reproducible and sensitive Ames test system.

1.10P.8

A Comparison of Bioassay Methods for Lepidopteran Species Using Mamestra brassicae

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Traditionally, the method of choice for many L*epidopteran* bioassays is contact through a leaf dip bioassay. However, due to restrictions dictating the length of these bioassays (i.e. leaf freshness) this method may be unsuitable for longer term sub-lethal monitoring. Furthermore, in past reviews of *Lepidopteran* bioassay methods it has been suggested that methods using artificial diet are better suited to high throughput assays due to their ease of storage and labour efficiency. For this experiment, three methods were trialled using Dimethoate dissolved in acetone; · Incorporated diet method –Adding of pesticide into an artificial diet - 4th instar *M. brassicae* added to a modified Hoffmans diet (Smith, 1966) containing varying concentrations of Dimethoate solution · Topical exposure – Application of Dimethoate solution applied on the second segment behind the head · Surface dosing Method -Dimethoate solution applied on the surface of the artificial diet An initial test of the incorporated diet method showed that Dimethoate is toxic to *M. brassicae* and have a low survival rate above 100mg/Kg of diet consumed. Whereas, when using the surface dosing method results were largely inconsistent between surface dosing trials. This method also seemed to indicate that *M. brassicae* have a low sensitivity to Dimethoate. No topical applications have impacted survival of *M. brassicae*. The replication of these initial tests are ongoing, therefore, there is no statistical data at present. If results at the end of the experiment reflect those of the initial test it is expected that the Incorporated diet method may prove the most reliable in terms of replicability. Results shown in these initial incorporated diet tests are also more reflective of previous literature into Organophosphate insecticides, such as Dimethoate. This suggests that *M. brassicae* exposed using topical or surface dosing applications are not in receipt of the same dose as those exposed via the incorporated diet. Furthermore, unlike topical applications and surface dosing, incorporating pesticides directly into artificial diet allows a consumption rate to be calculated. This can have longer term practical implications for models such as Dynamic Energy Budget (DEB) to be applied to the species.

1.10P.9

Exploration of Molecular Initiating Events (MIEs) in the Adverse Outcome Pathways (AOPs) relevant for poorly studied novel flame retardants

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Flame retardants (FRs) are broadly used in diverse consumer products and building materials. The most studied polybrominated diphenyl ethers (PBDEs) are under a strict ban but they are being replaced by a wide range of novel FRs like organophosphate FRs or novel brominated FRs. Despite of life-long exposures, the risks and toxicity pathways of these replacement chemicals have not been properly evaluated. In the present study, we first analyzed available toxicological information on 52 replacement FRs (in vivo studies, in vitro data from literature and US EPA ToxCast), which has been linked to relevant Adverse Outcome Pathways (AOPs) and used for categorization and strategic prioritization of the FRs (https://doi.org/10.1186/s12302-019-0195-z). For the nine highest priority FRs – i.e. tris-2-chloroethyl phosphate (TCEP), tris(1,3-dichloropropyl)phosphate (TDCIPP), triphenyl phosphate (TPhP), Tricresyl phosphate (TMPP), tetrabromobisphenol A (TBBPA), tri-n-butyl phosphate (TNBP), tri(2-butoxyethyl) phosphate (TBOEP), tris(1-chloro-2-propyl) phosphate (TCIPP), and 2-ethylhexyl diphenyl phosphate (EHDPP), the most relevant health outcomes identified were decreased male fertility, neurobehavioral effects and hepatotoxicity/hepatocarcinogenicity. However, there is a complete gap in the information on potential interactions of the priority FRs with putative molecular targets (Molecular Initiating Events - MIEs) beyond the relevant AOPs. Our follow-up running research, therefore, addresses in detail: (i) in vitro studies of interferences of novel FRs with signaling of nuclear receptors, namely androgen- and glucocorticoid (AR, GR) underlying the male fertility disruptions, and (ii) modulations of hepatic metabolism (mechanisms beyond the liver steatosis) using the human 2D and 3D in vitro models. The importance of the mixture effect of novel FRs as well as development of quantitative AOPs for the above outlined health outcomes will be discussed in detail [The research is supported by the EU H2020 Programme under grant agreement No 733032 HBM4EU].

1.10P.10

Human stem cell-based 3D in vitro models to study cyanotoxin-induced chronic hepatotoxicity and liver disease

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In vitro models based on monolayer (2-dimensional, 2D) cultures of immortalized hepatic cell lines are often known to poorly recapitulate the key hepatocyte-specific functions, which significantly limits their use for hepatoxicity assessment. In contrast, 3-dimensional cultures more closely mimic in vivo-like tissue microenvironment and physiological cellular interactions, which can stimulate cell differentiation and induction of hepatocyte-specific characteristics and functions. In our research, we used 3D scaffold-free spheroid culture system, both static microtiter plate-based cultures, alllowing for higher throughupt and screening, as well as spheroid cultures grown in microgravity bioreactor system, allowing for scaling up the experiments and dynamic long-term studies. These culture systems were used to prepare and characterize hepatospheroids derived from hTERT immortalized human adult liver stem cell line HL1-hT1, and also from human embryonic stem cells CCTL14. These stem cell-derived hepatospheroids showed improved heptocyte-specific characteristics and functions compared to monolayer cultures, and also increased sensitivity to cyanotoxins microcystin-LR (MC-LR) and cylindrospermopsin (CYN). MC-LR and CYN represent common aquatic contaminants and potent hepatotoxins, linked both to acute human intoxications and liver injuries, as well as associated with the development of chronic liver diseases, such as fatty liver diseases or liver cancer. However, such in vivo effects of MC-LR and CYN are difficult to study in vitro using traditional 2D cultures of hepatocellular carcinoma derived cell lines, since they are usually lacking expression of specific membrane transporters required for cellular uptake of MC-LR, or activity of phase I biotransformation enzymes implicated in CYN-induced toxicity. Using our 3D stem cell-derived hepatospheroid cultures, we were able to assess both acute liver cell damage induced by these cyanotoxins, as well as assess alterations of key cellular events involved in the development of liver steatosis/steatohepatitis. Thus, 3-dimensional (3D) culture techniques in combination with stem cells seem to represent a promising strategy to improve physiological relevance of liver in vitro models for evaluation of key molecular and cellular events relevant for adverse outcome pathways for liver toxicity and diseases. Acknowledgements: Research is supported by the Czech Science Foundation project No. GA19-19143S.

1.10P.11

Human 3D hepatic spheroids for in vitro assessment of hepatotoxic and steatogenic key events in response to endocrine disruptors.

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Liver steatosis or nonalcoholic fatty liver disease (NAFLD) is manifested by accumulation of fat by the liver cells, and represents the most common chronic liver disease in population. If not reversed, NAFLD can progress to steatohepatitis, and eventually into irreversible liver diseases (fibrosis, cirrhosis and hepatocellular carcinoma). There is increasing evidence suggesting links between NAFLD and chemical exposures, namely exposures to endocrine disruptors (EDs). However, current validated *in vitro* testing methods have only a limited ability to assess steatogenic- and metabolism-disrupting potential of EDs and related health hazards.In this study, we optimized 3-dimensional in vitro model based on human hepatic cell line HepG2 to evaluate hepatotoxic and steatogenic activity of selected EDs. Mature HepG2-derived spheroids showed increased expression of key hepatospecific genes, e.g. albumin, connexin32, drug-transporting proteins (OATP1B1, 1B3), or drug-metabolizing enzymes (CYP1A2). Hepatic spheroid cultures were adapted for 96-well microplate format and multiparametric evaluation of spheroid growth, morphology, viability/metabolic activity, membrane integrity and ATP content. These assays were complemented by high content fluorescence microscopy of cellular processes involved in development of liver steatosis and steatohepatitis, such as lipid accumulation, oxidative stress and mitochondrial toxicity. This setup was compatible with (semi-)automated workflow (liquid handling, automated readouts/imaging, image analysis) and suitable for (semi-)high throughput screening. For further mechanistic assessment of selected ED compounds and concentrations, the key molecular events central to hepatic steatosis and steatohepatitis (e.g. fatty acid and triglyceride metabolism, ER stress, or inflammatory responses), were further evaluated by gene expression analyses, functional biochemical and/or immunochemical assays. This study demonstrates that such 3D hepatic spheroid model can be used for *in vitro* assessment of key molecular and cellular events of AOPs for hepatic steatosis and steatohepatitis, and for evaluation of steatogenic potential of EDs or other environmental toxicants. Acknowledgements: This work was funded by the European Union's Horizon 2020 research and innovation program under grant agreement no 825712 - OBERON project (https://oberon-4eu.com), that is part of the European EURION cluster (http://eurion-cluster.eu/).

1.10P.12

Testing the acute toxicity of an alternative to glyphosate - pelargonic acid

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Glyphosate-based herbicides were used as plant protection product globally for several decades. However, glyphosate is discussed as showing genotoxicity and many other side-effects such as inhibiting the mitochondrial succinate dehydrogenase, leading to a decreased ATP production. Therefore, finding alternative active substances is necessary. Pelargonic acid (nonanoic acid) and its ammonium salt (saponified form), which are used as alternatives to glyphosate-based herbicides, are biological derived substances considered as environmentally friendly herbicides. To test the effects of pelargonic acid in its acidic form (active substance in TopGun) and its saponified form (in Finalsan Plus) on the aquatic ecosystem, students of the master study program "environmental management and ecotoxicology" compared the toxicity levels of these substances using zebrafish embryos within the scope of a student research course. The project was developed by applying the 7-step problem-based learning method which allowed the students to design their experiments independently with the guidance and feedback of the lecturers. Acute toxicity was determined according to OECD test guideline 236 in D. rerio which revealed a LC50 of 1.55 mg/L of pelargonic acid, a LC50 value of 0.93 mg/L pelargonic acid in TopGun and a LC50 of 36.37 mg/L of Finalsan Plus. Neutral Red Uptake assays were performed on the rainbow trout-derived gill cell-line RTgill-W1 to determine the acute toxicity according to the OECD test guideline 129 which revealed the IC50 value of 12.4 mg/L pelargonic acid in TG. Due to solubility limits, no reliable IC50 could be obtained. The results of the tests indicate differences in the acute toxicity of the pelargonic acid-based formulations TopGun and Finalsan Plus. However, more investigations have to be done in order to analyse if the acidic form is more toxic than the saponified form. Furthermore, detailed ecotoxicological risk assessments and an evaluation of the genotoxicity of both herbicides has to be performed before any conclusion can be drawn. Financial support from the City of Vienna project PBL in Molecular Life Science (21-06) is gratefully acknowledged.

1.10P.13

Whole-tissue gene and protein expression predict adverse outcomes of 17α-

ethinylestradiol in early-life stage fathead minnows (Pimephales promelas)

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Emerging chemical contaminants (ECC) pose significant environmental concern because of their potential adverse effects towards non-target aquatic organisms in exposed ecosystems. However, current approaches to assess the environmental risks of chemicals such as ECCs rely on extensive live animal testing, which is expensive, time consuming, and of significant ethical concern. To address these issues, there is increasing pressure to shift the current paradigm of chemical risk assessment towards one that is rooted in mechanistic toxicity data through the examination of the mode of action of a chemical and use this information to predict an apical outcome of regulatory relevance. However, there is significant need to calibrate and validate this approach by conclusively linking this mechanistic information to toxic outcomes. This study utilized non-target transcriptome and proteome approaches to characterize molecular toxicity pathways to predict adverse apical outcomes. Early-life stage fathead minnows (*Pimephales promelas*) were exposed to graded concentrations of EE2 (4, 20, 100 ng/L) and a solvent control immediately after fertilization. A subset of exposed larvae were collected four days post-hatch for whole transcriptome and proteome analyses. Another set of larvae were continuously exposed to EE2 until 28 dph for histopathological examinations. Genes and proteins associated with coagulation, a variety of proteins involved with cellular processing and metabolic processes, and necrosis were significantly dysregulated. Trans-omics analyses revealed core response modules that may be linked to observed histological observations. Hepatocyte basophilia and the abundance of eosinophilic proteinaceous fluid within the sinusoids of the liver parenchyma were observed. Similarly, larger tubules with dilated lumens and distorted structure, hypertrophied epithelial cells with vacuolated swelling, and swollen glomeruli with eosinophilic fluid were observed in the kidneys. These observations are likely to be the results of estrogen-induced severe upregulation of VTG, as well as increased energy demand. Results showed that trans-omics integration improved the biological interpretation of molecular perturbations by providing evidence of conservation of toxicity pathways across biological levels. This approach proved to be a powerful tool in furthering our mechanistic understanding of and predicting phenotypic responses of contaminants. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

1.10P.14

Low dose gamma radiation induce chromatin accessibility changes in zebrafish embryos

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Epigenetic mechanisms allow gene regulation in a developmental context and as a response to environmental stimuli and we have previously reported epigenetic effects of low dose gamma radiation in zebrafish (*Danio rerio*). At environmentally relevant dose rates of gamma radiation, exposure of zebrafish embryos (2.5-5.5 hpf) revealed significant dose response on differentially expressed genes. To elucidate our findings, zebrafish embryos were exposed to 10 mGy/h gamma radiation from a ^{60}Co source. Chromatin accessibility assay (ATAC-seq) were analysed with GUAVA, a graphical user Interface for the analysis and visualization of ATAC-seq data (Divate and Cheung, 2018). It revealed 58 gained closed and 123 gained open regions. Interestingly, no accessible chromatin were localized in the promoter regions and 68% in distal intergenic regions. Lindeman et al., 2019. *PloS ONE,* Vol 14, Issue2*, DIO:* 10.1371/journal.pone.0212123Divate and Cheung, 2018, *Front Genet*. 2018;9:250. doi:10.3389/fgene.2018.00250

1.10P.15

"Bellow the eye": using eDNA for the evaluation of environmental water quality in a tropical urban estuary

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Coastal regions are important areas for the development and reproduction of species. Estuaries play an essential role as areas of high diversity, place of refuge, protection, food and reproduction. It favours the establishment of fishing activities and provides the intense human occupation, as approximately half of the world's population lives in areas coastal areas, including the estuarine areas. Given the importance of these areas, pollution in estuarine regions becomes a matter of concern as anthropic factors, driven by disorderly urban and industrial growth, Located in Aracaju, the capital of the State of Sergipe (NE- Brazil), the Sergipe River estuary lies within a densely populated area with a poor basic sanitation, and receives a large quantity of raw an treated domestic sewage. Other activities that contribute to the poor water quality include shrimp¢_s aquaculture and deforestation of mangroves. These activities contributed to increase the organic matter that may led to a reduction in dissolved oxygen and increased levels of eutrophication throughout the Sergipe River estuary. There are high levels of anthropogenic activities upon the banks of the Sergipe River. These range from plastic, textile, ceramic and engineering industries. Studies to this date investigating water quality about the effects on the resident biota are scarce. This research aims to investigate the effect human activity has upon the ecosystems. The study will use water collected from the Sergipe River and a close by non-contaminated river (Real River) filtered through DNA filter units. The objective was to extract environmental DNA, perform PCR amplification and downstream Illumina sequencing. The PCR amplification targeted algae, bacteria and fish species. Allowing species to be identified and compare biodiversity across the sampling sites. The presence/absence of species was correlated with water physical-chemical parameters and contaminants, and acute toxicity was tested using *Mysidopsis juniae*. The obtained results show a relationship between different biota communities, the contaminants analysed and the toxicity assays, showing the susceptibility/resistance of some species to species contaminants. This work is funded through a MSCA Fellowship (H2020 – COFUND, SIRCIW > MINT), by EU, Welsh Gov. and Cardiff Univ. and through "TATU" by CONFAP, Royal Society, Newton Fund, Academy of Medical Sciences and British Academy for Humanities and Social Sciences.

One Health and the Environment (P)

1.11P.2

Effects of boat noise on the Lusitanian toadfish Halobatrachus didactylus: evidences from oxidative stress and energy metabolism parameters and linkage to reproductive success

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Marine traffic has increased in recent decades, becoming the most common and chronic source of ocean noise pollution. Important studies show that the presence of noise can drastically reduce the perception of sound by aquatic organisms, but the direct impact on individual fitness is poorly known. The Lusitanian toadfish Halobatrachus didactylus is a common species in southern Portugal. Males rely on advertisement calls to attract females to their nests, a fundamental process for successful reproduction. The aim of this study was to evaluate the impact of boat noise on the reproductive success of this species and to further assess the effects of this noise stressor using biomarkers of: 1) oxidative stress – activity of the antioxidant enzymes superoxide dismutase and catalase, and levels of oxidative damage (lipid peroxidation and DNA damage); 2) energy metabolism – measurement of cellular respiration by the activity of the electron transfer system (ETS), and activity of the enzymes lactate dehydrogenase (LDH) and isocitrate dehydrogenase as a measure of anaerobic and aerobic metabolisms, respectively. For two consecutive mating seasons two sets of 12 concrete nests were exposed for two fortnights to either control (playback of ambient noise) or playback of boat noise. The noise treatment simulated the passage of 10 ferries and of 4 small boats per hour, 18 hours per day, similar to what fish may experience in the Tagus estuary. In each fortnight, a nest-set was either exposed to ambient noise or boat noise treatment, so that the two nest-sets would receive both treatments to control for site effect. At each fortnight, nests were checked for the presence of eggs and the attending territorial males. Males with eggs in the nest were sacrificed and muscle and liver samples were taken for the biochemical analyses. The eggs found in the nests were removed and randomly distributed for either control or playback of boat noise for the next 2 weeks, after which eggs and embryos were collected for biomarkers evaluation. Our results demonstrated that boat noise induces biochemical alterations in different life stages of toadfish. The potential of the selected biomarkers of oxidative stress and energy metabolism as sensitive endpoints to evaluate effects of noise in the Lusitanian toadfish Halobatrachus didactylus is discussed.

1.11P.3

Use of RNA sequence analyses to identify novel modes of action of oil toxicity to early life stages of fish.

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Polyaromatic hydrocarbons (PAH) from oil impairs heart development in early stages of multiple fish species. RNA sequencing of mRNA and microRNAs have indicated disruption in the expression of genes important in ionic transport and cholesterol biosynthesis that correlate with morphological changes in cardiac development. Injections of zebrafish embryos with microRNAs have replicated 3-ring PAH-like cardiotoxicity including decreases for the cardiac, specific potassium ion transporter, *kcnh6* and the calcium ion transporter *ryr2*. In addition, significant reductions in cholesterol with hearts were also observed in zebrafish embryos treated with the 3-ring PAH phenanthrene and the cholesterol-statin agent, atorvastatin. Pre- or co-treatment of phenanthrene with cholesterol could not restore cardiac function. These results demonstrate the utility of genomic and epigenomic analyses in better understanding the mechanisms of PAH toxicity in early life stage fish. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

1.11P.4

Histopathology lesions linked to pop levels in tissues from blue shark

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The constant loading of marine ecosystems with persistent organic pollutants (POPs) is one of the biggest issues in the present Era. Quantification of pollutants' concentrations alone, although providing important knowledge about the environment's contamination levels, does not give insights to the way organisms are affected. For this, linking these measurements with biological endpoints such as histological damage is essential. By bioaccumulating and biomagnifying pollutants from their prey and their environment, big, long-lived predators tend to amass pollutants in greater quantities than other organisms. Sharks, like *Prionace glauca,* are extremely important parts of marine ecosystems. This species' worldwide distribution and the fact that is the most abundant species of shark in the World has drawn scientist's attention to its potential as a sentinel in biomonitoring studies. Based on previous research and to further inquire about the suitability of *P. glauca* as a sentinel species in marine pollution monitoring surveys, blue sharks were sampled aboard a commercial swordfishing boat operating in Portuguese waters. An extensive set of organs/tissues were sampled to assess contaminant body burden (i.e. POPs) and perform a descriptive study of injuries. The most important histopathological findings found were: in brain (neuronal vacuolisation, neuronal degeneration -satellitosis and neuronophagia-, demyelination and presence of perivascular cuffings), in gills (epithelial hyperplasia of the lamellae, chloride and mucus cells proliferation and capillary vessels dilation), in kidney (Bowmann´s capsule thickening, Bowmann´s space dilation, degeneration and necrotic changes in the epithelial cells of the distal tubules) and in liver (massive steatosis phenomena and connective tissue proliferation). Some of these histopathological alterations could be correlated with the contaminant body burden, emphasizing the negative effects of POPs, as well as the huge potential of *P. glauca* to be used as a sentinel of marine pollution.

1.11P.5

Skin as an alternative to traditionally invasive tissues in biomonitoring campaigns using Scyliorhinus canicula as a sentinel

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When it comes to marine pollution, sharks are some of the most affected organisms due to their top position in the trophic chain and their biological characteristics, such as long lives. This, along with the fact that they are consumed by humans makes them interesting subjects for environmental contamination assessment. Traditional studies assessing the impacts of contamination on sharks rely on very invasive (at times lethal) sampling. With the aim of improving animal welfare and to allow long term repeated sampling campaigns, less-invasive alternatives are needed. Since skin biopsies have already shown promise with large endangered animals (i.e. cetaceans), this work aimed to assess the potential of this tissue to be used in the assessment of metal contamination and biochemical responses in sharks. The Atlantic lesser-spotted dogfish (*Scyliorhinus canicula*) was chosen as a test species, and a total of 74 specimens were captured aboard a commercial trawling vessel operating in Portuguese coastal waters. Muscle and skin were sampled from each individual, and a total of 13 metals, along with several energy metabolism and oxidative stress related biochemical responses were measured. Overall, contamination by metals was greater in skin than in muscle. Nevertheless, several positive correlations were identified between both tissues for the tested metals. Biochemical responses, mainly energy metabolism related, also seemed to be similarly influenced by the same metal contaminants in both tissues. All correlations and interactions found in-between metals and biomarkers point to a pattern of similar response both in deeper muscle and skin biopsies. Overall, the correlations found throughout this study indicate that skin has the potential to replace muscle in future biomonitoring and non-lethal campaigns using this shark species.

Perspectives on Data Driven Biology: Applications and Safety Assessments (P)

1.12P.1

EcoToxDB: a toxicogenomics knowledgebase to query, browse and interrogate chemical-gene-exposure networks

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The EcoToxChip project aims to develop, test, validate, and commercialize quantitative PCR arrays (EcoToxChips) and a data evaluation tool (EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures of regulatory concern. EcoToxDB is a knowledgebase that provides convenient access to all gene expression profiles generated using the EcoToxChip (as well as a range of other data such as metabolomics, proteomics, transcriptomics, chemical residue values, histology). EcoToxDB was purposefully designed through careful consultations with project partners (primary focus are Canadian regulators) and the user community. It is thus designed to be a resource (FTP and web-based) to query and browse results initially from 52 unique chemical exposure studies (8 chemicals across 6 species and 2 lifestages) plus dozens more being realized through project partners on key models (i.e., fish-fathead minnow; bird-Japanese quail; amphibian-Xenopus laevis) and ecological species (fish: rainbow trout; bird: double-crested cormorant; amphibian: Northern leopard frog) of relevance to the Canadian and international regulatory community. Currently, EcoToxDB holds data from six species, eight chemicals, 47 exposure studies, 409 samples and annotations for thousands of genes. Via EcoToxDB, users will be able to not only access raw or summarized data but also build chemical-gene exposure networks and thus explore deeper scientific questions. EcoToxDB will be hosted on a MongoDB server and will be accessible through an intuitive web interface. A set of application programmable interfaces (APIs) will also be developed for efficient loading of data into other related applications. Through an associated FTP site, protocol files, and raw and processed versions of EcoToxChip internal data will be available for download. EcoToxDB.ca will contain demonstrations, FAQs, tutorials, and other resources to best assist all levels of users. The database is designed to help researchers and scientists explore results of exposure studies on ecological species. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

1.12P.2

EcoToxChip: A toxicogenomics tool for chemical prioritization and environmental management

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Here we provide an overview of the EcoToxChip project which is a Canadian effort that aims to develop, test, validate, and commercialize quantitative PCR

arrays (EcoToxChips) and a data evaluation tool (EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures of regulatory concern. In Project Phase 1, EcoToxChips are being developed for laboratory model species representing the most important vertebrate groups in ecological risk assessment (fish-fathead minnow; bird-Japanese quail; amphibian-*Xenopus laevis*). Model species (adult and early-life stage, ELS) are being exposed via standardized tests to 8 environmental chemicals representative of natural resource/environment sectors of Canadian concern and also ones that impact a wide biological space (EE2, chlorpyrifos, benzo(a)pyrene, lead, fluoxetine, selenomethionine, trenbolone, HBCD) (Activity 1). An integrative systems approach based on functional 'omics (combined global transcriptomic and proteomic profiling, targeted metabolome) and physiological analyses across levels of biological organization is being applied to characterize relevant toxicity pathways including adverse outcome pathways, AOPs (Activity 2); from this, and other resources, species-specific EcoToxChips consisting of 384 environmentally-responsive genes of regulatory concern are being informed, built, tested, and optimized (Activity 3). EcoToxChip performance is currently being validated (and further optimized) with our collaborators with respect to technical, biological, and user experience factors (Activity 4). Under Activities 5-7, knowledge from Phase 1 is being translated to 3 native species (i.e., fish: rainbow trout; bird: double-crested cormorant; amphibian: Northern leopard frog). EcoToxXplorer.ca is live now and provides intuitive bioinformatics support. To position the team advantageously with regard to the commercialization and institutionalization of the deliverables, our GE3LS research will produce and leverage social science knowledge about the phenomenon of "institutional entrepreneurship". The anticipated socioeconomic benefits associated with the adoption of our deliverables include, more focused animal testing, improved regulatory decision-making, and cost-efficiencies. Here we provide a 42-month update of our project (www.ecotoxchip.ca), specifically showcasing the technical performance results of the EcoToxChips from the validation tests.

1.12P.3

Semantic data access for toxicological research.

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The integration of data is a challenge in many toxicological research tasks. We aim at integrating the required data for the specific task of ecological risk assessment into a semantically annotated knowledge graph. This Toxicological and Risk Assessment (TERA) knowledge graph integrates large knowledge bases of chemical effect data, species taxonomy and compound classifications. We have automated the process of integrating and mapping these sources by the use of Semantic Web Technologies. We demonstrate powerful data inference and access using a suite of software tools over the knowledge graph. The tools are openly available at in a repository.

Wildlife Ecotoxicology: From Sub-lethal Responses to Adverse Effects at the Individual and Population Level (P)

1.13P.1

Levels of Polychlorinated Biphenyls Are Still Associated With Toxic Effects in Harbour Porpoises (Phocoena phocoena) Despite Having Fallen Below Proposed Toxicity Thresholds

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Polychlorinated biphenyls (PCBs) are toxic, persistent and lipophilic chemical compounds that accumulate to high levels in harbour porpoises (Phocoena phocoena) and other cetaceans. It is important to monitor PCBs in wildlife, particularly in highly exposed populations, to understand if concentrations are declining and how levels relate to toxicological thresholds and indices of health like infectious disease mortality. Here we show, using generalised additive models and tissue samples of 814 UK-stranded harbour porpoises collected between 1990 and 2017, that mean blubber PCB concentrations have fallen below the proposed thresholds for toxic effects. However, we found they are still associated with increased rates of infectious disease mortality such that an increase in PCB blubber concentrations of 1 mg kg-1 lipid corresponds with a 5% increase in risk of infectious disease mortality. Moreover, rates of decline and levels varied geographically, and the overall rate of decline is slow in comparison to other pollutants. We believe this is evidence of long-term preservation in the population and continued environmental contamination from diffuse sources. Our findings have serious implications for the management of PCB contamination in the UK and reinforce the need to prevent PCBs entering the marine environment to ensure that levels continue to decline.

1.13P.2

An overview of the last 35 years of research into lead contamination in European raptors

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Lead (Pb) is a widely distributed toxic heavy metal that affects wild birds. The ingestion of Pb from hunting ammunition has been shown to be the main source of exposure and raptors are particularly at risk due to their feeding habits. Given the evidences of Pb toxicity and its health impact on birds, several EU countries have implemented national regulations to ban the use of Pb ammunition in wetlands and other protected areas. However, Pb remains an important health problem for raptors around Europe. As part of the European Raptor Biomonitoring Facility (ERBF) COST Action (https://www.cost.eu/actions/CA16224), we performed a systematic review on Pb contamination in raptors in Europe. We aimed to compile all the published data reporting lead contamination in European raptors and, through a meta-analysis, analyse how Pb levels vary between species and whether there are clear spatial and temporal trends in contamination. We searched Web of Science, PubMed, ScienceDirect and Scopus and we found 113 articles related to Pb and raptors in Europe which were published between 1983 and 2019 (search done until February 2019). We included 50 papers in the meta-analysis which fulfilled specific inclusion criteria. Almost half of the studies were published in the last decade and many were from Spain. Little specific information was available on Pb in raptors from Eastern Europe (except for Poland) or from Scandinavia. Published studies covered 36 raptor species. The common buzzard (*Buteo buteo*), Barn owl (*Tyto alba*) and Tawny owl (*Strix aluco*) were the most studied bird of prey and owls respectively. Liver was the matrix that was analysed most frequently although feathers have been increasingly used for monitoring since 2010s. Half of the published studies reported Pb levels above the thresholds described to cause (subclinical and clinical) effects on birds (thresholds: liver and kidney > 6 µg/g dw; bone > 10 µg/g dw; blood > 20 µg/dL). Finally, this work will also inform on the species with the highest Pb levels and will provide a pan-European evaluation of the spatial and temporal trends of Pb contamination and poisoning in raptors.

1.13P.3

Wide scope screening of emerging contaminants in peregrine falcon Falco peregrinus in The Netherlands

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Terrestrial species are exposed to multiple environmental stressors but there is a paucity of long-term monitoring data for them compared with marine species. Long-term monitoring of contaminants in apex predators and their prey can be used to assess the effectiveness of chemicals risk mitigation measures and identify emergence in the environment of substitute chemicals which may present similar levels of hazard. This in turn can support better chemicals management. The Naturalis Biodiversity Center working with the Dutch Peregrine Working Group has initiated long-term contaminant monitoring of breeding peregrine falcon in The Netherlands. Annual sampling of peregrine chick down, prey remains, eggshell fragments and pellets from nests during routine ringing was started in 2015, together with opportunistic sampling of adult carcasses, with a view to assessing exposure to legacy and emerging contaminants. The peregrine is a protected species and sampling has been restricted to non-invasive methods with minimal disturbance to breeding birds. In the past, the peregrine has shown high sensibility to legacy contaminants such as DDT and there is a possibility of toxic effects from the large number of emerging contaminants used in a multitude of products in Europe. Analyses of samples from top predators and their prey using high-throughput analytical methods such as high-resolution mass spectrometry (HRMS) and non-target screening (NTS) allows for identification of a theoretically unlimited number of chemical substances in a sample. Validated generic sample preparation protocols able to retain compounds with wide physicochemical properties were utilized to extract both polar and non-polar emerging contaminants. The extracts were analyzed by LC-ESI-QTOFMS and by GC-APCI-QTOF for more than 2400 emerging contaminants. Furthermore, chromatograms were digitally archived in NORMAN Digital Sample Freezing Platform for further retrospective suspect screening. In this study, we present preliminary results from adult liver samples collected in 2017. We set this within the content of long-term data on the reproductive success of peregrine and on peregrine ecology in The Netherlands, to shed light on possible contaminant interactions with other environmental stressors.

1.13P.4

Environmental health assessment based on a complementary approach using metal quantification, oxidative stress and trophic ecology of two sympatric gull species (Larus michahellis & Larus audouinii)

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Metal pollution is currently a major issue in marine ecosystems, as organisms are exposed and accumulating increased levels from several anthropogenic sources. Seabirds have been proved to be suitable bioindicators of marine environmental health, providing valuable information on trophic relationships and quality of resources. This study aimed to: (1) assess metal contamination in two gull species breeding in sympatry, and in two distinct colonies separated by ca. 400 km; (2) relate their trophic ecology with metal concentrations; and (3) evaluate their oxidative stress in relation to their specific trophic ecology and metals' concentration. Data were collected during 2017 in the western and southern coasts of Portugal. Blood and feathers were collected from Yellow-legged gulls *Larus michahellis*, in the two sites, and Audouin's gulls *Larus audouinii* in the southern coast. A set of 13 (essential and non-essential) metals were quantified and oxidative stress was measured through four biomarkers (DNA damage, LPO, OXY and d-ROM assays). Additionally, stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) were used to link metal contamination and oxidative stress with the trophic ecology of each species/population. There was a clear segregation of metal contamination between the two species and to a lesser extent between colonies. Overall, Audouin's gull was the most contaminated species for most metals, presumably because this species relies mainly on fish and other marine resources. The Yellow-legged gull feeds regularly on other food sources besides fish, including those from terrestrial environments, which may dilute contamination levels. However, Yellow-legged gulls from two distant colonies exhibited similar values for most metals. Oxidative stress responses were related with birds' trophic ecology and foraging habitat, but apparently not with metal contamination. In an environmental health assessment perspective for the North Atlantic, particularly along the Portuguese coast, this study showed a substantial exposure of these gull species/populations to some specific metals.

1.13P.5

Embryonic exposure to persistent organic pollutants and mercury in marine birds and investigations into associated alterations in hepatic gene expression

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Marine birds can bioaccumulate considerable persistent contaminant body burdens, and maternal transfer to eggs may expose sensitive embryos to high concentrations. Yet, deleterious effects inferred from tissue residue concentrations suffer from uncertainty, and high-throughput techniques to assess biological effects caused by persistent bioaccumulative contaminants or potential environmental disasters like oil spills are lacking in wildlife sentinel species. Our research focuses on two wildlife sentinel species, the rhinoceros auklet (*Cerorhinca monocerata*) and the double-crested cormorant (*Phalacrocorax auritus*), which occupy an offshore-nearshore habitat gradient, and aims to demonstrate the utility of hepatic gene expression for linking embryonic contaminant burdens to transcript-level effects *in vivo*. To quantify embryonic body burdens, we measured the concentrations of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), brominated and chlorinated flame retardants (FRs), and total mercury (THg) in developed (pre-hatch to pipping) sentinel species embryos from breeding colonies in the northeast Pacific Ocean from 2017-2019. Some THg observations in *C. monocerata* (0.3-0.6 µg g^{-1} ww) fall within literature values for embryotoxicity in this order of seabirds (LC_{50} 0.3-4.3 µg g^{-1} ww), and contaminant burdens are considerably higher in *P. auritus* embryos from Vancouver harbour than *C. monocerata* from offshore colonies. We are currently profiling hepatic gene expression in the sampled embryos with ToxChip qPCR arrays to determine whether contaminant exposure is associated with dysregulation of genes involved in pathways including xenobiotic metabolism/biotransformation, oxidative stress, thyroid hormone function, lipid metabolism, and many others. Together, these data will signal whether an embryotoxicity hazard from THg and other persistent halogenated contaminants to marine birds in and around the Salish Sea exists, if any spatial patterns exist, and if these correlate to liver gene expression profiles.

1.13P.6

Effect of Diluted Bitumen on the Survival, Physiology, and Behaviour of the Zebra Finch (Taeniopygia guttata)

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Bituminous petroleum dominates crude oil production trends in Canada. Given the ongoing production and transport of diluted bitumen (dilbit), including projected increases to shipment by sea from Vancouver, British Columbia, there is a need for *in vivo* toxicity data to assess the potential hazard of dilbit spillage to wildlife. Peer reviewed literature on dilbit toxicity is limited to teleost fish, despite the importance of coastal lands and waters as important habitat for bird fauna, an ecological receptor vulnerable to oil spills. In this study we used the zebra finch (*Taeniopygia guttata*) as a tractable avian model system for preliminary oral exposure studies on Cold Lake blend dilbit. Objectives were to 1) determine appropriate methods of establishing dilbit toxicity to birds, 2) determine a range of lethal and sublethal doses, and 3) obtain meaningful physiological and behavioural responses. We conducted three 14-day oral gavage trials resulting in an LD_{100} at 12 ml kg^{-1} (body weight) d^{-1}, and an LD_{50} at 10 ml kg^{-1} d^{-1}. Mortality was associated with significant mass loss, incidental plumage oiling, elevated total antioxidant titers in blood plasma, and decreased relative pectoral muscle mass. In addition, we found evidence for sub-lethal effects at dilbit doses less than 10 ml kg^{-1} d^{-1}, such as elevated hepatic EROD biotransformation enzyme activity. Further sub-lethal effects include changes in the activity behaviours of treated birds on day 6, such as increased sleep-like behaviour and decreased self-maintenance behaviours such as preening. Surprisingly, we found no effect of dilbit on hematocrit or hemoglobin even though anemia or erythropoietic response is often reported in conventional crude oil-exposed birds.

1.13P.7

Repellency, anorexia, and aversion by neonicotinoid-treated seeds and cotyledons on captive eared doves (Zenaida auriculata, Columbidae)

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Granivorous birds are exposed to neonicotinoids used as seed treatment for both treated seeds that remain unburied after sowing and, since they are systemic insecticides, for cotyledons from treated seeds. As the avoidance of contaminated food is a behavior that can result in a reduction in the amount of toxic ingested, the aim of this study was to evaluate the avoidance (repellency, anorexia, and aversion) to sorghum and soybean seeds, and cotyledons treated with neonicotinoids on a captive South American granivorous bird (eared doves, *Zenaida auriculata*) by one-choice tests in individual cages. After acclimatization of the birds to individual cages and a period to accustom the birds to the untreated food, the doves were exposed to the treatments in two periods of 3 or 5 days of duration separated by a rest period, and, in the end, they were exposed to untreated test food to assess whether neonicotinoids induced specific seed aversion. With sorghum, the three neonicotinoids produced a marked repellent effect, decreasing 98.2% (imidacloprid), 99.1% (clothianidin) and 97.4% (thiamethoxam) the consumption compared to control birds. However, this repellency was not enough to prevent intoxication signs and the death of 3 of the 8 (37.5%) and 1 of the 8 (12.5%) of the doves exposed to imidacloprid and clothianidin, respectively. Only 12 of 40 doves consumed soybean, of which only three birds ate more than three soybeans per day. In them, imidacloprid-treated soybeans caused a significant repellent effect. Instead, the repellent effect of imidacloprid and thiamethoxam was not significant in cotyledons. The birds did not learn to avoid the test food (absent conditioned aversion), suggesting the importance of the color to produce the rejection effect that neonicotinoids give to the seeds. Signs of anorexia were observed only against the neonicotinoid-treated sorghum. In conclusion, with the commonly used doses, the rejection caused by the neonicotinoids was strong in the seeds but failed to prevent intoxication and death in the case of treated sorghum on eared doves. In contrast, soybean cotyledons failed to produce a rejection effect and the birds showed no signs of intoxication. These results show the need to assess the risk of each neonicotinoid separately, considering the bird's food preferences, doses and moments, and the crops in which they are applied.

1.13P.8

The ingestion of copper treated seeds during winter has lagged effects on reproductive output of red-legged partridges

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Due to reductions in winter food resources, newly sown cereal seeds have

become a key component of many bird species' diet, but these seeds are often treated with pesticides that may cause toxic effects. Copper has been used as a fungicide in agriculture since the beginning of S XIX and its first documented use was as a seed treatment. Nowadays it is still widely used and its use is accepted in organic farming. However, its effects on wildlife continue to be poorly studied. The aim of the present experiment is to test the effects of the ingestion of seeds treated with copper oxychloride on red-legged partridge (*Alectoris rufa*) physiology, fitness and reproduction. Partridges (n=11 to 13 pairs per treatment) were fed with wheat seeds treated with 0% (C), 20% (LD) and 100% (HD) of the copper oxychloride application rate during 25 days in late winter. Exposure doses were estimated to be 112 and 22 mg/Kg bw/day in HD and LD groups respectively. Exposed partridges did not reduce food consumption or body condition during the experiment compared with controls but partridges in the HD group presented reduced plasmatic levels of triglycerides (p=0.03), total proteins (p=0.02) and tocopherol (p=0.02) and increased levels of magnesium (p=0.03), the latter effect occurring in females only. Phenotypical effects (a reduction in the carotenoid-based pigmentation of the eye ring) were also detected in the HD group (p=0.02). Exposure of partridges finished 50 days before the first egg was laid, but reproductive effects were observed in the HD group. The fertile egg ratio (mean±SE) was reduced in the HD group (58.2±13.4%) compared with the control group (85.7±9.2; p=0.07). The effect in the total success of the reproduction (% hatched eggs from the total eggs) was even more pronounced; mean success was 61.4 ± 10.6% in the control group and 32.8 ± 10.4 % in the HD group (p=0.06). These effects finally resulted in a very reduced brood size in the HD group (2.7 ± 1.0) compared to the control group (6.0 ± 0.9; p=0.05). Our results show that exposure to high doses of copper, outside the reproductive period, leads to physiological alterations and to a lagged effect that becomes apparent later during reproduction. Although the effects are only visible in the worst case scenario, field studies have shown that this is a realistic scenario. Once again it is demonstrated that seed treatment is a risky practice for farmland birds that depend on newly sown seeds to survive in winter.

1.13P.9
Do court sentences reflect the reality of wildlife poisoning in the natural environment? The Spanish case.

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Although poisoning of wildlife is considered, since 1995, as a crime by the Spanish Penal Code, it is a deeply rooted habit of the population. The illegal use of poisoned baits to kill "unwanted" animals, such as predators, and specially birds of prey is a great threat for both domestic and wild animals. Poisons to kill animals are not selective; therefore animals poisoned by baits or other legally used pesticides, may suffer secondary poisonings. In Spain, according to WWF, between 1992-2013, the number of animals poisoned reached 18,503 individuals, including kites, vultures, eagles, wolves and bears. According to the Life+Veneno Project, 80 Court sentences about wildlife poisoning have been pronounced in Spain between 1994 and 2019. The aim of this study was to review these sentences comparing the data with epidemiologic data reported by the regional authorities, in order to evaluate if the court sentences reflect the real situation of wildlife poisoning in Spain. The compounds involved in poisonings in those sentences partly match with those found in the document published in 2016 by WWF and SEO/Birdlife. Aldicarb and carbofuran are the compounds most commonly used to kill wildlife, followed to a lesser extent, by two convulsivant compounds (strychnine and endosulfan), other cholinesterase inhibitors and a second generation anticoagulant rodenticide, bromadiolone. The court sentences handed down in Spain include 439 poisoned animals, mainly birds (72%) and mammals (25%). Fifty percent of these animals (n=215) are necrophagous: 114 Black kites (Milvus migrans), 42 Red kites (Milvus milvus), 41 Griffon vultures (Gyps fulvus), 10 Cinereous vulture (Aegypius monachus), 6 Spanish Imperial Eagle (Aquila adalberti), 1 Bearded vulture (Gypaetus barbatus) and 1 Golden Eagle (Aquila chrysaetos). The mean time elapsed between the crime and delivery of the judgment of the Court of First Instance is close to 3 years (median, 2 years). According to the Spanish Penal Code, this offence carries a maximum prison sentence of two years and/or economic fines. Moreover the poisoner can be disqualified from exercising the right to passive suffrage and the right to practice hunting activities. The vast majority of the Court sentences concluded with guilty verdicts (n=74, 92.5%). Imprisonment sentences were imposed in 32 of the trials; while economic fines and disqualification to hunting activities were imposed in 60 of them. Funded by Seneca Foundation, CARM (20945/PI/18).

Innovative Assessment Tools and Criteria for the Protection of Ecosystems and Human Health (P)

1.14P.1
Smart genomic technology to prompt identification of pollution sources in aquatic ecosystem when extreme weather events happen.

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Global environmental change cause negative effects on aquatic ecosystems that might affect also human health. European Directives define microbiological and chemical parameters, limit values and methods for the assessment of water quality to be used in the monitoring programmes (e.g. surveillance, operational) while there are not criteria or methodologies available for the emergency monitoring when extreme weather event happen. In particular, currently available methods for surface water microbiological monitoring are based on the analysis of a limited number of cultivable marker species and requires laborious and time-consuming microbiological approaches. In recent years, several researches have been focused on the exploration of the potentiality of smart technologies to characterize the microbiological contaminants with the purpose to prevent human health risks. "Development and application of Novel, Integrated Tools for monitoring and managing Catchments" (INTCATCH) is a HORIZON2020 project funded by the European Union, which aims to deliver new innovative tools for monitoring and managing of surface waterbodies in Europe. One of the goals of the project is to develop innovative smart technologies able to provide in real time relevant informations on the microbiome of surface waters. The autonomous boats included in the project were equipped with a filtration system and connected with the portable genomic laboratory. The filtration system allows the concentration of water samples necessary for the metagenomics analysis, based on Next-Generation-Sequencing (NGS) for the characterization of microbiome in different surface waterbodies. The comparison of results obtained by metagenomic method vs those based on microbiological approach were performed analyzing water samples from four sites of the Garda lake. The results have showed that the proposed system allows an effective detection of microorganisms. The use of smart technologies developed by the project will help to promptly identify pollution sources and detect microorganisms relevant for human health, providing rapid responses in the case of extreme weather events, such as flooding. In addition, the proposed methodology, which is able to link microbiome changes to the effects of chemical contaminants or other stressors, could provide an effective support for the investigative monitoring under the Water framework directive.

1.14P.2
Can macrophytes be a novel tool for trapping microplastic in aquatic ecosystem?

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Microplastics (MPs) can occur into aquatic ecosystem through atmospheric fallout, indiscriminate disposal or mishandling of plastic waste and incomplete screening of wastewater effluent. To deepen the knowledge about environmental fate of these new contaminants, the aim of this study was to investigate their presence and interaction with aquatic macrophytes. They are aquatic plants that grows in rivers or on the bank and are either emergent, submerged or floating. Furthermore, they are one of biological element for the ecological status assessment according to Water Framework Directive 2000/60/EC. Two typologies of rivers, Farfa River and Almone River, were selected to perform this study. The Rivers are located in Latium Region (Central Italy), one in near natural conditions area and the other in the urban area of Rome. A total of six sampling sites were studied twice a year. Sampling and analysis Protocols for MPs recovery in water, sediment and aquatic Macrophytes were set up.Preliminary results, showed that MPs were found in all studied sites: for water and sediment samples an increasing gradient of MPs was observed from upstream to downstream. In macrophytes sample, higher concentration of MPs than in other matrices was recovered.The analysis of the three components could be useful to better understand the environmental fate of MPs in aquatic ecosystems and the possible interaction between MPs and biotic communities. These results are indeed very interesting because Macrophytes root and/or leaf can work as a trap for MPs particles.

1.14P.3
Application of Effect-based Methods (EBMs) in a river basin: a preliminary study in Central Italy

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The aim of the study was to evaluate the water quality of an Italian river ecosystem. Sampling was conducted in the final stretch of the Tiber River basin (Central Italy) in two different seasons. Effect-based methods (EBMs) were applied for assessing potential ecotoxicological effects caused by chemical pollutants and contaminant mixtures, using two *in vivo* bioassays: Daphnia sp. Acute Immobilization assay (OECD 202/2012) and the Fish Embryo Acute Toxicity (FET) test (OECD 236/2013) with zebrafish. In addition, sublethal endpoints were investigated for zebrafish embryos such as spontaneous coiling tail (STC), morphological deformities, cardiological parameters. The sublethal effects in fish embryos are considered innovative and a very promising tool in water quality research and their application in short-term ecotoxicity detection has been recently introduced in European projects. Three sampling sites were chosen: two in the urban part of the Tiber River in Rome and one as a reference site in the Farfa River, a tributary upstream of the city. The sites in the Tiber River are potentially affected by different pollution sources, including urban and industrial wastewater discharges, release of pesticides, livestock waste products, and waste dumps. The results of the study showed differences between the two selected species and the three samples. Zebrafish embryos were generally more sensitive than *D. magna* in detecting even low effects in all the water samples, especially related to sublethal endpoints. The results of this research confirm the effectiveness of EBMs in investigating and monitoring water chemical pollution, and stress the need to perform further studies, e.g. chemical analyses and other bioassays, to improve the knowledge of the health status of the Tiber River basin. Further results will aim to support the local authorities in adopting measures to reduce and to eliminate the sources of chemical pollution in this area.

1.14P.4

Alternative methods to animal testing: the zebrafish earliest life-stages as a tool for the detection of chemical pollution effects in the context of Water Framework Directive

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The Water Framework Directive (WFD) addresses the European Union (EU) member countries to achieve a good quality status of all water bodies. The WFD measures have allowed the reduction of the discharge, release, and emission of several priority substances, with the aim of mitigating the risks that chemical pollution poses to the environmental and public health. Moreover, a Watch List (WL) of emerging pollutants was adopted in this context for the monitoring of new chemical substances. However, a monitoring plan for chemical mixtures is missing, and only a tiny fraction of substances widespread in the ecosystems is regularly monitored. The chemical analysis alone is not sufficient to face the complex consequences of chemical pollution for the organisms living in the water bodies. Therefore, innovative monitoring strategies must take into account new tools for the detection of effects arising from environmental pollutants. The effect-based methods (EBMs; i.e. bioassays and biomarkers) are essential in filling these gaps and reach the WFD goals. In vivo bioassays allow the evaluation of the chemical pollution effects directly on a living organism. However, an important issue related to these methods is the requirement of using living animals for the experimental analysis. According to the EU Directive 2010/63/EU, the earliest life-stages do not fall into the regulatory frameworks dealing with animal testing and meet the principle of the 3Rs. The zebrafish (Danio rerio) earliest life-stages, i.e. embryos and early larvae, have been considered as a replacement tool for animal experiments. Zebrafish represents an emerging vertebrate model to detect the effects of chemical substances. Particularly, the use of zebrafish earliest life-stages allows the analysis of multiple lethal and sublethal endpoints as well as the assessment of specific modes of action (MoA) induced by chemicals. Despite the use of this model has been increasing, a broad literature review on zebrafish earliest life-stages for the WFD regulatory purposes has still been missing so far. We reviewed a large number of studies on the effects for zebrafish earliest life-stages of exposure to the chemical substances included in the WFD and the related WL. We totally found 30 endpoints due to 10 different MoA that affect embryo development. Considering the great advantages of using zebrafish earliest life-stages, this model should be encouraged in the screening of chemical pollution of water bodies.

1.14P.5

A preliminary study comparing a toxicity assessment instrument output to traditional laboratory assays using Daphnia magna

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The continual monitoring of water quality can be challenging with the wide range of pollutants present in the environment. An ecological approach to this utilises an indicator species monitoring system, to observe changes in target species. The Daphnia Toximeter (DT) is a commercial application of this, and is an instrument that has been designed to used with wastewater treatment work (WWTW) outflows as a tool to measure the water quality, however there is very little literature available discussing the use of this instrument for research applications. The DT has been configured to trigger an alarm if the through flow water in the instrument causes a change in the daphnia behaviour. Although the application of this instrument is typically for field monitoring of water sources, in this study we are comparing the outputs of this instrument to lab-based assays. The aim of this is to compare the end points and increase the resolution of the data of the 48-hour OECD 202 test with daphnia. We aimed to determine if the DT had similar toxicity outputs for both a dissolved chemical, Sodium Lauryl Sulphate (SLS) and dispersed nano materials (gold nano particles). SLS is a commonly used surfactant which has been shown to have an EC50 of 1.2-7.5 mg/L during the OECD 202 test with daphnia. Gold NM (both spheres and rods with cationic surfaces) have previously been shown to change daphnia behaviour, specifically in terms of the carapace moult, indicating that that daphnia may divert energy from moulting to deal with oxidative stress caused by the surface interaction with the charged particles. This study aims to see if the DT can detect this change in movement due to the charged particles, which has been anecdotally observed as a jerky movement of the daphnids. Both the traditional laboratory assays and the DT experiments were conducted using controlled laboratory medium and temperature, and pH and dissolved oxygen were monitored throughout. WWTW often don't have the capacity to remove NMs and therefore NMs have the potential to be present in the outflow in varying concentrations, so it is interesting to see the DT detection limits for the sublethal effects of this type of water contamination compared to a more simplistic chemical exposure.

1.14P.6

A new structural alerts scheme to predict the absence of well-known endocrine disruption modes of action

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Endocrine disruption (ED) potential of substances is of high concern for human health and environment, as reflected in the updated chemical regulations. Under increasing regulatory pressure to reduce animal testing and due to the complication and cost of studies to determine ED properties, in silico methods may be used advantageously. We are developing an in silico test battery to predict with high probability that a test substance doesn't act through the best understood ED modes of action, i.e. related to estrogenic, androgenic, thyroidal and steroidogenic (EATS) modalities and thus is unlikely to meet the criteria to be classified as an ED compound according to the EFSA/ECHA guidance. Our tiered approach comprises: 1) 2D structural alerts schemes (internal and external), 2) 3D molecular docking, 3) 3D molecular dynamics. This communication presents the strategy and the preliminary results obtained with the internally developed 2D structural alerts scheme. This scheme identifies fingerprints in the chemical structure responsible for the ED MoA, based on in vivo and in vitro data for humans and different animal species, aiming to cover both human health and environmental toxicity. Data sources include, but are not limited to, the EDKB database, Danish list of EDCs and published papers. This first model is operational to assess ligands of oestrogen and androgen receptors with a subset of more than 1400 substances, currently reaching a specificity of 0.96 and a concordance of 0.86, where a true positive is defined as 'a non-EDC predicted as a non-EDC'. The model is also able to predict several thyroid and steroidogenesis disruption mechanisms, but this part still needs some expansion and is yet to be validated. The first results of this risk assessment-driven model are encouraging, and the further development and validation of this model, together with its compilation with other models included in our tiered approach will lead to a high reliability consensus prediction of the best understood ED modes of action. If the consensus prediction gives no alerts for ED MoA, it means high certainty that the substance does not act with the well understood ED MoAs, and can be labelled as "substance of low ED concern". If alerts occur, literature searches or further testing to assess the ED properties of the substance is advised. The results of our in silico assessment will help orient testing by providing clues of which biological target will likely be disrupted.

1.14P.7

Assessment of ecological state (health) of aquatic ecosystems on the base of cardiac activity monitoring system using Bivalves as bioindicators

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Freshwater bivalves were collected in a few study sites (differ in anthropogenic pressure) in recreational areas of the Neva River estuary (St-Petersburg, Russia). Laser fiber-optic monitoring system was used (Kholodkevich et al., 2009) to study characteristics of their cardiac activity. To test adaptivity of mussels we exposed them to salted water (6 g/L NaCl). The latter serves as a short-term (1-hour) hyper-osmotic stress load. In compensatory cardiac response we study the heart rate (HR) recovery time (Trec) after removal of test-load. Trec was calculated as the time needed to restore normal cardiac rhythm after removal of load. Rapid HR recovery of mollusks after loading testifies a good physiological status of the organism and subsequently provide the decision on a good ecological status of aquatoria of their dwelling. Prolonged Trec could be linked with the decline in mollusk's health because of the alterations in the status of their habitats. According to ranging proposed previously (Kholodkevich et al., 2019) ecological state in Dubki (35-60 mins), Ushkovo (70±8 mins) and Repino (98±12 mins) could be attributed as "high", "good" and "moderate", respectively. The technology used complements the modern methods for indication of surface water quality and contributes to the environmental safety of recreational water areas. It can be recommended for use in regional environmental monitoring programs for aquatic ecosystems assessment. Prospects of using the technology developed at the SRCES RAS are discussed as a Best Available Technology, which could be applicable in environmental risk assessment of early signs of threats to coastal recreational areas.

Chemical Safety Assessment of Surfactants: Current Challenges in Regulatory Science and Future Prospects (P)

1.15P.1

Assessment of ecological hazards and environmental fate of disinfectant quaternary ammonium surfactants

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Quaternary ammonium compounds are cationic substances that have diverse uses in a variety of consumer and commercial products, such as pesticides, herbicides, biocides, and cleaning products. Quaternary ammonium surfactants (Quats) exhibit antimicrobial activity through their interaction with the cytoplasmic membrane of microorganisms. Disinfectant Quats in antimicrobial products are regulated in Europe as biocides by the European Chemicals Agency under the Biocidal Products Regulation, and in the United Stated they are regulated by the U.S. Environmental Protection Agency under the Federal Insecticide, Fungicide and Rodenticide Act. Among the earliest used disinfectant Quats are the alkyl dimethyl benzyl ammonium chloride (ADBAC) compounds and the dialkyl dimethyl ammonium chloride (DDAC) compounds. ADBAC was first registered as an active antimicrobial ingredient in the United States in 1947 and DDAC in 1962. As part of the registration process in the United States and Europe, regulatory agencies solicit submission of ecological toxicity and environmental fate and transport data from manufacturers of antimicrobial active ingredients and formulated antimicrobial products, as well as from other stakeholders. The majority of submitted studies are unpublished reports that have been prepared by independent laboratories. Available data and regulatory evaluations of the ecotoxicity and environmental fate of ADBAC and DDAC were reviewed to understand the ecological hazards and potential environmental exposure. Focus was placed on aquatic organisms given the down-the-drain, end-of-life disposal for Quats in consumer and commercial cleaning products. There are substantial data regarding the acute and chronic aquatic toxicity of ADBAC and DDAC to algae, aquatic invertebrates, and fish for both chemical compound families, such that they are classified as hazardous to the aquatic environment, which is not unexpected for biocidal compounds. However, there are similarly robust environmental fate data that demonstrate that these compounds are highly removed from wastewater (99.8% based on a *weight of evidence* approach), strongly adsorb to soils, and are therefore immobile, and have low potential for bioaccumulation.

1.15P.2

Biodegradation studies with biocidal cationic surfactants: how to mitigate toxicity and secure bioaccessibility?

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Cationic surfactants are used as key ingredients in fabric softners and hair conditioners, but many are also applied as antiseptics in all kinds of products and industrial processes, because they effectively disrupt bacterial cell wall formation. In order to assess the environmental impact of cationic surfactants, the biodegradability of these chemicals is of high relevance in determining the environmental persistency of such chemicals with antimicrobial activities. To study biodegradability, full mineralization to CO_2 is recommended to be measured. As a consequence of high background CO2 signals from the presence of active microbial inoculum in biodegradation studies, relatively high concentrations of ~10 mg/L of test chemical is required. For many cationic surfactants, this concentration is toxic to the microbial inoculum, which hampers biological degradation processes. The OECD testing guideline already provides the option to mitigate toxicity by adding silica sorbent, but clear guidance on how to select such mitigating sorbents is currently lacking. We have performed a series of studies to develop and evaluate a systematic testing approach to incrementally adapt ideal settings for biodegradation studies with cationic surfactants. This directly incorporated the gained insights on the sorptive potential and expected toxicity of cationic surfactants, and recent developments of automated continuous digital manometric detectors. Potentially more efficient biodegradation test designs could be defined compared to the undesirable common alternatives, i.e. a need for expensive radiolabeled chemical standards, and/or laborious test designs with a priori uncertain outcomes.

1.15P.3

Can adjuvants affect Eisenia fetida growth or reproduction?

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Adjuvants are widely used in order to improve pesticides activity. It is generally believed that they are innocuous substances as they were not created to be toxic per se, what has led to few research about their direct impact on the environment. The aim of this study was to analyse the effect of 6 adjuvants on *Eisenia fetida* growth and reproduction. Six commercial adjuvants were tested in two concentrations, the recommended one and another 10 times higher, using an OECD (2004) modified protocol (400 gr of soil test substrate/container, positive control was boric acid, and earthworms were disposed 3-6 hours over moistened filter paper in order to evacuate their gut content before registering their weight). The considered adjuvants were mainly integrated by soybean oil, fatty acid methyl esters, nonylphenol ethoxylate, ammonium sulfate, hepamethyltrisiloxane, and nonylphenol poliethoxylate + sodium dodecylbenzenesulfonate, using 5 replicates per treatment. Data was analysed first with ANOVA, then a Tukey test was conducted to compare treatments. The analysed variables were relative growth rate (RGR), number of hatched and unhatched cocoons, number of juveniles, and hatching rate. Results showed that exposure to adjuvants did not have an effect on earthworms, as none of the treatments differ from the control considering the parameters described previously. Only one statistical difference was found between two treatments, the lower concentration of nonylphenol ethoxylate who produced the major number of juveniles and the higher concentration of fatty acid methyl esters that presented the lowest amount of juveniles. An interesting outcome is that both treatments showed a reverse response while seeing RGR and juveniles production. A possible interpretation is that under moderate stress, earthworm reproduction become a priority over growth. This phenomena involves reallocation of energy that has already been proved under other stressful circumstances. Overall, adjuvants did not produce effects on growing and reproductive parameters assessed in *E.fetida*, during the experimental period.

1.15P.4

Disclosure and data availability of co-formulants and their relative toxicity to active ingredients in viticultural pesticides.

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p margin-bottom: 0.25cm; line-height: 115%; background: transparent none repeat scroll 0% 0%; The protection goals for pesticides in regulatory frameworks and guidelines in the European Union have focused on the active ingredients (a.i.). In academia, however, the lack of openly available information on co-formulants of pesticides hampers the research progress that could be made in terms of toxicity to non-target communities. In view of the various compounds listed in the many pesticide patent applications, it is understood that the undisclosed co-formulants are mixtures of compounds that could potentially be more harmful than concluded in an amendable regulatory framework. This research was done to further contribute to the awareness of co-formulants toxicity to non-target communities of organisms. Being in a wine region of Germany, we were curious about the co-formulants found in the products used in vineyards. The Julius-Kuhn Institute reports almost yearly, a list of a.i. applied by winegrowers in Germany. We found 70 different compounds in 69 out of 132 pesticide formulations that had disclosed additional compounds in the product safety data sheets (SDS), of the top four most applied fungicides, herbicides and insecticides. The disclosure of co-formulants in SDS varies among countries as there was a challenge identifying the products just by the name because often there is no common identification or patent code. Approximately 75% of the co-formulants found were at least corrosive, a health hazard or a hazard to the environment, many are poorly tested compounds and there are those not considered for registration under REACH unless they are used for non-pesticide uses as well (ECHA, 2017). The EC50 data that was available for only 31 of them, indicated that several of them are relatively more toxic to *Daphnia magna* than the a.i. of the examined formulation. Moreover, 17 of these more toxic compounds did indeed coincide with similar EC50 values of the pesticide formulation. The investigation on pesticides as a whole was pursued for a shift away from limiting the risk assessment to mainly a.i. and metabolites. Among other findings are patterns such as the mysteriously consistent undisclosure of the formulations containing MCPA, dicamba, mecoprop-P and 2,4-D and substances found such as unapproved a.i and biocide a.i. The goal was also to emphasize the importance of combined ecotoxicity of not just multiple a.i. in a product, but of the complex mixtures of co-formulants from the point of manufacturing the compounds, to production and application of the pesticides.

1.15P.6
Toxicity of 3 surfactants: alkylbenzene sulfonate, lauryl alkylsulfonate and triton x in 4 different cladoceran species
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The surfactants are synthetic compounds used in the manufacture of detergents. Generally anionic surfactants are the most used, but the use of cationic and non-cationic surfactants is increasing because they are part of many personal care products such as shampoos, facial cleansers and bath gels. In this study an evaluation of the toxicity of 3 surfactants (Alkylbenzene sulfonate (ABS), Lauryl alkylsulfonate (LAS) and Triton X) was carried out on 4 cladoceran species: Chydorus sp. Daphnia magna, Daphnia exilis and Simocephalus mixtus, to determine their sensitivity to these toxic. Static bioassays were performed where neonates were exposed to 5 concentrations of each xenobiotic, for 48 hours to determine their lethal concentration 50 (LC50) by means of the Probit method. The sensitivity of the species was determined by comparing their LC50 values, by means of a "t" test. The degree of lipoperoxidation in your tissues and the activity of the AchE enzyme also were evaluated. The results obtained indicated that the most toxic surfactant for the 4 species was LAS, LC50 values varied from 1.3 to 6.31 mg L-1. The surfactant which caused the greatest oxidative stress was LAS. Significant differences in AchE enzyme activity of organisms exposed to surfactants were observed compared to the control group. AchE activity decreased to 48% in tests with ABS. The species most sensitive to surfactants was Daphnia exilis. The surfactants tested had deleterious effects in sublethal concentrations.

1.15P.7
Utilising membrane partitioning coefficients in physiologically-based kinetic modelling
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The partitioning between *n*-octanol and water (log K_{OW}) is commonly used as a surrogate for estimating passive partitioning through cell membranes. This makes it a useful input to calculating tissue:plasma partitioning coefficients, which are used to parameterise physiologically-based kinetic (PBK) models for estimating internal organism toxicant concentration distribution for risk assessment. The tissue:plasma partitioning coefficients describe how a chemical distributes into different tissues in the body and are a key set of parameters in PBK models. A number of methods for extrapolating from log K_{OW} or membrane-water partitioning (log K_{MW}) are available, but previous work has showed over 10-fold differences in predictions between algorithms when using the log K_{OW} based predictions. Developments in both *in vitro* and *in silico* methods have led to a significant increase in the accessibility and accuracy of estimation of membrane-water partitioning (log K_{MW}). Its use promises to offer much greater mechanistic insight and accuracy, compared to log K_{OW}, when used as an input to PBK models. This is especially relevant for ionizable molecules, including surfactants, as the specific interactions of polar, charged, or amphiphilic compounds with the ordered three-dimensional structure of biomembranes cannot be adequately described by isotropic solvent such as octanol. Here we report on a comparison using different methods for extrapolating log K_{OW} or calculating log K_{MW} on predicted tissue:plasma partitioning coefficients and subsequently the outputs of a PBK model: maximum plasma concentration after exposure (C_{max}) and the integral of plasma concentration against time (AUC). These results are compared with observed clinical data for a set of mixed neutral and ionizable molecules. The work provides guidance on when different methods could offer benefits, and be used to help guide parameter generation, based on chemical space, for input into PBK models and predicting internal chemical concentration distribution. Whilst this initial investigation focusses on a human PBK model, the results have implications for other organisms, including fish. Keywords: membrane-water partitioning, physiologically-based kinetic

1.15P.8
A proposed Hofmeister series for anionic surfactants
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Anionic and surfactants are large-scale produced chemicals which are present in many consumer products and consequently also in the environment as organic contaminants. Basic parameters that can describe the environmental fate and behavior of most neutral chemicals, such as the octanol-water partitioning coefficient (K_{OW}), are not yet available for surfactants and current predictive models are therefore unreliable. A better understanding of the behavior of these compounds in water may help with understanding their behavior in the environment. The molecule of an anionic surfactant interacts with the bulk water phase mostly through hydrophobicity, hydrogen-bonding and electrostatic interactions. These molecular properties are a result of the charge distribution of the surfactant molecule and together determine the number, orientation and density of water molecules that are directly surrounding the surfactant. This is also referred to as the `hydration` of a molecule. The Hofmeister series is a well-known classification of ions according to their hydration and allows to describe and compare the interactions between different ions in a system. In this study, we propose a Hofmeister-like series for anionic surfactants and rank the hydration of five surfactant headgroups: carboxylates, sulfates, sulfonates, perfluorcarboxylates and perfluorosulfonates. Previously published parameters from retention measurements and literature data from various sources were used to determine the order of hydration for these surfactants. The ranking can be used to explain differences in affinity of anionic surfactants for environmental phases and may aid in understanding how to remove these contaminants from the water phase.

Improving Exposure Characterization of In Vitro Testing to Support Quantitative Extrapolations to In Vivo Systems (P)

1.16P.1
ECO 36 Paving the way for QIVIVE: from nominal to free to cellular concentrations in in vitro assays
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This poster gives an overview on the ECO 36 Project entitled “Paving the way for QIVIVE: from nominal to free to cellular concentrations in *in vitro* assay” funded by CEFIC’s LRI. We developed experimental approaches and models to derive dose-metrics amenable for *in vitro* to *in* vitro extrapolation (QIVIVE), such as freely dissolved and cellular effect concentrations. High volatile losses and crossover were seen for semi-volatile and hydrophobic test chemicals in *in vitro* assays. Due to its high sorptive capacity, the medium served as passive dosing device but also decreased the bioavailability. The time-resolved freely dissolved concentrations in *in vitro* bioassays in 96-well plate format were measured for a suite of neutral and ionic organic chemicals with solid-phase microextraction. The experimental effect concentrations were used to validate a mass balance/partition model that can predict the freely dissolved and cellular effect concentrations for any well-plate format, medium and cells, provided that lipid- and protein content of medium and cells are known. Kinetic resolution of the model was provided for 2D reporter gene assays and more complex 3D cell models were explored for use of repeat-dose techniques.

1.16P.2
BE-SPME as a predictive tool within the in vitro space (Verification testing against historical datasets). Reduced volume BE-SPME testing and method development.
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Biomimetic extraction using solid phase microextraction (BE-SPME) passive sampling has been demonstrated to be a predictive analytical tool for the environmental toxicity of complex petroleum substances across a range of aquatic vertebrate, invertebrate, and plant species. A limitation of the method, in its current format, is the sample volume (20 mL) required to perform the analysis. This makes it impractical to extend its application beyond whole organism toxicity testing. The current sample volume and PDMS volume on the passive sampler have been optimized to maintain non-depletive, equilibrium sampling conditions mimicking the exposure of aquatic organisms to dissolved, bioavailable hydrocarbons. The development of a reduced volume BE-SPME method will permit extension of this technique to *in-vitro* and high-throughput reduced volume assays. This work focuses on reducing the necessary sample volume to perform BE-SPME analysis from 20 mL to 4 mL along with the concomitant reduction in PDMS volume on the passive sampler to maintain the necessary non-depletive ratio. To validate the reduced scale BE-SPME method, a water accommodated fraction (WAF) using a cracked gas oil was prepared at a nominal loading of 50 mg/L. BE-SPME kinetic data was developed using the conventional 30 µm PDMS (0.132 µL) fibers and 20 mL samples in parallel with the reduced volume method of 7 µm PDMS (0.028 µL) fibers and 4 mL samples. Fiber uptake kinetics were measured over 15, 30, 60, 100, 300, and 600 minutes of orbital agitation to compare measurements between 7 and 30 µm PDMS fibers. As expected, uptake kinetics onto the 7 µm PDMS fiber were faster than the 30 µm PDMS fiber. Overall, 7 µm PDMS fiber measurements align with 30 µm PDMS fiber data; however, 7 µm PDMS fiber measurements had slightly elevated BE-SPME values. Near-term work will expand test substances to include a broader range of UVCBs and incorporate well-plate sampling for automated analysis application. Long-term work will further reduce volume requirements (≤1 mL) for adaptation to 96-well plates. A leap in current technology will be required to achieve desired application to 96 or 384 well-plate assays as PDMS coated fiber technology does not currently suit the small volumes found in these higher number well-plates.

1.16P.3
SNAPFISH - Searching for refined in vitro approaches to predict

bioconcentration in fish

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The ecotoxicological assessment of chemicals requires information about their physical-chemical characteristics. Regarding hydrophobic organic substances, information about their biotransformation and potential bioaccumulation is important because they can accumulate along the food chain. Besides the direct determination of steady-state bioconcentration factors (BCFs) in fish (e.g., via OECD TG 305), these data can also be obtained by the use of *in vitro* depletion methods to minimize the use of live animals in the context of the 3R-targets (replace, reduce, refine). The aim of the present study is to obtain *in vitro* metabolic rates of deltamethrin, 4-n-nonylphenol, methoxychlor, and pyrene as a reference compound and to analyse the enzymatic turnover of these chemicals. To achieve this, assays with rainbow trout (RT) hepatocytes and RT subcellular S9 fractions are used. Here, partitioning-based dosing methods are being applied to improve the exposure control of low dissolved concentrations of the test substances and to avoid additional organic solvents which could affect the enzymes. Furthermore, the metabolic rates can be used for *in vitro-in vivo* extrapolation to predict steady-state BCFs and to improve existing BCF prediction models. Perfused liver experiments will provide metabolic rates that can be used as an intermediate step in the extrapolation. A refinement of the models would result in more reliable BCF estimates and hence an improved predictability of the environmental risk of hydrophobic chemicals. We will present *in vitro* metabolic rates for the above mentioned test compounds, as well as results of the equilibrium dosing based adaptation of the test system.

Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment (P)

2.01P.1

A small-scale setup for testing algal toxicity of nanomaterials and other difficult substances

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The algal toxicity test is one of three mandatory tests and international guidelines have been developed for this test to ensure high reliability of test results. However, maintaining stable and uniform test conditions during algal testing (e.g., in terms of pH, temperature, carbon dioxide levels and light intensity) is generally challenging and specifically difficult for nanomaterials (and other difficult substances). This severely affects the reliability and reproducibility of algal test results for nanomaterials and hence their regulatory acceptance. To improve this situation, we have developed a testing platform, LEVITATT (LED Vertical Illumination Table for Algal Toxicity Tests), that counters issues of intra-sample shading of samples and ensures a homogeneous distribution of light for all tested samples using LED illumination from below. Furthermore, LEVITATT enables pH control during the incubation by a steady influx of atmospheric CO_2 as well as the use of a fixed CO_2 concentration in vial headspace (e.g. for volatile substances), versatile biomass determination methods, versatile choice of test container material, tightly defined light conditions which can be varied according to needs, sufficient replicate volume to allow sampling for characterization and quantification of nanomaterials before during and after testing. Additionally, the setup allows for determination of advanced sub-chronic endpoints such as changes in algal pigmentation as a result of nanomaterials exposure. LEVITATT is due to its compact design, appropriate for high-throughput screening testing. The test setup complies with ISO standard and OECD guidelines for algal toxicity testing and has successfully been validated with reference toxicants as well as been applied for testing of JRC repository nanomaterials (TiO_2, ZnO, CeO_2 and $BaSO_4$). This contribution will show the practical applicability of LEVITATT and main outcomes of the tested nanomaterials.

2.01P.2

Maintaining the exponential growth rate of algae in a 96-hour exposure

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Algae are internationally established test organisms in chemical risk assessment. Toxicity test guidelines incorporating algae are largely focused on traditional apical endpoints such as growth rates following a 72-h chemical exposure as per OECD 201 guidelines. However, depending on the country of registration, the OCSPP 850.4500 might be required, which looks at endpoints over 96-h. In a number of 96-Hour algal tests it has been noted that the algal growth rate in control groups and uninhibited test groups has a tendency to drop off after 72 hours. The reason for the decline is suspected to be due to the algal growth entering the stationary phase. The OECD and OCSPP test guidelines give minimum algal density increases in the control of 16 x over 72 hours and 100 x over 96 hours respectively; however, typical algal density increases have been recorded of 362 x over 72 hours and 438 x over 96 hours. It is therefore likely that the failure to maintain exponential growth has been caused by a limiting factor such as nutrient availability or insufficient gas exchange. Here we present the findings of a series of experiments to investigate modifications to the 96 hour test design to ensure exponential growth is maintained throughout the 96 hours of exposure. A reduction of growth rate in the control groups which is not observed in the inhibited test groups may result in an under estimate of toxicity as the test groups catch up with the controls.

2.01P.4

Investigating the Toxicity of Zearalenone to Freshwater Macrophytes (Lemna minor) and Microalgae (Pseudokirchneriella subcapitata).

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Mycotoxins are an important class of chemicals of emerging concern, recently detected in aquatic environments, potentially reflecting the influence of fungicide resistance and climatic factors on fungal diseases in agricultural crops. The oestrogenic mycotoxin zearalenone has been measured in streams and lakes at levels of < 100 ng/L. Freshwater toxicity data for mycotoxins such as zearalenone exist primarily regarding zebrafish, there is a lack of zearalenone phytotoxicity data which is important given that other mycotoxins have been shown to cause phytotoxicity in *Lemna* spp. Here we investigate the phytotoxicity of zearalenone as a chemical of emerging concern, beginning with the standardised OECD test guidelines for the macrophyte *L. minor* and the microalgae *P. subcapitata*. In the algal static study (72 h at 24 ± 1°C), exposure to zearalenone gave average specific growth rate (cell density) EC50 and yield (cell density) EC50 values of > 3.1 and 0.92 mg/L, respectively. Zearalenone was less toxic in the macrophyte static study and after 7 d (at 24 ± 1°C*)* *L. minor* growth was significantly reduced (38-67%) based on frond number and frond area at only the highest exposure of 11.4 mg/L. *L. minor* showed a higher tolerance to zearalenone than reported for other mycotoxins with *Lemna* spp. with reported growth inhibition due to deoxynivalenol, nivalenol, T-2 toxin and verrucarin A being 38 - 72% at concentrations in the range of 0.5 - 4.6 mg/L. Following the growth inhibition studies potential modes of action were investigated, initially via physiological measures of photosynthetic performance and biochemical analysis of lipid peroxidation and catalase activity. Chlorophyll fluorescence parameters indicated zearalenone induced electron transport inhibition in PSII of macrophytes. Since the biochemical data were not consistent with regard to the concept of this leading to photo oxidative stress, further work is required to establish a full adverse outcome pathway for zearalenone in plants.

2.01P.5

Is the aquatic effect assessment for herbicides protective in the EU ?

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In Europe the risk assessment for plant protection products is based on a tiered approach. The principles of such an approach include that lower tiers are protective of higher tiers and that higher tiers can be used to validate lower tiers. Van Wijngaarden and Arts (2018; Environmental Toxicology and Chemistry 37 (1): 175–183) undertook a study to validate the standard first-tier in the aquatic effect assessment for herbicides (Tier 1-Regulatory Acceptable Concentration (Tier 1-RAC)) with results of higher-tier micro-/mesocosm studies (Ecological Threshold Option (ETO-RAC)). In this study, 14 compounds were identified for which aquatic threshold concentrations could be derived. The study concluded that the Tier-1 RAC for herbicides is protective for aquatic communities as based on the higher-tier ETO-RAC for most herbicides included in this analysis. Since then, several other studies were undertaken to verify these results and to expand the database. Therefore, the current study intends to have a closer look at the higher tier endpoints that were used in the several assessments and that have been published in the Draft Assessment Reports and List of Endpoints at the EU level. Special attention will be given to a couple of issues that are regularly raised in the scientific discussions when these focus on the protectiveness of the primary producer risk assessment: 1. The Assessment Factors used in the risk assessment in order to conclude on an ETO-RAC; 2. The consideration of an ERO-RAC based on an Effect Class 3 with an appropriate Assessment Factor, as in van Wijngaarden and Arts only the ETO-RAC was considered; 3. The evaluation of higher tier endpoints and the appropriate level at which they should have been deduced, that is at the population and ecosystem level; 4. The evaluation of fungicides in this assessment and check if their mode of action causes primary producers to be the most sensitive. Results will be presented and discussed.

2.01P.6
Effects of artificial ultraviolet B radiation on macrophyte Lemna minor: a conceptual study for toxicity pathway characterization
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The macrophyte *Lemna minor* may be grown in hydroponics or aquaponics for feed, treatments of wastewaters, and ecotoxicological studies, where elevated artificial UV-B irradiances are applied for disease control or toxicity testing. Although high UV-B irradiances can be harmful, there is limited knowledge on dose-dependency and effects of UV-B in aquatic plants acclimated to supranatural UV-B irradiance. The present study was therefore conducted to shed light on the toxicity mechanisms associated with growth inhibition and lethality in non-UV-B-acclimated *L. minor* under elevated UV-B irradiances (0.008-4.2 W m^{-2}) together with constant UV-A (4 W m^{-2}) for 7 days. In this respect, a suite of bioassays was deployed to assess oxidative stress responses, photosynthesis-related effects, DNA damage and gene expression associated with antioxidant biosynthesis, DNA damage, programmed cell death, pigment metabolism, photosynthesis and respiration. UV-B triggered both dose-dependent and target-specific effects that were related to growth inhibition, whereas UV-A alone did not cause any effects. UV-B inhibited photosystem II performance and reduced carotenoid levels at 0.23 W m^{-2}, whereas growth inhibition, excessive reactive oxygen species production, lipid peroxidation, cyclobutane pyrimidine dimer formation in the DNA, mitochondrial membrane potential reduction and chlorophyll depletion were observed at UV-B irradiances between 0.5 and 1 W m^{-2}. Potential linkages between the responses at different levels of biological organizations were proposed and summarized in a conceptual toxicity pathways network as an initial effort to develop Adverse Outcome Pathways (AOPs) for elevated UV-B radiation in aquatic primary producers.

2.01P.7
Modes of action and adverse effects of gamma radiation in an aquatic macrophyte Lemna minor
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High dose rates of ionizing radiation have been reported to cause adverse effects such as reduction in reproduction and growth, and damage to protein and lipids in primary producers. However, the relevant effects of ionizing radiation are still poorly understood in aquatic plants. This study was intended to characterize the biological effects and modes of action (MoAs) of ionizing radiation using gamma radiation as the prototypical stressor and duckweed *Lemna minor* as a model organism. *Lemna minor* was exposed to 1, 14, 24, 46, 70 mGy/h gamma radiation dose rates from a cobalt-60 source for 7 days following the testing principles of the OECD test guideline 221. A suite of bioassays was applied to assess the biological effects of gamma radiation at multiple levels of biological organization, including detection of reactive oxygen species (ROS), oxidative stress responses (total glutathione, tGSH; lipid peroxidation, LPO), DNA damage, mitochondrial dysfunctions (mitochondrial membrane potential, MMP), photosynthetic parameters (chlorophyll a, chl *a*; chlorophyll b, chl *b*; carotenoids; Photosystem II (PSII) performance; CO_2 uptake), intercellular signaling (Ca^{2+} release) and growth. Gamma radiation increased DNA damage, tGSH level and Ca^{2+} content together with reduction in chlorophyll content, maximal PSII efficiency and CO_2 uptake at dose rates between 1 and 14 mGy/h, whereas increases in cellular ROS and LPO, inhibition of MMP and growth were observed at higher dose rates (≥24 mGy/h). A network of toxicity pathways was proposed to portray the causal relationships between gamma radiation-induced physiological responses and adverse outcomes to support the development of Adverse Outcome Pathways (AOPs) for ionizing radiation-mediated effects in primary producers.

2.01P.8
Combined effects of gamma radiation and ultraviolet-B on Lemna minor: mechanistic insights and quantitative assessment
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Elevated ionizing radiation level (e.g. gamma (γ) radiation) are typically observed at nuclear accidental sites such as Chernobyl and Fukushima, representing a risk to aquatic organisms. However, current approaches in risk assessment of γ-radiation are primarily focused on single stressors which do not reflect the environmental conditions where a multitude of stressors (multiple stressors) co-exist. Other environmental stressors, such as non-ionizing (UV-B) radiation can also affect living organisms due to periodic events or changes in climate conditions. As γ-radiation and UV-B display both similar and dissimilar mode of action (MoAs), when simultaneously exposed, γ-radiation and UV-B might induce cumulative hazard and risk. Therefore, to assess the risk of γ-radiation in natural aquatic ecosystem, it is crucial to clarify the potential interactions between UV-B and γ-radiation on aquatic organisms. In this respect, aquatic floating macrophyte Lemna minor was used as an indicator and forecaster species to assess adverse effects and interactions. In the presented study, non-UV acclimated L. minor was exposed to elevated □-radiation (12, 20, 47 mGy/h) and UV-B at ecologically relevant level (0.5 W/m2) for 7 days, either alone or in combination. To clarify the interactions on toxicity mechanisms, endpoints were assessed at different levels of biological organization, including oxidative stress (ROS level and lipid peroxidation), photosystem II (PSII) performance, oxidative phosphorylation (OXPHOS), energy depletion (ATP content) and growth. Genes expression associated with antioxidant biosynthesis, DNA damage, programmed cell death (PCD), pigment metabolism, photosynthesis and respiration were investigated as well to fill in data gaps in the toxicity pathways. 2W-ANOVA and modified independent action (IA) modeling were placed to evaluate the interactions. When exposed alone, elevated □-radiation induced dose-dependency adverse effects on DNA damage, PCD, oxidative stress, pigments, PSII, glycolysis, oxidative phosphorylation (OXPHOS) and growth, while UV-B induced impacts on PCD, oxidative stress, OXPHOS, and PSII. In combined exposure, UVB induced interactions were presented as target-specific. Antagonistic interactions were observed on oxidative stress, DNA damage, OXPHOS and glycolysis. Additivity interactions occurred on PSII preformation and frond development (size and weight). Whereas, synergistic interactions were observed in non-photochemical quenching, activity of antioxidants and reproduction inhibition. Data obtained were summarized into a conceptual framework to improve the mechanistic knowledge for the risk assessment of γ-radiation when co-exposed with UV-B at the ambient level.

2.01P.9
Is glyphosate-based management of invasive aquatic plants detrimental to nearby native at-risk macrophytes?
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Invasive aquatic plants disrupt wetland communities, often reducing the biodiversity of native macrophytes. The herbicide glyphosate can control invasive plants and hence mitigate their effects, but it can also be toxic to nearby non-target macrophytes. If one goal of invasive plant management is to maintain native macrophyte diversity, then the most effective practices are those that consider potential non-target effects of glyphosate applications. Our study assessed growth and survival of *Ammania robusta*, a macrophyte native to North America with populations at risk of extinction in Canada, post-exposure to low level glyphosate concentrations that may arise following the control of neighboring invasive plants. We evaluated three 'realistic' pathways mimicking unintended exposure: spray of $4 \cdot 10^{-7}$-5% glyphosate drifting from nearby foliar applications; pulse and continuous exposures of 2 and 41 µg/L glyphosate residues in surface water that may result from nearby applications; and rhizosphere contact with invasive plants hand-wicked with 5% glyphosate. At 14 days post-exposure, glyphosate spray concentrations as low as 0.1% reduced *Ammania* shoot and root growth, and plants sprayed with 1.34 and 5% glyphosate showed almost complete decay. Our assessed endpoints 'plant height' and 'root dry weight' were more sensitive than 'total shoot length', 'side shoot number' and 'shoot dry weight' to glyphosate spray. Conversely, low level pulse and continuous glyphosate residues in water did not affect *Ammania* growth and survival. Likewise, *Ammania* with rhizosphere contact to a glyphosate-hand-wicked invasive plant grew as well as *Ammania* without neighbors. Our data suggest that not all glyphosate exposure pathways at 'real-world' concentrations negatively affect at-risk macrophytes. Adjacent hand-wicking of invasive plants or low level surface water residues appear to pose no risk; however, glyphosate spray drift at concentrations much lower than the commonly used 5% rate could impair growth and survival of sensitive macrophytes. Thus, we recommend that glyphosate-based invasive plant management near at-risk macrophytes implements measures to limit off-target spray drift and considers the feasibility of more targeted applications, such as with a paint brush or squirt bottle. Implementing these recommendations into best

management practices for invasive plants can support both the conservation of at-risk macrophytes and recovery of biodiverse wetland communities.

2.01P.10

Assessment of the effects of 3 non-steroidal anti-inflammatory drugs on the macrophytes Lemna gibba L. and Egeria densa Planch. 1849

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The non-steroidal anti-inflammatory drugs are free sale products that are often eliminated in aquatic systems. They have been detected in wastewater in concentrations of ppm and in natural waters in ppb and ppt . These drugs can cause harmful effects on aquatic organisms, as they are designed to have a physiological effect at very low concentrations. The aim of this study was to evaluate the toxic effect of three commonly used drugs (Aspirin, Paracetamol and Naproxen) on the macrophytes: *Lemna gibba* and *Egeria densa*. bioassays of 96 hours duration were conducted to determine the EC_{50} (effective concentration 50), is the concentration which inhibits population growth. In the bioassays also were evaluated: chlorophyll and carotene production, the level of lipoperoxidation (Tbars) and the concentration of total sugars. The EC_{50} varied from 17.42 to 1471.6 mg/L the most toxic compound for macrophytes was Naproxen. It was observed a decrease in the levels of chlorophylls, between 3 to 40%. In the tests with Paracetamol the lowest concentration of chlorophylls was observed. Tbars levels were high in organisms exposed to drugs compared to the control group. The lowest total sugar concentrations were obtained in the Paracetamol tests. Because in Mexico only 14% of the wastewater produced receive some form of treatment, and has not evaluated the concentrations of these drugs in sewage waters, it is important to continue to make assessments of the effects of these drugs for the purpose of proposing appropriate management measures to reduce the risk for the presence of these compounds in aquatic systems.

2.01P.11

Developing a tool to apply in the risk assessment for Plant Communities in the vicinity of agricultural fields/orchards

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The risk assessment of pesticides is currently based on standardized limit greenhouse tests with a minimum of six plant species from six different families including mono- and dicotyledon species. In case of herbicidal or plant growth regulator activity a dose response test on a selection of 6 to 10 monocotyledon and dicotyledon plant species providing an ER50 value is required. Higher tier tests considering plant abundance and biomass are not standardized. As in Europe landscape level risk assessment is in development, the question arises how such a risk assessment can be performed for terrestrial plants. When considering the landscape level, the level of biological organization is not the species, but merely the plant community. Therefore, at the request of European Crop Protection Association (ECPA), the ultimate aim of this project is to deduce an appropriate number (presumably 5 – 10) of representative plant communities in terms of habitats, functionalities and structures at the European level to be used in the risk assessment and/or testing for herbicides. As a first step the year 2019 was dedicated to exploring data that could be used to identify plant communities at the landscape level that are characteristic of agricultural areas in Europe. For this purpose, we applied the tool Quickscan (http://www.QUICKScan.pro), which is a spatial modelling environment that combines expert knowledge with spatial and statistical data. The results of this application are visualized in interactive maps, summary charts and trade-off diagrams. We collated a summary chart with boxes in which each box represents an existing database. In the tool, the boxes are linked with knowledge rules. As a central box we considered the anthropogenic vegetation classes as described by Mucina (2016). Predictors for the occurrence of these vegetation classes are climate, topography and soil factors as well as biogeography. These classes are linked with information about important plant traits (life form, nitrogen sensitivity, reproduction, fruit type from the TRY database) that play a role in the overall vulnerability of plants. Distribution of agricultural crops are linked to herbicide use and mode of action of the herbicide resulting in small, medium or large effects or no effects on biomass. The 10 anthropogenic vegetation classes will be further explored by application of the QUICKScan model in order to identify plant communities to be used in the risk assessment of pesticides.

2.01P.12

Closing the gap between lower and higher tier testing in NTTPs: assessing effects of a sulfonylurea herbicide on an experimental plant community in containers under semi-field conditions

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Standard non-target terrestrial plant (NTTP) guideline tests are done in greenhouses on potted crop species grown as monocultures (OECD 227&208). As it is the case for many lower tier tests, studies are done under worst case as well as highly standardized conditions to get a high margin of safety while at the same time keeping variability as low as possible. NTTP guideline tests have been critizised for lacking realism: if results of the lower tier tests show a concern for NTTPs, higher tier testing may be needed to assess whether this concern may occur under more realistic conditions in the field. Accordingly, a need for suitable higher tier tests is identified. So far no agreed NTTP higher tier method has been established. In 2019, we presented a method for conducting higher tier NTTP field studies using commercially available seed mixtures as the source for establishing experimental plant communities on study sites (Isemer et al., oral presentation SETAC Europe 2019). As it was expected, we found that the increase of realism in the field study method compared to the greenhouse standard tests is inevitably accompanied by an increase in variability. Furthermore, field testing is resource and time consuming. In order to overcome inevitable shortcomings of lower and higher tier tests and to close the gap between standard lower tier testing in greenhouses and a full scale field test, we present a method assessing effects of herbicides on an experimental plant community grown in containers under semi-field conditions. The used herbicide, application and assessments corresponded to the previously presented field study to allow for comparisons between study types: the same seed mixture comprising 11 garden-species used to establish the experimental plant community in the field was sown in large containers (430 x 430 x 360 mm) and grown in an outdoor area protected by a cage. Three sulfonylurea herbicide rates plus a water control were applied after four weeks of cultivation. Vegetation cover according to the Londo scale, number of plant individuals and dry biomass per species, symptoms of phytotoxicity as well as BBCH stage were assessed before as well as two and four weeks after application. It was found that effects of the herbicide in semi-field conditions were similar as those seen in the field study confirming suitability of the method for refining the risk assessment for NTTPs and serving as a higher tier study method.

2.01P.13

Higher tier testing in non-target terrestrial plants - Steps towards increased realism

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The authorization of plant protection products under Regulation 1107/2009 requires an assessment of the product`s potential to affect plants occurring outside the field (non-target plants). This applies particularly to herbicides as many of them can influence plants even at low doses found in spray drift. Routine testing with non-target terrestrial plants (NTTP) is conducted in standardized greenhouse studies[1,2]. Results from these studies are used as basis for the lowest tier of the ecological risk assessment. When needed, the next risk assessment step may be based on testing under more realistic conditions in semi-field and field studies. Although the Guidance Document[3] mentions higher tier testing as a refinement option in the NTTP risk assessment, no agreed standard protocol has so far been established[4]. Here, we give an overview of the diverse potential options for higher tier testing in NTTPs (e.g. testing of wild instead of crop species, testing of reproductive parameters in addition to vegetative parameters, testing of plant communities instead of single plants, using different growth stages for application, extended study duration, testing under outdoor or field conditions). As an example for higher tier testing, a case study is presented: Three different products were tested on seven crop species grown in monoculture in large containers under outdoor conditions. In general agreement with available lower tier testing guidelines, the application was done at the 2-6 leaf stage and plants were assessed 7, 14, and 21 days after treatment. Vegetative vigour endpoints (plant survival, shoot dry weight, phytotoxicity) were recorded and compared to the corresponding endpoints of the same species and test items obtained under standard greenhouse test conditions. Sensitivity to all three products was overall lower under outdoor conditions by a factor ranging between 1.19 and 3.60. These differences can be explained with more realistic exposure conditions and more robust growth of the plants under outdoor conditions. 1 OECD (2006) Terrestrial plants test: Seedling Emergence and Seedling Growth Test (208) 2 OECD (2006) Terrestrial plants test: Vegetative Vigour Test (227) 3 EC (2002) Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC4 EFSA PPR Panel (2014). Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants. EFSA Journal 12, 3800

2.01P.14

Reproductive plant testing - What has to be considered Review of published data and summary of identified open questions to implement reproductive phases in non-target plant testing for registration purposes

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In 2014, EFSA has published a Scientific Opinion on risk assessment of plant protection products for NTTP's. This document questions the protectiveness of the current testing scheme (OECD, EPA Guidelines) involving early plant growth stages. The inclusion of potential effects on reproduction in the risk assessment scheme is proposed while no guidelines for reproduction studies are available. A selection of points that need further consideration and discussion is presented: - Seed batches with constant germination rates are needed. Many non-crop species have a fluctuation in germination rate over the year. In addition, they are not bred to show a uniform growth, which complicates the conduct and assessment especially of long-term studies. - Most of reproduction studies in the literature used artificial soil mix with higher content of organic matter. Higher organic matter might be useful for long term growing but might affect the availability of test items. - Seed production requires some kind of pollination. Under greenhouse conditions the use of insects might not be possible, thus limiting the range of possible test species. - Some species need vernalisation. Placing test species outside the greenhouse to provoke generative phase may be possible but difficult to harmonize between labs and climatic zones. - So far application timing is based on pre – emergence versus applications at BBCH 12-14. It is unclear whether it is more relevant to extend these tests to the reproduction phase or to use later application timings (e.g. at flowering). - Do we have to exclude biennial or perennial plants from tests in order to have a test design which is feasible under greenhouse conditions? - The definition of when species reached a specific growth stage is fairly difficult within the generative phase. Many plant species show flowering, fruiting and shedding at the same time on the same individual over weeks. - The influence of the test item may delay flowering and fruiting. This does not necessarily reduce the total fruit and seed production if the termination date of the study is flexible. - The endpoint 'number of seeds' needs methods to prevent seed loss by shedding. - Counting of fruits instead of seeds may be more appropriate for certain test species. - Can we reach enough statistical power to gain reliable statistical endpoints? In order to evaluate the feasibility of developing standardised tests for plant reproduction, a ring-test is recommended in several laboratories. Contributors: Mihan, Christine (Bayer CropScience AG), Davis, Jo (Syngenta); Geiger, Michael (BASF AG)

2.01P.15
Historical Sensitivity of Common Species Used In Non-Target Terrestrial Plant Testing
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Non-target terrestrial plant (NTTP) studies are used to assess the potential effects of agrochemicals on seedling emergence and vegetative vigor. Seedling emergence studies assess the exposure effects on emergence and growth in accordance with OECD 208 or U.S. EPA 850.4100 guidelines. Vegetative vigor studies assess the exposure effects on young plants in accordance with OECD 227 or US EPA 850.4150 guidelines. Dependent on the regulatory objective, these studies often require defining a no observed effect rate or various EC_x values and may include testing up to 10 species of common plants. The inherent variability in this testing coupled with other challenges (hormesis, frequent shallow dose responses, lack of standardization for replication or planting density) presents significant issues in conducting this testing and interpreting the data. While increasing replication or testing additional application rates can increase the statistical power of these tests, this is often not feasible due to the resources required to conduct larger studies. In this poster, we summarize an extensive historical database for both seedling emergence and vegetative vigor studies. Trends in sensitivity across common species used in testing with respect to plant protection product mode of action will be assessed. The basic objective of this examination is to provide a potential framework for *a priori* determination of species sensitivity for future testing. This may allow for improved design of NTTP studies and more efficient use of resources toward the goal of providing the most useful data set for regulatory purposes.

2.01P.16
Phytotoxicity of sugarcane vinasse biodigested in an acidogenic reactor
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Vinasse is the main liquid waste of ethanol and sugar production's process. The main destination currently given to vinasse is the fertirrigation, which can cause several environmental problems, among them the inhibition of seed germination. Two-stage anaerobic digestion process is an environmental and energetically attractive method for vinasse treatment before fertirrigation. Thus, the purpose of this study was to evaluated the toxicity of vinasse, to 3 plants species, before and after the acidogenic step of a two-stage anaerobic digestion process. The influent and effluent of a lab-scale acidogenic reactor treating vinasse were used in the phytotoxicity tests. The plants used were: *Brassica oleracea*, *Lactuca sativa* and *Eruca sativa*. Ten (10) seeds of each species were placed over a filter paper in glass Petri dishes and 5 different dilutions (0.1, 5,10, 50 and 100%) of acidified vinasse (Effluent) and vinasse *in natura* (Influent) were used. All tests (including a control with filtered water) were performed in triplicates. The dishes were placed in the dark, during 96 h, at 20± 2ºC (USEPA, 1996) and root relative growth and seed germination were evaluated. Comparatively to *in natura* vinasse (pH 6.5 ± 0.11), the acidic characteristic of the acidified vinasse (pH 4.90 ± 0.06) promoted greater toxic effect on root growth (%) for the 3 plants species tested. With the influent, 20% of germination for *E. sativa* species on the 100% dilution was observed, while on the effluent there was 10% of germination for *L. sativa* species on 50% dilution. The dilutions of vinasse (EC50) for influent and effluent were: 3.32 and 0.0015% (*B. oleracea*); 13.14 and 2.68% (*L. sativa*) and 21.49 and 12.2% (*E. sativa*), respectively. In *B. oleracea*and *L. sativa* tests statistically significant differences between influent and effluent ($p=4.6\ 10^{-12}$ and $p= 1,9\ 10^{-18}$) were observed. *E. sativa* showed no statistical difference between influent and effluent (p=0,35) (Tukey's test). Greater toxicity was observed with acidified vinasse, demonstrating the importance of coupling the acidogenic reactor to a methanogenic reactor in vinasse treatment. Furthermore, our results suggested that phytotoxicity tests are an effective tool for assessing the impact of vinasse (treated and untreated) on soil fertirrigation.

Bees, Bugs and Beneficials in Environmental Risk Assessment and Testing (P)

2.02P.1
Increased Pollinator testing demands: What we know by now from the rush
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With the European Food Safety Authority (EFSA) Guidance Document on the risk assessment of plant protection products on bees (EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295), a number of changes and impacts on assessing the potential risk for pollinators has moved into focus. While some of the newly requested test setups and endpoints have undergone development and in some cases multiple ring-test phases, other designs still phase lack of data or time to develop the methodology properly. However, are these additional data sets indeed helpful and increase the safety of the given compounds, or is it just a tick the box procedure without a serious use for risk assessment? The here presented data is intended to give a detailed insight on endpoints tested across the three main pollinator species required for current risk assessment scheme. Data sets are accomplished from one specific laboratory over the recent years, further endpoints are included from public available literature sources, such as publications or Draft Assessment Reports on the specific compounds. Further on, details and difficulties on the different methodologies and species selected are discussed. Main point raised for discussion shall be the benefit and also the undisputed need for additional species in pollinator testing in the light of practical achieved results and outcomes on compounds of different chemical classes and with different modes of action.

2.02P.2
Practical and regulatory experience in the conduct of bee residue trials
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To ensure the safe use of agrochemicals, today's regulatory system requires an assessment of the environmental risk to bees, as well as an assessment of the dietary risk to humans following the consumption of honey and other bee products. Field trials can provide valuable data to assess the potential exposure of foraging honey bees to agrochemical residues and hence the potential for residues to reach honey consumed by humans. With increasing requests for pesticide residue data on honey and other bee products, field trial teams and risk assessors alike must find workable procedures to collect and analyse appropriate samples and understand how such data can be used in a regulatory context. For the past several years, Staphyt's field team has conducted experimental GLP field and tunnel residue trials, testing different methods for the collection of various apicultural matrices for subsequent residue analysis. These trials have included studies on primary and succeeding crops, across several Central and Southern European Member States, with collection of matrices including pollen and anthers, nectar, mature honey, soil cores and guttation fluid. Having gained considerable practical experience in the setup of these studies, here we will present our tested field methods to share our expertise. In particular, we will discuss the advantages and disadvantages of various sampling techniques, such as manual- versus honey bee-collected sampling. We will discuss practical considerations for trial sites distributed across different European zones, including the importance of uniformity of tunnel setup, equipment and sampling techniques, as well as the choice and cultivation (e.g. sowing time and irrigation) of appropriate pollinator-attractive crops. With the combined expertise of Staphyt's Bee Team, consisting of regulatory, scientific and field specialists, together we aim to provide both a practical (field) and regulatory (consultancy) perspective on the conduct of pan-European field and tunnel residue studies for environmental and consumer risk assessments.

2.02P.4
Current challenges and future perspectives of the bee risk assessment under

the new EFSA guidance for cereal crops
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A risk assessment for bees is carried out for the registration of active substances and Plant Protection Products in the EU. A new EFSA guidance has been proposed to cover more risk scenarios and bee species compared to the traditional risk assessment. This scientific review provides an insight into the current challenges and future perspectives of the bee risk assessment under the new EFSA guidance for cereal crops. The most representative active substances used for the control of pests in cereals were extracted from statistical reviews on the use of Plant Protection Products in the EU. The acute risk assessments for oral and contact exposures were carried out in line with the new EFSA guidance assuming three levels of toxicity for a range of spray applications. Most of the examined active substances are for use as sprayed fungicides and herbicides on cereals. Insecticides exhibit the highest acute toxicity to honey bees compared to fungicides, herbicides and plant growth regulators. Of the 53 active substances investigated, 11 have had their approvals for use in the EU renewed. In the renewal processes of these active substances, toxicity data gaps were identified for the effects on hypopharyngeal glands and accumulation, larval toxicity, data on metabolites occurring in pollen and nectar and exposure via contaminated water. Several observations were apparent in the analysis of the outputs of acute risk assessment. The risk to all bee species was firstly identified for the 'weeds' scenario in the oral and contact exposures, as the main route of exposure to bees from the application of Plant Protection Products to cereals is through foraging on weeds in the treated field. Several risk mitigation measures are proposed in the new EFSA guidance to address the risk to this scenario. The risk for the 'treated crop' scenario was also identified in the acute risk assessment. Bumble bees and solitary bees were the most sensitive species in the risk assessment. In the absence of toxicity data for bumble bees and solitary bees, the new EFSA guidance recommends using the toxicity endpoint for honey bees as a surrogate with an assumption of 10-fold greater toxicity. However, this would result in a doubly conservative approach since much lower trigger values are proposed for the risk assessments for bumble bees and solitary bees. Further research is needed on these species and their routes of exposure to validate their proposed parameters for the risk assessment.

2.02P.5
Progress on the Osmia acute oral test - findings of the ICPPR Non-Apis subgroup solitary bee laboratory testing
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The publication of the proposed EFSA risk assessment guidance document of plant protection products for pollinators highlighted that there are no study designs for non-apis pollinators available. As a result the risk assessment of non-apis pollinators uses apis pollinator data with so-called assessment factors to compensate for the lack of knowledge on other species. To fill part of this knowlegde gap an acute oral test for solitary bees was developed within the ICPPR non-apis group. Ringtests have been conducted in 2018 to validate and improve the suggested protocol. And in 2019 a standardized protocol has been tested by all participants once more. The tests have been performed with *Osmia bicornis*, *Osmia cornuta*, *Osmia lignaria* and *Osmia cornifrons*. A summary of the ringtest results of both years will be given and further recommendations will be presented.

2.02P.6
Comparative Toxicity of Dimethoate to Honeybees, Apis indica and Apis mellifera
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A comparative toxicity of dimethoate (technical grade) was studied in two species of honeybees, *Apis indica* and *Apis mellifera* when exposed through oral and contact route. OECD test guidelines 213 and 214 were followed for oral and contact tests, respectively. Group of ten bees with three replicates of both *Apis indica* and *Apis mellifera* were exposed at 0.05, 0.075, 0.113, 0.17, 0.255 and 0.383 µg a.i./bee along with a control group through oral and contact route separately. The LD_{50} was determined using Finney's probit method and the results are presented as follows. **Honeybee species 24 h LD_{50} (µg a.i./bee) Lower fiducial limits Upper fiducial limits *Apis mellifera* (mean values) Oral** 0.159 0.133 0.191 **Contact** 0.116 0.104 0.129 ***Apis indica* Oral** 0.092 0.037 0.147 **Contact** 0.067 0.056 0.081 The oral and contact toxicity of dimethoate to *Apis indica* is 1.7 times lesser than *Apis mellifera*. The difference in toxicity of dimethoate between *Apis mellifera* and *Apis indica* could be due to difference in their body mass. When the ratio of body mass measured was 2:1 between *Apis mellifera* (mean body weight-117 mg) and *Apis indica* (mean body weight-59 mg). Sensitivity of *Apis indica* to dimethoate is marginally higher than *Apis mellifera* and similarly, *Apis indica* can be employed for assessing the risk of chemicals.

2.02P.7
Combined exposure of the red mason bee Osmia bicornis to three insecticides reveals significant interactive effects
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There are many plant protection products used in agriculture, including a range of insecticides applied to mass-flowering crops, such as oilseed rape. Besides direct effects of single pesticide, which are evaluated following legal requirements, pesticides may potentially interact with each other, causing unpredictable effects on pollinators, different from those predicted by a simple summation of individual effects. Such combined effects of pesticides on bees are still poorly recognized. The solitary bees from *Osmia* genus are not included in any standard procedures for ecological risk assessment of pesticides. Nevertheless, due to their beneficial role for ecosystem services, effects of pesticides on *O. bicornis* are recently getting more scientific attention. In the present study, we tested three insecticide formulations, namely Dursban 480EC containing the organophosphate chlorpyriphos, Sherpa 100EC containing the pyrethroid cypermethrin, and Mospilan 20SP with the neonicotinoid acetamiprid, for their mixture effects on survival of adult female *O. bicornis*. Three concentrations equivalent to no effect (0), 48-h EC_{10} and 48-h EC_{25} of each pesticide were used in a full factorial design with topical exposure (1 □l/bee). The results showed significant effect of each insecticide alone ($p< 0.0001$) and in mixture: Dursban significantly increased mortality in the bees treated with Sherpa or/and Mospilan (interaction term p‿ɤ0.007). A very high toxicity was observed for Dursban with LT_{50} of 1-4 d for all possible treatments with this pesticide except three: Dursban at EC_{10} alone (LT=48 d) and in combination with Mospilan at either EC_{10} (LT_{50}=53 d) or EC_{25} (LT_{50}=38 d). Surprisingly, the LT_{50} for bees treated with Sherpa alone was higher for EC_{25} (48 d) than for EC_{10} (22 d) but did not exceed 9 d for all possible combinations with other pesticides. Mospilan was less toxic for bees when applied alone (LT_{50} of 38 and 28 d for EC_{10} and EC_{25}, respectively) or in combination with Dursban at EC_{10} (see LT_{50s} above), but reduced survival drastically ($LT_{50} \leq 9$ d) when applied with Dursban at EC_{25} and/or any concentration of Sherpa. The obtained results for topical exposure will be compared with those from similar experiment with oral exposure and the effects of significant interactions between studied insecticides will be discussed in the context of changes needed in pesticide risk assessment. This study was supported by National Science Centre, Poland (2017/26/D/NZ8/00606)

2.02P.8
Effect of agricultural landscape structure on pesticides residues and pollen diversity in the nests of red mason bee Osmia bicornis
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Due to agricultural intensification, the land use has changed substantially in the last few decades, shifting from small-scale farms with diverse cropping system towards large-scale monocultures where landscape heterogeneity disappears. While many large-scale monocultures are not attractive to pollinators due to the lack of floral resources, some mass flowering crops, such as oilseed rape, may be important for them. Such crops, however, are treated with a range of pesticides, with insecticides being of particular concern because of potential effects on pollinators which are attracted to the blooming crops and use the contaminated nectar and pollen as food for themselves and the larvae. Pollen diversity and accumulation of pesticides in pollen collected by red mason bees as a food for their larvae were assessed in 12 nests located in 2 contrasting landscapes (10x10 km): dominated by large (L) or small fields (S). Nests (6 per landscape) were allocated on the perimeter of oilseed rape fields and represented different oilseed rape coverage (% land cover). The local landscape structure of each site was characterized within a 100, 200, 500 and 1000 m radius from the nest using 15 different landscape characteristics which were then reduced by non-metric multidimensional scaling to two axes: nMDS1 characterized the dataset primarily according to the field-to-field border length and main crop, whereas nMDS2 captured the prevalence of ¢_natural" landscape features (e.g. length of borders between fields and natural habitats) as confronted with more "urban" features (e.g. vegetation by infrastructure). Each site was also described by Shannon-based landscape diversity index. Pollen diversity was positively (p=0.01) related to nMDS2 with significant interaction between landscape type and nMDS2 (p=0.0008) but only for the 1000 m radius area. We found residues of 34 pesticides in bee-collected pollen at concentrations from 0.015 to 320 ng/g. Acetamiprid, chlorpyrifos-ethyl and flonicamid were the most frequently detected

insecticides. Toxic unit for pesticides in pollen was positively marginally significantly (p=0.06) related to nMDS2, but again for 1000 m radius only. These results indicate that *O. bicornis* foraging range is pretty large, and both the landscape diversity and landscape structure within ca. 1000 m radius around the nest is important for this species. This study was supported by National Science Centre Poland (2015/19/B/NZ8/01939)

2.02P.9

Effect of agricultural landscape structure and pesticides residues on population parameters of the red mason bee Osmia bicornis

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The decline of pollinators in recent decades has led to intensification of research on the causes of this phenomenon. It is assumed that one of the reasons is the intensification of agriculture, including the widespread use of pesticides and changes in land management. This study investigated how the structure of agricultural landscape and pesticides affect population parameters of the solitary red mason bee *Osmia bicornis*. Two research areas (10x10 km) represented two contrasting types of landscape, dominated by large-scale (L) or small-scale (S) agriculture but with similar percentage coverage of the arable land area (80-85%). In each landscape, 6 nests, each with 200 reed tubes and 400 cocoons, were placed at borders of oilseed rape fields in early spring 2017. The local landscape structure was characterized within 1000 m radius around the nest by 15 different landscape characteristics which were then reduced by non-metric multidimensional scaling to nMDS1 which characterized the dataset primarily according to the field size and main crop type and nMDS2 capturing the prevalence of ¢_natural" landscape features as confronted with more "urban" features. To quantify the exposure to pesticides, the residues of pesticides in provisions collected by *O. bicornis* in ca. 100 reed tubes were analyzed for each site. The remaining 100 tubes per site were used to measure life history parameters of the bees: average number of cocoons per tube, average cocoon mass, adult emergence rate, sex ratio and body mass of adults after enclosion and their survival. In addition, susceptibility of the bees to Dursban 480EC was tested, with LT50 as an endpoint. None of studied parameters depended on pollen contamination, but significant negative relationship of cocoon mass (p=0.008) with nMDS1 and positive with nMDS2 (p=0.01) was found, showing that cocoon mass decreased in landscapes dominated by small fields with cereals and increased in more urbanized landscapes but with high share of borders between fields and natural habitats. Similar relationships were found for the mass of emerged adults (p=0.04), while sex ratio negatively depended on both nMDS1 (p=0.001) and nMDS2 (p=0.01). The data suggest that pollen contamination at the levels found in the studied areas is of minor importance, while landscape structure affects some life history traits of the bees. This study was supported by National Science Centre Poland (2015/19/B/NZ8/01939)

2.02P.10

Honey residue semi-field studies first experiences and open questions

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On September 2018 the new guidance document for the conduct of MRL (maximum residue levels) studies in honey was published (SANTE/11956). Studies are needed for consumer safety assessments of residues of plant protection products in honey. Now, after the first season with semi-field studies conducted according to this document, the publication would like to present what have we learned so far and what questions have arisen. Questions are not only on the performance of the studies but also concerning the regulatory framework. From a practical point of view, possible crop choices, influence of the hive strength on the amount of honey will be discussed. An open point in the guidance is that from each tunnel 100g of honey should be sampled. However, in our opinion this amount is not based on real experiences with this study type. Bees are foraging in the tunnels in a restricted environment. Additionally, only a small fraction of this sample size is needed for residue analysis. Data for honey amounts from different crops i.e. Phacelia, oilseed rape and buckwheat are presented to give a more realistic estimation of achievable amounts. A further recommendation in the guideline is that if cells are not capped within the flowering period in the tunnel, the hives should be moved to a remote location and stay there until they are capped or a water content ≤ 20% is reached. However, in the regulatory context there might be authorities who may question this approach. Bees will continue to forage and dilution may occur, which would not represent a worst-case scenario for a region with large fields of the target crop. The results presented will help to develop the existing knowledge about bee residue studies with honey further. **EC (2018) Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9)**

2.02P.11

Pollinator monitoring for standardized evaluations of wild bee communities in agroecosystems and its potential in a risk assessment context

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Extensive knowledge of the occurrence, condition and population changes of wild bee communities in agroecosystems is important. The knowledge is needed to understand the complexity of potential exposure routes to plant protection products in specific crops or to evaluate possible impacts at a landscape scale taking into account other influencing parameters like the cultivation system or management practices. Pollinator surveys that focus on the wild bee diversity and abundance can be useful to make spatial and temporal comparisons in a multifaceted context to allow conclusions regarding the causes of community and development changes. They can therefore provide an important database for the design and evaluation of strategies and concrete measures to support and conserve wild bee communities in agroecosystems. To evaluate a wide range of pollinator species occurring in a specific crop several methods are available. We recommend using a combination of different types of sampling methods: non-selective methods with two types of traps (vane traps and bee bowls) which can be installed at different locations: i.e. in the centre of the fields, at the borders of the fields and outside in adjacent field margins or in non-cropped areas. Additionally a selective method should be used like sweep netting and observations via standardized transect walks in defined distances and time intervals.We investigated with this methodology the abundance and species richness of natural occurring wild bees in a crop considered to be not attractive as foraging and nesting habitat. Furthermore the adjacent field margins were also assessed to compare the in-crop and off-crop as utilized food source during the flowering and reproduction phase of the crop plant. The results show that wild bees are not distributed equally in agricultural regions. The highest diversity and abundance could be found in the surroundings of the investigated fields. Species richness and abundance directly in the crop was very low in comparison. The results indicate only a low preference for those crops which supply no nectar or pollen and pose therefore a low risk for wild bee exposure. The results show that selective and non-selective methods in pollinator surveys help to understand pollinator-plant (crop) interactions in a risk assessment context but are also a useful tool to evaluate the impact of mitigation measures i.e. planting of flowering strips, etc.

2.02P.12

"A buzz of silence": identifying native bee species sensitivity to pesticides in a high pollination dependent area.

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Plant-pollinator interactions are critical for food production specifically of fruits. The interaction is mutually beneficial: pollinators gain food and plants get fertilised. Bees play a vital role in the pollination process. Although most agricultural pollination is commonly attributed to honeybees, solitary bees also contribute decisively to crop pollination. In the past years, declines in bee diversity have been documented in various regions of the world in relation to habitat destruction and fragmentation, insufficient floral resources and pesticide use. The São Francisco Valley (SFV) is a crucial fruit production area accounting for 85% Brazil's export of mango and passion fruit, crops that depend on bee pollination. A total of 187 species of bee have been described in this area; most of them are endemic and rare taxa. The food production of the SFV is very important to Brazil's economy and the agricultural system is fundamental for sustainable production, therefore, protection strategies are of extreme importance. Previous studies associated reduced survival and longevity of bees to pesticide exposure. In addition, contamination with pesticides of bee products widely used by the population, such as honey or the fruits produced is another serious issue. The paucity of data available about pesticide use and effects on bee hampers establishment of effective management and conservation actions preventing bee and crop yield decline. A more than usual number of bee deaths was registered in the past months in the SFV and is creating a huge concern among the local farmers who simultaneously reported a decrease in fruit production. In order to study the high mortality, we focused on solitary bees. In the SVF, we assessed the species most affected and most resistant in the area, along with a screening of pesticides in water and soil samples. A metagenome and metatranscriptome analysis were performed in organisms from the previously selected species. The results showed relationships between pesticides residues and bee's species being affected. The metagenome/transcriptome analysis showed the importance of microbiome in solitary bees and raised the importance of studying the neglected species. This project was supported using seed funding from Cardiff University's GCRF QR Funding from the HEFCl for Wales. Dr Nuno Ferreira was supported by a MSC COFUND Fellowship (H2020-COFUND-SIRCIW>MINT-512202) through Cardiff University, Welsh Government and the EU.

2.02P.13

Quantification of pollen intake as a behavioral endpoint for foraging activity in ecotoxicological risk assessment

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To date, there is no appropriate tool available to objectively assess sublethal and behavioral endpoints as part of the ecotoxicological risk assessment for pollinators. The current examination through expert assessments implicates a number of shortcomings, as the measurements are subjective, not simultaneously examined and sampled sparsely. However, a tool suitable to provide the basis for reliable risk assessment must be able to provide both continuous and comparable data of several hives. In an effort to provide such a tool, a camera-based visual monitoring device has been developed by apic.ai. The system, which can be installed in front of honeybee or bumblebee hives uses Neural Networks to automatically extract features from video footage. This enables the generation of new insights with the potential to measure sublethal effects and changes in behaviour on a grand scale. Among the features which can be extracted are pollen intake, movement patterns and physical characteristics. If they were usable and accepted as endpoints in ecotoxicological testing, they could contribute to reducing the uncertainty of risks to pollinators. This research focuses on pollen intake as an indicator for the intensity of foraging activity.To assess the influence of plant protection products on foraging activity, video data of honey bee hives during an Oomen feeding study will be analysed. Footage of eight hives will be processed, out of which four were fed 200 µg imidacloprid /kg in addition to the 500 g of 50% sugar solution which all received. Focal point of the investigation is the correlation between foraging activity and exposure to a neonicotinoid.The video data has been captured between the months of May and September 2019. The amount of collected pollen will be analysed by March 2020.

2.02P.14

Comparing model predictions to measured diversity of and pesticide residues in pollen samples, collected in the citizen science based INSIGNIA project

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In the INSIGNIA EU project, a protocol is developed and tested for citizen-science based monitoring of pesticides using honeybees. As part of the project, biweekly pollen samples are obtained from sentinel apiaries over a range of European countries and landscapes, validating different devices and methods. The samples are analysed for botanical origin, using state of the art molecular techniques, and for residues of a large number of agricultural pesticides and veterinary products, both authorized and unauthorized. The data collected are used to develop and test a spatial modelling system aimed at predicting spatially-explicit pollen diversity and pesticide mixture exposure and risk at the apiary level, from basic landscape data. The system consists of model components for spatial and temporal pollen resource distribution, application and environmental fate of pesticides, and honeybee landscape-scale pollen foraging, with a common underlying geo-data base containing European land-use and land-cover data (CORINE) supplemented with national data sets on agricultural and (semi-)natural habitats. It requires data on the use of agrochemicals and on the phenology and other characteristics of the crops and wild flowers constituting the main pollen resources. The first results of applying the modelling system will be presented, comparing model-predicted pollen composition and pesticide residues to sampling data obtained in the first year of the INSIGNIA project, from apiaries in Austria, Denmark, Greece and the UK. This comparison is the first step in an iterative process of refinement of models. It will indicate to what extent apiary exposure can realistically be predicted from relatively coarse-grained Europe-wide landscape data using models of intermediate complexity, and point out the steps required to arrive at more precise predictions.

2.02P.15

Characterizing pesticide residues in pollen and nectar collected from plants: The case study of Ireland

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Most pesticides applied in Ireland, in terms of volume, are Plant Protection Products (PPPs), including herbicides, fungicides and insecticides. These PPPs are applied via spraying or as seed treatments. To ensure sustainable pesticide use, there is a need to understand where these PPPs are found, both within and beyond the target plants, whether it is feasible to evaluate any impacts on bees and other beneficial insects, and the extent to which potential strategies can mitigate these impacts. Recent evidence suggests that PPPs found in soils (or seed treatments) can also be translocated through plants, and can be found in the nectar and pollen of both crops and wildflowers. Pollen and nectar are the main food source for bee pollinators and this oral exposure, along with direct contact through spraying, is of concern for their health and for the delivery of pollination services. Therefore, this study is an effort to evaluate the residues of PPPs found in pollen and nectar of insect-pollinated plant species in Ireland. Systemic PPPs, extensively used in Irish agricultural systems, which potentially have negative impacts on pollinating insects, were selected for study. Pollen and nectar from two plant species from the Irish farmland (one crop and one hedgerow species) were collected and analysed using validated extraction protocols of these pesticides. The frequency of detection and the concentration of the target pesticides in nectar and pollen samples from each site was determined by the appropriate validated GC-MS or HPLC-MS method in order to estimate the pesticide exposure risk for nectar and pollen feeding pollinators. In this presentation, preliminary findings will be discussed.

2.02P.16

Residues in landscale plants and sublethal effects on bumblebees and 2 specie of butterflies

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Neonicotinoid insecticides are commonly used in landscapes and nurseries in the US, but levels of residue in ornamental plants is rarely measured, Neonicotinoid residue was determined for trunk injections of trees, soil drenches of trees and herbaceous plants, and nursery plants. Residue from landscape plants were compared to residue levels in plants grown near agricultural fields. Residues in plants were compared to lab and field studies on sub lethal doses on bumblebees and butterflies. Research on bumblebees, Eastern bumblebee, *Bombus impatiens*, demonstrated that 20 ppb clothianidin (neonicotinoid) and 4 ppm Acelepryn (bee friendly clorantraniliprole) had different effects on BB colony health. Data showed that the bee friendly insecticide clorantraniliprole had no effects on colony weight, consumption, brood production or movement, when the neonicotinoid clothianidin affected all of these parameters. Higher levels of foliar applied clorantraniliprole at field relevant doses did cause mortality of bumblebees. Two species of butterflies, *Danaus plexippus*, the monarch butterfly, and *Vanessa cardui*, the painted lady butterfly, could tolerate higher levels of residue in LD 50 studies as well as chronic 5 week studies. Sub lethal effects on butterflies were reduced flight and proboscis extension, but no effects were found on fecundity. This research provides correlative data on the status of the rust patched bumblebee, *Bombs affinis*,that commonly occurred in urban areas and is now on the endangered species list.

2.02P.17

Interactions Between Bombus terrestris and Glyphosate-Treated Plants: Are Bees at Risk of Herbicide Exposure?

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Over recent years the effects of herbicides on bee health have been receiving increasing attention. However, it is unclear how bees are exposed to herbicides in the environment, and whether it is through direct spray during application, foraging on treated plants or contact through residues in the environment, amongst other routes. In this study, we aim to determine if bees will avoid foraging on herbicide treated plants, therefore reducing their contact with herbicides in the environment. We observed the interactions of bumblebees (Bombus terrestris audax) with Phacelia tanacetifolia plants, which were treated with the herbicide glyphosate or water, and whether these interactions changed depending on the time after spraying i.e. when the herbicide had translocated further through the plant. Observations where conducted during two time periods, post spray: either plants freshly treated with glyphosate or water (within 24 hours) or after glyphosate had time to translocate within the plant (48 hours). Observations of individual foragers were carried out in an exclusion cage containing four randomly placed Phacelia plants, two treated with glyphosate and two treated with water, and observers were blind to the treatment of each plant. The frequency and duration of interactions were recorded during a 25 minute period using behavioral logging software (BORIS). Interactions observed included: nectar feeding, pollen collection and investigation (inspection but not foraging) of plants. We found that most bees had a foraging interaction (pollen collection or nectar feeding) with glyphosate treated plants, and we preliminarily conclude that bees indiscriminately forage on glyphosate treated plants.

2.02P.18

Exposure of the bumble bee Bombus terrestris to anthropogenic pollutants in urban and agrarian environments

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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 modern agriculture. Natural habitats for pollinators such as honey and bumble bees are being lost due to fragmentation, land-use changes and the application of pesticides. This drives pollinators into urban surroundings, where they find small scale habitats in parks and gardens. In urban, as well as agrarian landscapes, various anthropogenic activities lead to diverse pollutant emissions. In urban areas, chemical pollution is characterized by traffic pollutants resulting from the incomplete combustion of fossil fuels or the abrasion of tyres and roads, as well as the use of biocides on buildings, in parks and in private gardens. The agricultural landscape in contrast, is more contaminated with various pesticides. 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 focused on honey bees, while data on pollutant loads of non-*Apis* pollinators is rare. *Bombus terrestris* is an important pollinator in cities and agrarian landscapes and has a more spatially restricted foraging behaviour. This makes *B. terrestris* well suited as a biomonitoring tool for representing the exposure of pollinators to various chemical profiles in anthropogenically influenced landscapes. In this project, living individuals of *B. terrestris* were exposed in different outdoor sites, and then collected and extracted using a modified QuEChERS method. Hives were placed in close proximity to major traffic roads in Aachen (Germany) as well as in agricultural fields situated around the city between March and September. Polycyclic aromatic hydrocarbons (PAH) and trace metals are being investigated as markers for traffic pollution. A selection of pesticides (amongst others acetamiprid, boscalid, chlorotoluron, indoxacarb, prosulfocarb) are being quantified to measure the exposure from different pesticide sources. In total, concentrations of approximately 40 organic contaminants are being quantified using LC- and GC-MS/MS, trace metals are measured using ICP-MS. To understand possible differences in the bumble bee exposure, passive samplers (polyurethane foam disks) are being simultaneously exposed at the same locations and also being analysed for the target substances.

2.02P.19

Bee Pollinator Toxicogenomics: Understanding the Biochemical and Molecular Interactions of Managed Bee Pollinators with the Novel Butenolide Insecticide Flupyradifurone

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The toxicity of insecticides to bees is routinely assessed according to officially published guidelines and guidance documents using the honey bee as the primary surrogate species to fulfill regulatory requirements. In contrast, deeper knowledge on the molecular and biochemical interactions of bee pollinators with insecticides is largely lacking, but would be of great interest to mechanistically understand pollinator-pesticide interactions. To address this knowledge gap, Bayer AG, Crop Science Division, initiated the project “Bee Pollinator Toxicogenomics” with the particular aim to elucidate the molecular basis of selectivity of insecticides against managed bee pollinators with special reference to a comparative functional genomics approach covering different bee species in cooperation with external partners. Within this project, the molecular and biochemical mechanisms underlying the bee-selective profile of the novel butenolide insecticide flupyradifurone was investigated. Flupyradifurone is a systemic insecticide that targets the postsynaptic nicotinic acetylcholine receptor (nAChR) of insects` as a partial agonist and is applied to control a broad range of sucking key pest species while displaying an overall favorable ecotoxicological profile. A large number of laboratory, semi-field and field studies have demonstrated that flupyradifurone poses no unacceptable side-effects on honey bees when it is used according to the proposed field-rates, even when the product is applied during bloom. We holistically studied the in vivo metabolism and detoxification mechanisms of flupyradifurone in honey bees and identified three key cytochrome P450 monooxygenases (P450s) involved in the rapid detoxification of flupyradifurone, thus conferring tolerance. A comparative analysis of the acute contact intrinsic toxicity of flupyradifurone to other managed bee pollinators including Bombus terrestris, Osmia bicornis and Megachile rotundata revealed, that the alfalfa leafcutter bee is 170-fold more sensitive compared to the other bee species and furthermore lacks the ortholog P450 enzymes involved in the oxidative metabolism of flupyradifurone, thus explaining the elevated intrinsic toxicity. Our study mechanistically assess pollinator-insecticide interactions and suggest phylogenetic analyses of key detoxification enzyme families in combination with functional analyses as a predictive tool for species sensitivity.

2.02P.20

Disturbed energy metabolism after neonicotinoid exposure in relation to altered homing flight activity of honey bees

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Neonicotinoids are suspected to be implicated in the decline of honey bee populations. As nicotinic acetylcholine receptor agonists, they disturb acetylcholine receptor signaling, leading to neurotoxicity. Several behavioral studies have shown links between neonicotinoid exposure and adverse effects on foraging activity, homing flight performance and reproduction but the molecular aspects underlying these effects are not well understood. Besides possible sublethal neurotoxic effects of neonicotinoids leading to disorientation which might adversely effect homing flight time. Neonicotinoids may also disturb energy metabolism hence, causing longer homing flight time or exhaustion. To test the second hypothesis, pollen foragers were fitted with RFID chips, exposed orally to 1 ng/bee thiamethoxam applying single bee feeding and group feeding approach, released 1km from the hive and homing flight time was monitored using the RFID system. In the evening, all returned foragers were collected and stored at -80°C until further analysis. After homing flight data analysis, brain RNA of fast returning controls and slow returning exposed foragers of both feeding strategies was isolated and energy metabolism transcript expression was analyzed using quantitative PCR. We analyzed expression of *cox 5a*, *cox 5b*, *cox 6c* and *cox 17*, all transcripts of complex IV and *ndufb-7*, part of complex I of the oxidative phosphorylation. The expression of *cox 5a* and *cox 17* was significantly altered in pollen foragers exposed to 1 ng/bee thiamethoxam by applying single bee feeding approach and there was a strong correlation between the down-regulation of *cox 17* and the prolongation of homing flight time. No significant changes were detected by applying group feeding. In summary, this small study has two major findings. First, feeding strategy is very important as regards significant effects and single bee feeding approach should be used in future studies as dosing of bees is not throphallaxis dependent. Second, there is a clear link between prolongation of homing flight time and energy metabolism. Therefore, longer homing flight time may be not only due to disturbed orientation but also due to a lack of energy. We have elucidated the link between homing flight performance and expression of selected transcripts in the brain of honey bees in case of exposal to a neonicotinoid but further studies are needed to analyze the energy metabolism more in Detail.

2.02P.21

Body distribution and effects of the neonicotinoid insecticide clothianidin in bumblebees (Bombus terrestris)

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Bumblebees can be exposed to neonicotinoid pesticides through nectar and pollen collected from treated crops, which can cause lethal and sublethal effects. However, the bioaccumulative potential of neonicotinoids in bumblebees has not been studied before. Our study aimed to assess the accumulation of the neonicotinoid clothianidin in bumblebee workers and queens, and assess whether the accumulation could cause change in population dynamics (i.e. mortality and brood production) and foraging behaviour (i.e. nectar consumption and storage). Bumblebee colonies (*Bombus terrestris*, n = 48) were exposed to field-realistic concentrations of clothianidin through artificial nectar ranging from 1 µg/L to 13 µg/L, in a chronic exposure regime lasting nine days. Clothianidin showed a dose-dependent uptake in the head (< 0.2 – 2.17 µg/kg) and body (< 0.2 – 3.17 µg/kg) of workers, and in the body (< 0.2 – 2.49 µg/kg) of queens, although concentrations were below that measured in the nectar (BAF = 0.2). Exposure did not cause a change in mortality or brood production but showed a hormetic trend (first a positive then a negative response) in nectar consumption and increased food storage with increasing treatment levels, meaning the bumblebees preferred spiked nectar instead of their stored food. In a broader perspective, this could negatively impact the pollination efficiency of *B. terrestris*.

2.02P.22

The neonicotinoids clothianidin and imidacloprid differentially affect the brain of bumblebees at the cellular and molecular levels prohibiting the identification of biomarkers of general insecticide exposure.

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75% of all plant crops globally are dependent on animal pollination, much of which is carried out by insects. Sustainable food production and biodiversity maintenance in general is currently at risk due to the decline in insect pollinator numbers and diversity, driven by factors such as habitat loss, agrochemical use, disease and climate change. Although extensive research has been conducted to better understand these drivers, multiple gaps in our knowledge remain. This is particularly true for pesticides and their role in pollinator decline. In this study we investigated the effects of exposure to sub lethal doses of the neonicotinoids clothianidin and imidacloprid on the brain of the buff tailed bumblebee *Bombus terrestris*. Bees from 4 different colonies were acutely exposed to 2.5 and 10 ppb according to the OECD 213 guideline for oral tests, with minor modifications. Proteins were isolated from brains dissected from pesticide exposed bees after three timepoints (1h, 4h and 24h) and analysed on a ThermoFisher QExactive high resolution accurate mass spectrometer. An Olympus Fluoview Confocal Microscope was then used to analyse synapsin levels in specific neuropil areas of the brain. Protein identification, quantification, data normalisation and statistical and gene ontology analyses were conducted using MaxQuant and Perseus (v 1.5.5.3). Over 2,000 brain proteins were identified in all replicates after data processing and filtering. Functional annotation of the statistically significant differently abundant (SSDA) proteins ($p < 0.05$), was performed using the STRING platform, availing of the *Drosophila melanogaster* reference genome. Both neonicotinoids altered energy metabolism and cellular communication, with clothianidin specifically affecting cytoskeleton reorganization and intracellular transport whereas imidacloprid specifically affected nucleotide metabolism. This difference in effect made the identification of common bio-indicators of general neonicotinoid exposure difficult. The analysis of synapsin levels and distribution in the bumblebee brain also highlighted a differential response to both pesticides with imidacloprid resulting in higher synapsin levels, whereas clothianidin caused no effect. Taken together our results provide new insights into the molecular aspects of neonicotinoid exposure and the potential detrimental effects they have on brain molecular composition and neurophysiology at sublethal levels.

2.02P.23

Development of a standardized laboratory test for the mayfly Cloeon dipterum

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Insects within the orders Ephemeroptera, Plecoptera, and Trichoptera (the "EPT" insects) contain some of the most sensitive species to environmental contaminants and consequently are interesting to test under standardized laboratory conditions. However, many of these taxa were found to be difficult to keep in the laboratory because biological and physical requirements were often poorly known. However, a renewed interest in incorporating sensitive insect taxa in toxicity testing arose recently with an emphasis on mayflies as test species. Partly this is due to their high sensitivity of some mayfly species to a range of chemical classes. In order to generate good data to be used in risk assessment it is of paramount importance that a solid test methodology is available. For this purpose a comparison was made between two existing test methods. These methods comprised different designs (e.g., numbers of Cloeon per replicate), different food items and different test media. A matrix design was used to test these different factors, which was extended with the differing origin of the mayfly larvae. The results showed that test medium and test design were of lesser importance but that food played an important role in the development rate and survival of the mayflies in the tests. In addition, it was clear that transporting mayflies between participating labs resulted in poor test results as well.

2.02P.24

The pattern of interspecific sensitivity of carabid beetles towards cypermethrin in oilseed rape crops changes with exposure time

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Arthropod biodiversity in agricultural landscapes has decreased dramatically during the last few decades. The use of pesticides is considered one of the main causes of this trend. For understanding how pesticides may affect ecosystem services it is crucial to assess the exposure effect at the community level. Carabid beetles are considered one of the essential Ecosystem Service Providers (ESPs) in agro-ecosystems, being natural enemies of many crop pests, but the knowledge about their sensitivity towards commonly used pesticides is scarce. The main objective of our study was to measure sensitivity of Carabidae species collected from oilseed rape crops towards Sherpa 100 EC – a commonly used plant protection product with cypermethrin (pyrethroid) as an active ingredient. The possible changes of interspecific susceptibility patterns towards the insecticide over the exposure time were tested by comparing Species Sensitivity Distribution (SSD) curves established for 5 h, 24 h, 48 h and 96 h of exposure. In a set of dose response experiments different species of ground beetles were exposed to Sherpa 100 EC dissolved in acetone and distributed evenly on a Petri dish. The SSDs curves were established by fitting cumulative distribution functions to species-specific LD50s (median lethal doses) and were used to estimate the Potentially Affected Fraction (PAF) at certain doses of the pesticide and to derive the community level endpoint – hazardous dose for 5% species (HD5). The estimated HD5 values constitued only a fraction (≤0.1) of Sherpa 100 EC reccomended field dose in oilseed rape crops. Not only the HD5 values changed through the time of exposure, but also the order of species on the SSD curve. For instance, Bembidion lampros being the most resistant species after 5 h, became the most vulnerable one after 96 h of exposure. Therefore, time of the exposure is crucial for SSD interpretation and aplication in ecological risk assesment. The study was supported by the European Union's Horizon 2020 research and innovation programme, under grant agreement no. 773554 (EcoStack)

2.02P.25

Toxicity of Mamestra brassicae to Different Classes of Pesticide Compounds Exposed Through Artificial Diet

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The typical protocol for agrochemcical toxicity testing of Lepidopteran species is usually either a leaf disc bioassay or topical application. However, due to restrictions dictating the length of these tests (i.e. leaf freshness/moults) these methods are better suited to shorter term bioassays. Past reviews of *Lepidopteran* bioassay methods it have suggested that methods using artificial diet are more suited to high throughput assays due to their ease of storage and labour efficiency (Perez *et al*., 1997). To allow assessment of continuous exposure through diet, we developed a method incorporating pesticides into artificial diet. A variation of Hoffman's diet (Smith, 1966) was created with a composition suited to pipetting into multi-well plates. Pesticides carried in either water or acetone were added into the diet while it remained in a liquid state. 4th instar *M. brassicae* weighing 20mg ±5mg was added to each well and survival and weight monitored over a series of exposure times. Mortality was recorded at 0, 6, 24, 48, 72 and 96 hours. Weights were taken for *M. brassicae* on their introduction and after removal from the well, after 96 hours. Compounds tested for their effect on these endpoints included, insecticides (Imidacloprid, Cypermethrin, Chlorpyrifos, Sulfoxaflor and Cyantraniliprole), fungicides (Azoxystrobin, Propiconazole and Chlorothalonil), herbicides (Diflufenican and Glyphosate) and a molluscicide (Metaldehyde). Results indicate that *M. brassicae* were only sensitive to the insecticides Chlorpyrifos, Cypermethrin and Cyantraniliprole. The LC50 for Imidacloprid could not be determined but weight data showed a clear effect at 150mg/kg. *M. brassicae* showed low sensitivity to all fungicides, Molluscicides and herbicides and even at maximum acetone solubility, no weight loss was observed. Although probably not suitable for all *Lepidopteran* species, this incorporated diet method has provided a high-throughput bioassay system which allows sublethal effects to also be monitored. In addition, as the exact amount of diet consumed can be determined consumption rates can be calculated. This has implications for further study such as the application of Dynamic Energy Budget Models (DEB) to Lepidoptera.

2.02P.26

Precision farming - consideration of reduced exposure in the pollinator risk assessment

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In the course of the ongoing public and political discussions on the desired reduction of plant protection products (PPP) and the protection of insects and their divesity, especially pollinators, but also in the light of intended yield increase, process optimisation and cost reduction in agriculture, precision farming becomes more and more important. As there can be huge small-scale variability of insect, weed or fungal infestation of the crop, precision plant protection application will enable farmers an infestation-orientated and subarea-specific crop protection within a field. Currently, developments and research activities are mainly focussed on technical optimisation such as spatial recording of pests (weed species, insect pests, crop relevant fungal diseases), data processing and analysis as well as on the use of this information for the operating of precision application equipment. Agricultural devices used in precision application are currently under development (mainly research-orientated), but a few models are already in practical use. In principle, the use of precision techniques in plant protection should lead to reduced exposure of non-target organisms such as soil organisms, epigeous and epiphytic beneficial arthropods and bees. However, it is unclear to what extent this is the case for the different exposure pathways and where and how this can be taken into account in the European environmental risk assessment of PPP. We present exemplary precision application systems and discuss their potential influence on the exposure of honey bees and their colonies of partially treated fields. Furthermore, we suggest how precision plant protection application can be included in the environmental risk assessment scheme and present ideas to verify the theoretical assumptions.

Contaminants in Highly Exposed Wildlife: Interactions of Contaminants, Climate Change, and Other Environmental Stressors (P)

2.03P.1

Effect of agricultural management on males' reproductive status in Iberian hare (Lepus granatensis)

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The intensification of the agricultural sector during the 20th century has caused an important homogenisation of the landscape due to land consolidation and introduction of pesticides and chemical fertilisers to the environment. This fact has been related to the loss of biodiversity. Concerning pesticides, there are important shortcomings in the knowledge of secondary or sublethal effects on wildlife. In order to clarify if pesticides are responsible for wildlife reproductive impairment in the Iberian agroecosystems, male reproductive parameters of the Iberian hare (*Lepus granatensis*) were compared between areas with different agricultural management. Specifically, testes were collected from animals shortly after hunting in areas where pesticides are used (treated area, n=14) and others in pesticide-free areas (control area, n=9). At the laboratory, after biometrical measurements of testes, epididymal spermatozoa were collected to evaluate different quantitative and qualitative spermatic variables. The results obtained showed significant differences between animals from control and treated areas in: total motility (control *vs* treated, average values; 83.89% *vs* 47.79%); morphological abnormalities (2.56% *vs* 10.18%); viability (99.25% *vs* 98.29%); membrane functional integrity (78.39% *vs* 50.71%), and marginally significant differences in testicular weight (8.30 g *vs* 6.86 g). Hares from pesticide-free areas showed better sperm quality compared to those from pesticide-treated areas. This study is part of a more extensive project which will enable us to establish if the observed effects are due to a direct, or indirect influence of pesticides caused by, among other factors, nutritional differences related to the decrease of flora diversity associated with treated areas.

2.03P.2

Time trends of persistent organic pollutants and mercury in Canadian Arctic ringed seals and relationships with climate parameters

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The ringed seal (Phoca hispida) is the most abundant Arctic pinniped and has been a key biomonitoring species for assessing trends of persistent organic pollutants (POPs) and mercury in the changing Arctic. In Canada, ringed seal sampling has been done by local hunters during subsistence harvesting and coordinated by Hunters and Trappers Associations/Committees. The Northern Contaminants Program currently supports the monitoring of contaminants in ringed seals from the Beaufort Sea, Central Arctic, Hudson Bay and Labrador. Time trends of polychlorinated biphenyls (PCBs), organochlorine pesticides and mercury (Hg) have been analyzed in seals in relations with climate parameters such as mean ice coverage and ice type, air temperature, precipitation and climate indices (i.e., Arctic, North Atlantic and Pacific/North American Oscillations). Mercury is of particular concern given the elevated concentrations found in marine mammals, the importance of these animals as traditional country foods for Indigenous peoples, and the health advisories in place in specific Canadian Arctic regions. Results from this long-term study (1972-2017) indicated geographical differences in the contaminant concentrations in seals and the significant general decrease of most POPs (e.g. PCBs; dichlorodiphenyltrichloroethane, DDT; hexachlorocyclohexane, HCH) over time. Increases in concentrations of hexachlorobenzene (HCB) in seals from eastern Canada and β-HCH in the Beaufort Sea and Central Arctic were also observed. Mercury levels did not significantly change through time in seal liver, however, levels of total Hg in muscle significantly decreased over the time period in seals from East Baffin Island and eastern Canada. Hg concentrations in liver and muscle were found to significantly increase with trophic level values ($\delta^{15}N$). An increase in $\delta^{15}N$ values was also found overtime in seals from the Central Arctic. Isotopic markers of feeding ecology ($\delta^{13}C$) indicated a decrease of values through time in seal muscle at all sites suggesting a change in feeding habits and a shift in diet towards pelagic and/or offshore preys. Values for $\delta^{13}C$ were, however, not found to influence mercury accumulation in seals. Site-specific and contaminant-specific associations between climate patterns, air temperature, precipitation and ice type and mean sea-ice coverage were found suggesting the potential regional impacts of environmental factors on the accumulation of contaminants in seals. Continuous sources of legacy compounds and mercury as well as changes in food web composition and structure in relation to climate changes could also be influencing the very slow rates of decline, or stable levels, of contaminants found in seals at some sites. Further work is warranted to discern between covariation of climate changes and contaminant concentrations and cause-and-effect relationships.

2.03P.3

Effects of pesticides on wildlife: The conservation of the Iberian hare in Spanish agrosystems

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The gradual change from a traditional towards an intensive agriculture that has taken place in recent centuries has been translated, among other things, into an important land concentration and the entry of more and more pesticides and chemical fertilizers into the environment. Studies are now linking this agricultural intensification with the loss of plant and animal diversity. In the case of pesticide, although their mode of action is often known in the target species, their secondary or sublethal effects on wildlife are still unknown, particularly at the highest levels of biological organization: populations, communities and/or ecosystems. We face, therefore, the need to causally link knowledge about the molecular actions of pesticide to their possible interference with biological processes, in order to be able to develop reliable predictions about the consequences of the use of pesticides. Among the most consumed pesticides, in Spain, glyphosate, 2,4-D and MCPA stand out. Therefore, this project aims to study their effects on wildlife populations that inhabit agricultural environments, to make an assessment of the risk of exposure to pesticides and assess the effect they may have on the wildlife population dynamics. For this, the Iberian hare (Lepus granatensis) has been chosen as a model species because it is i) a species strongly linked to agrosystems, ii) herbivorous, and therefore susceptible to direct exposure of herbicides by ingestion of treated plants, iii) have a high ecological importance to be prey species of great interest for conservation, iv) and a high socio-economic relevance, being a hunting species. The results of this project will allow us to establish measures with which to reduce the exposure of wildlife to pesticides and thus favour the status of the species that inhabit that ecosystems.

2.03P.4

Silent Scottish ponds: Evaluating the health of natterjack toads (Epidalea calamita) in Scotland in relation to present and historic sites

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Amphibians are declining globally, with 32% of species threatened with extinction and 43% experiencing declines. In the UK, toad species are declining, particularly the natterjack toad (*Epidalea calamita*) which is threatened with extinction in Scotland. Major threats to their survival include changes in habitat management, climate change induced seawater inundation and possibly pollutants. This research sought to investigate the impacts of water quality and habitat characteristics on natterjack health using both field-based and laboratory techniques. Differences in water quality/habitat characteristics between Mersehead (viable natterjack population) and two sites with historical natterjack populations (Southerness/Caerlaverock) were investigated. Sites were visited twice a week to collect water and conduct physico-chemical measurements. Additionally, impacts of filtered water taken from these sites on hatching, tadpole growth and metamorphosis were analysed in the laboratory on eggs taken from a healthy population (Cumbria; 50 eggs from 6 spawn strings). In laboratory reared spawn, survival, hatch rate, timing of metamorphosis, weight/length of metamorphs, presence of chytridiomycosis/Ranaviurs as well as analyses of gonads/thyroid/thymus (histopathology), were undertaken. Water quality differed at Mersehead compared to Southerness/Caerlaverock, which included the site having higher temperature, higher dissolved oxygen, higher resistance and lower total dissolved solids. Higher resistance indicates fewer dissolved ions and relates to salinity, which was lower at Mersehead compared to Caerlaverock. Grass height was also significantly lower at Mersehead. In laboratory exposed tadpoles no evidence of chytridiomycosis or ranavirus was observed at any sites. There were differences between sites in health of offspring, and no hatching was observed in spawn reared in Caerlaverock water, potentially due to high salinity and total dissolved solids. Larvae reared in water from Mersehead and Southerness had high survival, but those from Southerness were smaller and developed more slowly. Further analyses are ongoing. This work suggests natterjack toads may be affected by various environmental stressors, including freshwater salinization, an effect of climate change. 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.

2.03P.5

Effect of temperature on a short-term exposure of common goby (Pomatoschistus microps) to an environmentally relevant mercury concentration

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Mercury (Hg) is a widespread pollutant across estuarine and coastal areas, raising concern for potential impact on aquatic organisms. Hg may enter into these ecosystems from natural and anthropogenic sources, being potentially toxic, persistent and may bioaccumulate and biomagnify through food webs, reaching concentrations many times higher than the levels in the surrounding water and ultimately representing a serious risk to human health. Hg toxicity may also induce reactive oxygen species (ROS) production in marine organisms, which are responsible for cell and tissue damage leading to cell death. Bioaccumulation of Hg in the different organs of fish depends, among other aspects, on the degree of environmental contamination. At the same time, temperature is certainly an important environmental factor to consider regarding bioaccumulation, due to its marked influence on the physiology and ecology of aquatic organisms. This study aimed to investigate the effect of different temperature scenarios (15, 20 and 25 ºC) on the Hg bioaccumulation on *Pomatoschistus microps* (Krøyer, 1838) muscle and liver, as well as on oxidative stress responses, after a short-term exposure to an environmentally relevant Hg concentration (1.2 ug g^{-1}). *P. microps* has been widely used as test organism, holds a key role in estuarine food webs as intermediary predator and is found in a large range of temperatures (8 – 24ºC). It is therefore pertinent to understand if their tolerance to Hg and bioaccumulation potential alters under different temperature scenarios. Our results showed that Hg bioaccumulation tends to increase along the temperature gradient in both muscle and liver. However, the activity of antioxidant enzymes and energy consumption was neither affected by Hg bioaccumulation nor by tested temperatures, suggesting a physiological adaptation to exposure conditions. These findings are ecologically relevant and highlight the importance of further investigation of combined effects of Hg and other stressors.

Ecosystem Functions and Services: Understanding and Managing Anthropogenic Impact (P)

2.04P.1

Use of ecosystem services concept in land degradation estimate in Russian Federation

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Land degradation and pollution lead to serious productivity loss of ecosystems. In terms of economy it causes a significant monetary loss to landowners and the society. The damage calculation of land could be a reasonable tool for environmental responsibility. Three types of expenses seem to be included to calculate the loss of land at degradation process: soil remediation, recovery to its initial price, as well as the consideration of lost ecosystem functioning and services. We proposed approaches to evaluate ecosystem functions in terms of economy. We collected and studied soil and waste samples on 8 land plots , disturbed by oil wells arrangement in Khanty-Mansiysk Autonomous Okrug-Yugra, Russia. The soil and waste samples were analyzed for the content of petroleum hydrocarbons (PHC), and chlorides. PHC were detected by gas chromatography, chlorides - ion liquid chromatography. The waste toxicity was determined by mortality of Ceriodaphnia affinis and infusoria Paramecium Caudatum. To assess the degree of soil contamination, the PHC content was compared with the existing established standards of soil quality for the region, which are different for selected types of soils (from 0,1 g·kg-1 for mineral soils of water protection zone up to 60 g·kg-1 for organic soils of peatlands). The chloride content in soils was compared with regional background content as 2 mg·kg-1. We counted the damage from land degradation according to the latest methodology, adopted in RF, which is based on the multiplication of taxes, correction factors, and indicators characterizing the damage in physical terms. An alternative way to count monetary damage is the calculation of expenses on restoration of damaged natural resources and lost ecosystem services. Here we have attempted to monetize the cost of lost ecosystem services - carbon dioxide sequesteration, purification of water in protection zones, and production services: wood products, biological stock of berries and mushrooms, hunting products. Comparing the results of economic evaluation of caused damage, done according to fixed taxes and expenses for remediation of 8 degraded land plots and lost ecosystem services, we can see that the evaluation of damage due taxes is almost always significantly overstated and does not correlate with the land remediation expanses. The consideration of ecosystem services leads to a some increase of land degradation damage values, that can indicate an underestimation of natural resources in Russia. Russian Federation is a global donor of ecosystem services, but the evaluation services are highly underestimated. The value of ecosystems will increasingly continue to grow in future, mainly due to the loss of ecosystems. It is necessary to further develop a methodology for assessing ecosystem services, thereby contributing to the restoration of damage caused by industrials.

2.04P.2

Biomonitoring in the Anthropocene: Rapid Assessment of Mining Remediation using Environmental DNA (eDNA) Metabarcoding

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To better understand ecosystem responses to anthropogenic stress, new biomonitoring methods are needed that assess biodiversity in a quick and cost-efficient manner. The emerging field of environmental DNA (eDNA) metabarcoding potentially offers such an approach, as it may provide high-throughput, low-cost identification of multiple species present in an area. To test the effectiveness of this new technology, eDNA was collected from various stream locations within The Wilds Conservation Center (Cumberland, OH). This area has active acid-mine drainage from historical coal-mining operations and the stream sites here include both remediated and unremediated sections. Water quality parameters (pH, metals, conductivity) were assessed at all sites to better understand differences in biotic indices across the range of AMD scenarios. The goal of this proof-of-principle study is to estimate biodiversity and community structure at each site using eDNA from water samples and leaf litter. These parameter estimates will then be compared to data from current and historical electrofishing and kick-net surveys in the area. Based on previous attempts to characterize macroinvertebrate communities via eDNA, leaf litter bags were used as a novel source of DNA to compare to the eDNA signal from water samples. The utility of leaf litter DNA serves two purposes as litter degradation rates can also be compared to community structures inferred from metabarcoding. Our working hypothesis is AMD reduces biodiversity and simplifies community structure at locations closest to an AMD source. These simplified community structures can then lead to reductions in ecosystem functions i.e. litter decomposition rates. Results from this study will provide a wealth of species information to be used in future remediation efforts by The Wilds. Knowing which levels of AMD correspond to desired community structures will help create AMD benchmark levels for successful management of remediation sites.

2.04P.4

Structural and functional changes in ground beetle communities of different ecosystems over a period of 25 years

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The decline of insects is an omnipresent topic that has attracted a great deal of attention in recent years, both in science and in the general public. Insects are essential components for the functioning of many ecosystems. Thus, it is of enormous importance to understand the real extent of the insect decline and the reasons for it in order to take targeted measures. As predators, ground beetles (Coleoptera: Carabidae) play an important role in the food web, but also herbivorous ground beetles make a major contribution to the maintenance of nutrient cycles. Due to their abundant occurrence and easy detection by pitfall trapping, they have long been a popular research object resulting in good knowledge of taxonomy and autecology. The differentiated ecological demands of many species make them very sensitive to stressors, which is why they are excellent indicators of the extent of changes in a site.

In this work, different ecosystems were investigated for changes in carabidocoenosis over the last 25 years. A total of 20 areas in forests, hedges, meadows and field margins were sampled from April to October 2018. All sites were located in the cultural landscape (agri- and silvicultural) and were thus under human influence.

Overall, a decrease in diversity was observed in all investigated ecosystems. In addition, the proportion of specialized species decreased consistently, which in some hedges and on the field margins was also accompanied by an increase in macropter and dimorphic species, i.e. species with a potentially larger range. Contrary to the expectation that the decline in the agricultural landscape is greater than in the forests due to the more intensive management, a greater percentage decrease in diversity was observed in forests and hedges.

2.04P.5

Survey of the Scarabaiedae (Coleptera) fauna of ParNa Iguaçu in Santa Tereza do Oeste - Paraná, Brazil

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The individuals of the Scarabaeidae family are detritivorous organisms that often bury the food resource as a substrate for nesting. Scarabids perform a number of relevant ecological functions, such as soil aeration and nutrient incorporation. National Park of Iguazu contains the largest remnant of the Atlantic Forest of southern Brazil, housing rich biodiversity of fauna and flora. However, there are no studies that mention the Scarabaeidae beetle fauna of the park. Thus, the objective of the present work was to perform a survey of the Scarabaeidae fauna within the National Park of Iguazu. In April 2019, 24 sampling points were defined, 12 at the forest's edge (~ 20 m from the edge) and 12 within the forest (~ 1 km from the edge). Five pitfall traps were installed at each sampling point for 48 hours. Twenty-eight scarabids were collected from the forest's edged belloging to three species - Anomiopus germari, Dichotomius bicuspis and Dichotomius sericeus. While inside the forest, 13 organisms were collected distributed between seven species - Anomiopus germari, Canthidium cavifrons, Canthidium dispar, Dichotomius bicuspis, Dichotomius sericeus, Eurysternus parallelus and Paracanthon sp. Works with this scope contribute to the knowledge of biodiversity, still unknown in tropical and subtropical ecosystems. In these ecossystems, there are a large number of species still to be described (Linnaeus Deficit), and little it is known about its distribution (Wallacean Deficit). In addition, the park is a federal conservation area and may represent a unique habitat for the occurrence of certain species. Only through the survey of fauna that it is possible to determine methodologies for the management of these animals and conservation policies.This project was supported using seed funding from Cardiff University's GCRF QR Funding from the HEFCl for Wales. Dr Nuno Ferreira was supported by a MSC COFUND Fellowship (H2020-COFUND-SIRCIW>MINT-512202) through EU, Welsh Government and Cardiff University.

2.04P.6

"Once upon a time in Brazil", Glyphosate, AMPA and Orthoptera lived together...

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Glyphosate is the most widely used non-selective systemic herbicide in Brazil and occupies 60% of the market worldwide sells. Recent studies indicate that glyphosate half-life is approximately three days, while that of its main metabolite, aminomethylphosphonic acid (AMPA), can reach a half-life of more than 900 days. Although its use is intensive in Brazil, there are disagreements about the effects they have on human health and ecosystems. In this study, we evaluated the effect of glyphosate and AMPA on Orthoptera species composition in four areas with different land use and occupation: urban zone, agricultural zone, forest edge and forest interior. In each of these locations, 12 sets of five pitfalls were placed 200m apart. The pitfalls remained in the field for 48h, being then removed, screened and the organisms identified. Next to each set of pitfalls was collected soil for glyphosate and AMPA analysis and quantification. Using Non-Metric Multidimensional Scaling (NMDS) we detected that glyphosate ($p = 0.550$) and AMPA ($p = 0.363$) was not the factor affecting species composition between areas, despite the fact that between areas species composition was significantly different ($p < 0.006$). The fact that no effect of glyphosate and AMPA on the composition was detected may be linked to high and constant concentrations of these pesticides in the sampled sites. Although there are specific laws in Brazil that prohibit the application of pesticides near cities and protected areas, these products were found meters within the National Park of Iguazu. Wind can be the main transport factor as it blows towards the park, carrying these products over long distances. As for composition, the observed difference is related to other unmeasured factors or even to pesticides, which has eliminated species in the regions where they are more frequent. This project was supported using seed funding from Cardiff University's GCRF QR Funding from the HEFC for Wales. Dr Nuno Ferreira was supported by a MSC COFUND Fellowship (H2020-COFUND-SIRCIW>MINT-512202) through EU, Welsh Government and Cardiff University.

2.04P.7

Ecotoxicological assessment of coal-mining associated soil environments: an earthworm bioassay

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Currently, it is unknown what impacts coal-mining activities may have on the ecosystem support functions of soils around South African coal mines, especially soils in areas that have been deemed "reclaimed". This study aimed to assess the support and habitat function of coal mining-impacted soils (stockpiles and reclamation soils) in South Africa by utilising earthworm bioassays. Soil samples were collected from post-coal mining chronosequences (ranging from 3-24 years), soil stockpiles (recently heaped and >10 years) and unmined areas from two coal mining sites in South Africa. Utilising Eisenia andrei as a bioindicator, endpoints such as avoidance response (AR), growth and reproduction success were measured. Growth, mortality and reproduction of earthworms in all soils did not suggest limited support functions of soils in both reclamation areas and stockpiles. However, analysis of the net AR of soils indicated significant differences (Kruskal-Wallis, $P < 0.05$) among "treatments" though net AR was only > 80% in a recently (3 years old) reclaimed soil. At all sites, earthworms generally preferred the unmined soil compared to the OECD control soil and test soils. General trends in AR suggested the negative effect of mining disturbance, general improvement in ecosystem support function with increasing age-of reclamation and the effect of variations in soil properties within and between sites. Of all endpoints measured, the avoidance test was the most sensitive and generally reflected the differences in soil physicochemical properties amongst test soils. Overall, although the study showed that coal mining soil environments are not ecologically derelict with regards to supporting biocoenosis, high intra-site variation in soil properties suggests lack of strict adherence to best practices for ensuring post-mining soil ecosystem restoration.

2.04P.8

Bringing an ecosystems perspective into ecological risk assessment: an overview

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The global call to preserve biodiversity means there is an urgent need to develop more robust approaches for conserving key ecosystem functions and services. Chemical pollution can be a key driver for ecosystem stress, so further approaches to ecological risk assessment (ERA) that could play a significant role in reducing the adverse ecological effects resulting from human impacts on the environment are needed.In ERA, effects on single species are traditionally used as proxies for the response of the ecosystem as a whole. However, this approach lacks ecological realism as single indicators, especially those based on laboratory assessments, may not necessarily be good indicators of overall ecosystem functioning. More inclusive approaches, accounting for what is happening throughout the ecosystem, could help to inform the development of future ERA that incorporate more robust indicators of ecosystem health and could provide 'early warnings' of sudden ecosystem changes. However, this depends on being able to identify indicators or markers that could serve as reliable and consistent proxies of responses in ecosystem function to chemical stress. Here, we assess the state of the science on the impacts of chemical stress on biodiversity and ecosystem function in freshwater systems, with a focus on the suitability of indicators of function for inclusion in ERAs. There has been an increasing frequency of studies over time with a heavy focus in developed countries and on temperate ecosystems. There have been few studies focussing on the impacts of a specific chemical or product, with most studies examining sources with multiple possible pollutants. Nitrates are the most commonly researched pollutants, with urban and industrial wastewater also widely studied. There is limited evidence to support the inclusion of specific ecosystem function measures in ERAs at present, with some of the ecosystem functions more commonly examined in the literature appearing to be relatively insensitive to the addition of chemical stressors. Of the ecosystem functions we considered, the rate of organic matter transformation best matched the requirements of a good indicator for use in ERAs. Based on these results, we discuss potential ways forward for ecosystem-based ERA, identify evidence and research gaps and make recommendations for future research and development on this topic.

2.04P.9

Assessing chemical risk within an ecosystem services framework: proof of concept case study with zinc

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INTRODUCTION ---- Recent multi-stakeholder workshops have identified advantages in using an Ecosystem Services (ES) approach to chemical risk assessment, as well as challenges in implementation [1,2]. CARES II is a proof of concept project based on 3 case studies to evaluate approaches for assessing impacts on ESs and the value this may bring to regulatory decision making. Here we describe a retrospective, site-specific study assessing the impact of zinc on a range of ESs in a lowland river in the UK, where concentrations of bioavailable zinc currently exceed the EQS of 10.9 ug/L. METHODS ---- This study was co-designed by representatives of the chemical industry, policy/regulatory agencies and academia during a stakeholder workshop. The study focuses on a lowland river in the Thames catchment, in which the following ESs are important: water quality regulation; recreational fishing and observing nature (e.g. bird watching). The retrospective assessment necessitated the use of the biotic ligand model to predict bioavailability from measured dissolved concentrations of zinc and associated water chemistry conditions [3]. Using published effects data for zinc,

we evaluated the susceptibilities of taxa expected to occur in the river according to habitat template models used under the Water Framework Directive (FCS, RICT, DARLEQ, LEAFPACS). The results were then verified against WFD monitoring data for fish, invertebrates, macrophytes and periphyton. Ultimately, the assessment of impacts from zinc on ESs was based on predicted and observed reductions (versus tolerable reductions [4]) in the range of functional groups (i.e. service providing units – SPUs) underpinning each prioritised ES. RESULTS AND DISCUSSION ---- Our results demonstrate the potential for practical application of the ES approach in retrospective chemical risk assessment, building on previous conceptual work. We also show how the ES approach could be used in conjunction with and add value to the WFD approach in terms of: i) evaluating the status of individual ESs; ii) understanding potential trade-offs and maximising ES delivery collectively and iii) helping to prioritise and identify where remedial measures are likely to have greatest benefit. REFERENCES ---- [1] Maltby et al (2018). Sci Total Environ. 621:1342-1351 [2] Faber et al (2019). Sci Total Environ. 651:1067-1077. [3] Santore et al. (2002). Comp Biochem Physiol C Toxicol Pharmacol. 133(1-2):271-285. [4] EFSA (2019). DRAFT Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Panel on Plant Protection Products and their Residues (PPR), Parma, Italy.

2.04P.10

Environmental risk assessment of plant protection products using the ecosystem services concept: Prospective proof of concept case study

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The objective of this case study was to explore the feasibility of applying an ecosystem services (ES)-based approach to environmental risk assessment using currently available data and methodologies. The output from the case study will be used to compare the qualitative outcome of current PPP ERA and an ES-based approach and to evaluate the added value of an ES-based approach to regulatory decision making. The study system is a European apple orchard managed according to IIntegrated Pest management (IPM) principles. The purpose of the orchard is to maintain long-term, sustainable and competitive cider apple production. Apple trees are a permanent crop and it is assumed that the trees are mature. Evaluation of ES delivery is focused on the orchard (approximately 30 hectares i.e. 600 x 500 m) plus a small boundary area (~10 m), which includes off-crop areas such as hedges but excludes aquatic systems. The organophosphate insecticide OP_CARES is used as the study chemical. OP_CARES is applied on two occasions, before flowering (i.e. 480 g a.i/ha on 15 April) and 6 weeks later (960 g a.i./ha on May 30) i.e. after flowering, which occurs in May. Four ES are included in this case study: soil quality regulation, pest control, pollination and recreation. Hayes et al (2018) have developed evidence-based logic chains describing the link between toxicant effects on soil organisms and ecosystem services. Based on their experience, a logic chain was developed for each ecosystem service. For the soil quality regulation ES, springtails and earthworms were used as focal organisms, for the pest control ES the ladybird, for the pollination ES the honey bee and for the recreation ES the Meadow Brown butterfly was selected. For all these species ecological models were used to predict the effects of the OP_CARES applications on their abundance and/or biomass or activity. When possible, ecological production functions were used to extrapolate these outcomes to the delivery of ES. *Acknowledgement* - The authors thank CEFIC LRI for funding this research under the ECO 45 grant.

2.04P.11

Assessing chemical risk within an ecosystem services framework: proof of concept case study with a laundry surfactant

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Recent multi-stakeholder workshops have identified advantages in using an ecosystem services approach to chemical risk assessment as well as challenges in implementation [1,2]. CARES II is a proof of concept project based on 3 case studies to evaluate the capability of assessing impacts on ecosystem services and the added value this may bring to regulatory decision making. Here we describe a prospective study assessing the impact of a surfactant used in domestic laundry products on a range of ecosystem services associated with the freshwater environment. The study, co-designed by representatives of the chemical industry, policy/regulatory agencies and academia during a workshop, prioritised the following ecosystem services: water quality, recreational fishing and swimming, observing nature, e.g. bird watching. An evidence-based logic chain approach was followed to link and assess chemical impacts on biological service providing units to changes in ecosystem service provision. Tools, such as ecological models, and qualitative directional trends between structural and functional groups were identified. Ecological models including Debtox models [3] linked to population models and the food web model, Aquatox [4], were identified as tools enabling assessment of impacts on abundance, biomass and other relevant ecosystem service-related metrics. Aquatox predictions of biomass for a range of trophic guilds could integrate impacts on many ecosystem service providing units, although additional tools are needed to link biomass to other attributes such as abundance and function. Evidence of directional relationships for ecological interactions that form the logic chains for ecosystem services provide mostly qualitative application. However, understanding the directional trends in ecological interactions helps point to potential trade-offs in ES. The ecosystem service assessment outcomes were compared to the conventional REACH risk assessment. [1]. Maltby et al 2018. Advantages and challenges associated with implementing an ecosystem services approach to ecological risk assessment for chemicals. Science of the Total Environment 621: 1342-1351 [2]. Faber et al 2019. Priorities and opportunities in the application of the ecosystem services concept in risk assessment for chemicals in the environment. Science of the Total Environment 651: 1067-1077. [3]. www.**debtox**.info [4]. AQUATOX v3.2. US Environmental Protection Agency https://www.epa.gov/ceam/aquatox

2.04P.12

Bridging the gap between environmental sciences and societal challenges aiming an integrated management of coastal ecosystems under the risk of salinization: SALTFREE and SALTFREE II

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Salinization of coastal terrestrial and freshwater ecosystems due to sea level rise is a major threat to biodiversity and, concomitantly, to the goods, functions and ultimately services provided by these ecosystems to the local communities. Nowadays, it is recognized that the larger involvement of the public in scientific processes helps researchers and science to develop more adequate risk assessment frameworks. Considering this key connection between environmental and societal components, the SALTFREE and SALTFREE II projects have been planned in order to develop successful integrated management strategies for coastal areas at the risk of salinization. The data collected within the SALTFREE project allowed the establishment of a database on safe salinity levels for a wide range of terrestrial and freshwater species belonging to key functional and trophic groups. The results showed that salinity levels corresponding to approximately 4.3% and 2.4% of seawater conductivity could induce major effects on the freshwater biota and on the productivity of coastal soils. The ongoing SALTREE II intends to validate those salinity levels under realistic environmental scenarios at the community and ecosystem levels. For that, it is necessary to perform an extensive evaluation of the value that biodiversity and ecosystem services have for the local community and stakeholders through presential workshops and meetings, ultimately aiming at efficient management actions for these coastal regions. The knowledge build-up within these two projects is a clear showcase example that the result of the combination of scientific knowledge with that of local communities is vital for integrated protection measures of biodiversity and ecosystem services of coastal ecosystems at risk of salinization.

2.04P.13

Embedding Ecosystem Services in Plant Protection Product Regulation

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Regulators at EU and Member State level face the challenge of having to make decisions on the registration of plant protection products (PPP) using environmental risk assessments, which do not include information on benefits and trade-offs between protection goals. In particular, evidence on how a particular PPP affects the socio-economics of farming is currently not part of the decision-making process. Regulation 1107/2009 states *'The purpose of this Regulation is to ensure a high level of protection of both human and animal health and the environment and at the same time to safeguard the competitiveness of Community agriculture'*. The latter element is recognition that a regulatory decision on a PPP should not made 'in a vacuum', either if there is no evidence of harm to non-target populations in the field resulting from its use according the Good Agricultural Practice or if a potential impact could or should be offset. Regulation 1107 provides for the protection of the environment in general terms. Specific protection goals (SPG) are recognised as a means for operationalising the legislation into measurable objectives in risk assessment and management. Given the SPG's regulatory nature and the important socio-economic and environmental impacts resulting from their selection, European Crop Protection Authority (ECPA) has initiated an Impact Assessment (IA) in line with the Better Regulation Guidelines of the European Commission to better understand the socio-economic and environmental trade-offs associated with SPGs. On behalf of ECPA, the authors are undertaking a number of case studies to evaluate the use of herbicides in a range of crop production scenarios. The focus of protection are non-target terrestrial plants (NTTP). A framework for defining ecosystem services, indicators and metrics for SPGs was developed by the authors and is based on The Economics of Ecology and Biodiversity (teebweb.org). The case studies are representative of European climatic regions and a range of cereal, vegetable and fruit crops. The case studies account for typical environmental settings of crop production in each country, such as field margin. Four scenarios are assessed in each case study. SPGs were developed for each ecosystem service under each scenario and for both in-field and off-field areas. SPGs can differ between the two field areas. In addition, socio-economic indicators, such as farm revenues, labour, machinery, herbicides, and change in land are valued and compared in order to derive net changes in services and socio-economics between scenarios in each case study. The project is underway and the findings will be used to illustrate the unintended consequences of decisions made without the consideration of the risks and benefits to ecosystem services provided by agricultural land and the socio-economics of crop production. And where impacts may outweigh benefits, to identify SPGs through scenarios that provide compensation for lost services or socio-economics.

Embedding Ecological Concepts in Ecotoxicology: Processes, Populations and Communities (P)

2.05P.1

Are forests providing their ecosystem service to guarantee the biological community?

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Forests and their edges are responsible for providing resources for maintaining biodiversity. In this work we evaluate how the ecological attributes respond to areas with distinct but contiguous physiographic structures: forest belonging to the National Park of Iguazu (F), its edge (E), the rural area of Santa Tereza do Oeste (R) and its urban area (U). Twelve points were sampled in each area for soil's physical-chemical properties and pesticide's residues. For each point, five Pitfall traps were also placed to determine biodiversity. After 48 hours, the traps were collected, sorted and organisms identified at an Order level. The ecological attributes of Wealth, Shannon Diversity, Pielou Equitability and Berger-Parker Dominance were calculated for each area and compared using generalised linear models. The diversity and richness values were significantly similar between F and E ($p > 0.05$) and higher than R and U ($p < 0.05$). Resource distribution, as assessed by Pielou Equitability, also showed higher F and E equitability ($p > 0.05$) and worse U condition when compared to F and E ($p < 0.05$). This result was corroborated with the dominance values, which were significantly higher in R and U ($p < 0.05$). The Rural and Urban areas presented the lowest values for diversity, richness and evenness, as well as greater dominance, which is possibly the damage resulting from pesticide overuse and fragmentation and/or complete removal of vegetation cover. The edge of National Park of Iguazu seems to act as ecotone between the Forest and Rural areas, as it has aggregated taxa from both locations, but this can be of concern as invasive species have been found on the border. The Forest area, even being close to the rural area, still preserved resources and conditions for the maintenance of the largest biological community.This project was supported using seed funding from Cardiff University's GCRF QR Funding from the HEFCl for Wales. Dr Nuno Ferreira was supported by a MSC COFUND Fellowship (H2020-COFUND-SIRCIW>MINT-512202) through EU, Welsh Government and Cardiff University.

2.05P.2

Stressor indication - to what extent are environmental impacts independent?

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As part of the nationwide pilot study of small streams a unique data set was assembled in 2018 and 2019. A total of 140 small streams with varying agricultural intensity were investigated. The aim of the nationwide monitoring was to identify the ecological effects of agricultural pesticides and to estimate the dependence from other environmental stressors such as pH deviation, flow velocity, habitat structure, temperature deviation, oxygen deficiency, trace elements and nutrients. The event-driven water sampling after rain events was taken to analyse the pesticide peak concentration in small streams. Macroinvertebrate samplings were performed in all streams in June after the main application phase of pesticides. In order to classify the biological effects of various stressors, several indices were applied: (i) pesticide pressure was identified by $SPEAR_{pesticides}$, (ii) stream degradation was quantified using the fauna index, (iii) oxygen deficiency was indicated by the saprobic index and (iv) several environmental stressors such as habitat structure, flow velocity and toxic pressure were identified by %EPT (proportion of abundance of Ephemeroptera, Plecoptera and Trichoptera species) and extrapolated biomass. For identifying the relevant stressors a multiple linear regression analysis were applied on all stressors for each endpoint. The event-driven sampling is best suited to assess peak concentrations that determine ecological effects, which is reflected by a strong corellation between $SPEAR_{pesticides}$ and TU_{max} (R^2=0.47, $p < 0.001$). Specific indices such as $SPEAR_{pesticides}$, fauna index and to lesser content the saprobic index react to their specific stressors. Given this, the unspecified %EPT index and extrapolated biomass react to several environmental stressors. Based on the $SPEAR_{pesticides}$ analyses, 71% of the investigated small streams have a "moderate" to "poor" ecological status in accordance to the Water Framework Directive. Toxic pressure, environmental stressors and an additional occurrence of pesticide mixtures in the field cause the macroinvertebrate communities to react more than two orders of magnitude more sensitively to pesticide pressure than under optimal laboratory conditions. Thus the small stream monitoring illustrates the relevance of pesticides and additional independent environmental stressors and thus the need for action in agricultural areas to maintain ecosystem integrity in small streams.

2.05P.3

Impact of multiple pressures on freshwater communities, using ecological and ecotoxicological indicators

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Pressure-Impact modelling in ecology aims to study the effects of multiple pressures (mainly land use and alteration of hydromorphology) on communities. This type of approach started lately to integrate chemical pressures with ecotoxicological indicators. Active biomonitoring, based on caging calibrated biota, measures bioavailable contamination and toxicity levels. The objective of this study is to quantify and understand the impact of multiple pressures, including chemical pressures (measured through caged gammarids), on macroinvertebrate communities at the national scale of France. Therefore, we created a Structural Equation Model (SEM) with multiple pressures (land use, sources of contaminants, hydromorphology and chemical toxicity) as inputs and indicators of invertebrate communities' state as outputs. For toxicity, inhibition of the feeding rate and the rate of gammarids' survival were used. To integrate other pressures, indicators of urban and agricultural landscape were considered. To represent the state of invertebrate communities, the Macroinvertebrate-based multimetric index (I2M2) and selected biological traits of invertebrates were adopted. The impact of multiple pressures on the state of the communities was quantified. Chemical pressure's impact on communities was confronted to the impact of other pressures. The quantification of the impacts based on the different indicators was compared. This approach highlights the importance of taking into account multiple pressures when studying the biological state of invertebrate communities. As well as integrating chemical pressures, measured through biota, in Pressure-Impact modelling. Furthermore, the effects of pressures on the state of

communities were compared with the effects demonstrated in other Pressure-Impact studies.

2.05P.4
Effects of antidepressant exposure on aquatic communities
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One of the emerging threats to aquatic ecosystems is pharmaceutical pollution. In the past decades, the consumption of antidepressants has increased. This group of pharmaceuticals pose a potential high risk to the aquatic ecosystem, both because of their high occurrence and environmental persistence. Fluoxetine is one of the most commonly prescribed antidepressants, and frequently detected in surface waters and sediments. Effects of fluoxetine have primarily been assessed on individual level, however, community-level risks associated with fluoxetine occurring in aquatic ecosystems are far from known. Therefore the aim of this study is to assess the effects of the chronic application of fluoxetine on key components of aquatic ecosystems including macroinvertebrate-, zooplankton-, phytoplankton- and microbial communities and organic matter decomposition, a measure of ecosystem functioning. We exposed 18 outdoor mesocosm (water volume of 1,500L and 10 cm of sediment) to five different concentrations of fluoxetine (0.2, 2, 20 and 200 µg/L) for eight weeks with adjacent an eight week recovery period. The endpoints we assessed included community metabolism, primary producers, macroinvertebrate-, zooplankton-, and microbial community, and decomposition rates. Preliminary results show responses on community metabolism parameters and macroinvertebrate community, mostly for the highest concentration of fluoxetine. We found a decrease in oxygen concentration and pH levels after exposure to fluoxetine in the two highest treatments. Regarding the macroinvertebrates, the snail Radix sp. and damselfly larvae (Zygoptera) showed highest sensitivity toward fluoxetine. Next to that, the PRC revealed significant treatment-related effects of the macroinvertebrate community composition to fluoxetine. Currently, we are still analysing the data of primary producers, microorganisms and zooplankton, to gain more insights into the community-level impacts of fluoxetine.

2.05P.5
Spatial variation in the sensitivity of freshwater invertebrate assemblages to chemicals
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Current ecological risk assessments of chemicals generally adopt a single threshold approach to the protection of natural communities. The species composition of assemblages varies spatially, but it is unknown how spatial variation in species composition relates to spatial variation in assemblage sensitivity. Consequently, adopting a single threshold approach may result in chemical risks to natural communities being overestimated or underestimated. A major challenge with relating spatial variation in the sensitivity of species assemblages to spatial variation in species composition is that toxicity data are not available for most species in natural communities. This study aimed to investigate: (1) how the sensitivity of untested species can be predicted; (2) how the sensitivity of assemblages varies spatially; (3) how the relative sensitivity of assemblages varies for different types of chemicals. The study investigated the sensitivity of more than 2,000 freshwater invertebrate assemblages from across England and Wales to pesticides and surfactants. The hierarchical species sensitivity distribution (hSSD) model was used to predict the toxicity of untested species and the concentration hazardous to 5% of species (i.e. HC5) was used as a measure of assemblage sensitivity. The sensitivity of freshwater invertebrate assemblages to specific chemicals will be compared and the spatial patterns of assemblage sensitivity will be discussed.

2.05P.6
Extrapolation of Metal Toxicity from Individuals to Communities in Three Daphnid Species: A DEB-based Approach
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There is growing evidence that, in order to effectively assess the risk of chemicals, it is crucial to take the role of species interactions into account. Due to the large number of possible species assemblies, it is desirable to develop predictive, mechanistic models that can be calibrated with standard toxicity data.Therefore, we have conducted life-table experiments with *Daphnia magna, D. pulex* and *D. longispina*, exposed to Cu, Ni and Zn, in order to calibrate Dynamic Energy Budget Theory Individual-Based Models (DEB-IBM). We derived DEB parameters from control data and calibrated modules for lethal and sublethal effects of Cu, Ni and Zn. Species were combined *in silico* into binary and tertiary communities and community dynamics under metal exposure were simulated.In the DEB-IBM, interspecific interactions emerge from physiological properties via competition for a shared resource. Each DEB parameter has direct or indirect consequences for resource utilization, and therefore for species interactions. Chemical stressors have the potential to alter these interactions, because effects are implemented as changes in DEB parameters.We modelled the effects of metals on two community-level endpoints, productivity and community structure. The two endpoints are inherently different because only productivity is subject to functional redundancy, leading to large differences in community-level sensitivity, based on which endpoint is chosen. While effects of metals on community-level endpoints can in principle be deduced from DEB theory, experiments to validate these predictions are still lacking, and are crucial to evaluate the usefulness of our approach in application. We believe that the use of DEB-IBMs to investigate effects of chemical stressors on higher levels of biological organizations can be fruitful, because data for calibration can be generated relatively easily and models can be developed from established, biology-based frameworks.

2.05P.7
Nutrient enrichment and predator stress influence the impacts of pesticides on D. pulex populations under Mediterranean conditions
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This study aimed to assess the combined effects of two pesticides with a different mode of action, nutrients, and predation on freshwater populations under Mediterranean conditions. Two cages containing *Daphnia pulex* (50 adults + 50 sub-adults/juveniles) were placed each of the 24 test mesocosms, one without predator and one with a predator (*Notonecta* sp.). The experiment was performed following a factorial design (n=3), with the following treatments: insecticide chlorpyrifos (1 µg/L, two levels: presence/absence), the herbicide diuron (18 µg/L, two levels: presence/absence) and nutrients (added/not added). Nutrients (P and N) were applied twice a week to reach a net addition in the water of 75 µg/L of P, and 750 µg/L of N. Nutrients addition started three weeks before the application of the pesticide, which was performed as a single dose. *D. pulex* abundance was assessed on days 2, 7, 14, and 21 relative to the pesticides application. The data analysis was divided into three independent blocks. In Block I, we assessed the interactive effects of nutrients, chlorpyrifos, and diuron. In Block II, we evaluated the interactive effects of nutrients, chlorpyrifos, and predation, while in Block III, the interaction between nutrients, predation, and diuron were assessed. Block I showed a positive effect of nutrients in the abundance of *D. pulex*, while reduced the number of adults, juveniles, and the total of surviving *D. pulex* towards the end of the experiment. Chlorpyrifos had more pronounced effects at the beginning of the test, but signals of recovery were observed on day 14 after the insecticide application. The interactions between diuron and chlorpyrifos were mainly additive, while the herbicide-nutrient interaction was negative antagonistic on days 7 and 21, and positive on day 14. Block II showed that predation was significant only in the first week of the experiment. The combination of insecticide and predation produced a more severe effect on the *D. pulex* population than predicted for both stressors in isolation. Predation-insecticide effects, however, were not affected by nutrient enrichment. Data from Block III showed no significant interaction of diuron, and predation, with no differential effects due to nutrient addition. Our findings suggest that nutrients might ameliorate the effects of pesticides on *D. pulex*, while predation is usually enhancing them, especially when co-occurring with pesticide pollution.

2.05P.8
Use of spatial ecology to assess exposure of red-legged partridges to pesticide-treated seeds
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Ingestion of pesticide-treated seed is one of the sources by which the use of Plant Protection Products (PPP) may affect granivorous birds. In fact, this exposure scenario is the main cause of wildlife incidences associated with approved uses of PPP. Experimental studies have evidenced a significant toxicity to red-legged partridges fed only with insecticide- or fungicide-treated seeds. However, no concluding information on exposure chances in the field exists. Captive partridges tend to avoid pesticide-treated seeds, but unpredictability of food location limits their ability to distinguish between treated and untreated seeds. In pesticide risk assessment, the proportion of contaminated food in the diet is extrapolated from the percentage of time (PT) the bird spends in treated fields, obviating the fact that habitat selection patterns depend on the activity, and the feeding habitat choice may not necessarily correlate to overall habitat choice. In order to determine the chances of exposure of red-legged partridges to pesticide-treated seeds in the field, we tracked 13 individuals using GPS loggers that recorded five to eight daily positions during the sowing season of cereals in central Spain. We identified fields with available treated seed and evaluated their frequency of use by partridges.

29.7% of all locations during the sowing season occurred in fields with treated seeds. Considering only locations between sunrise and sunset hours, when partridges may be feeding, this percentage increased to 32.7%. This evidenced the uncertainty associated with the PT approach and raised the need of refining the spatial analysis. With this purpose, we calculated the Kernel function of the home range of each animal and assigned a Kernel value to each location. Assuming that locations further away from the home range centroid (i.e. those with lower Kernel values) would have higher chances of not being casual (i.e. of being actively selected), Kernel values would be indirect indicators of habitat choice. Fields with available seeds had the lowest average Kernel values (262.6), ranging the rest of considered habitats from 296.3 in orchards to 367.9 in gardens. These results suggest that partridges would select fields with presence of sowing seeds as feeding habitats, and thereby using PT to characterize the exposure of partridges to treated seeds would lead to an underestimation of risks. Funded by the Spanish Ministry of Science (CGL2016-75278-R).

2.05P.9

Exposure to anthropogenic chemicals in wild carnivores: a silent conservation threat demanding long-term surveillance

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Top predators are fundamental drivers in maintaining ecosystems and preserving biodiversity. However, a number of species have experienced severe population declines and currently face critical conservation challenges. Although exposure to chemical contaminants has been recognized as a meaningful threat for wild carnivores, its population effect has been more rarely assessed. There are only a few case studies, based on monitoring across large temporal and spatial scales, that have been able to: identify the real thresholds at which chemical exposure adversely affect populations, demonstrate how populations have responded to such ecotoxicological challenges, or determine if chemical exposure has really been the cause of declines in wild carnivore populations. This work focuses on species in the mammalian order Carnivora to review some of the most relevant case studies that have led to identify chemical exposure as an anthropogenic force that may compromise the conservation of top predators. Some examples include both marine and freshwater carnivore species and their exposure to persistent organic pollutants (POPs) and methylmercury (MeHg); and terrestrial carnivores and their secondary exposure to anticoagulant rodenticides (ARs). The ongoing “chemical intensification” makes it critical to foster and keep efficient surveillance efforts on chemical exposure and their health impacts in carnivore populations long into the future. Thus, we also highlight the relevance of long-term monitoring surveillance in conservation programs, pointing out the challenges that should be considered in future ecotoxicological research on wild carnivores, including the need of establishing Adverse Outcome Pathways for different types of chemicals and the development of sensitive, precise and reliable biomarkers of exposure and effect by the use of non-destructive techniques. The inclusion of wild carnivores in ecotoxicological monitoring may deliver key and useful information for biodiversity conservation and One Health goals.

2.05P.10

Single and joint effects of recurring heat waves, increased temperature and a fungicide on zooplankton-dominated freshwater microcosms

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Changes of the environment associated with a global warming climate, henceforth Global Climate Change (GCC), may cause rapid alterations of distinct environmental variables in aquatic ecosystems, such as temperature and dissolved CO_2. With an expected increase of the severity and frequency of extreme events until the end of the 21st century, including higher mean temperatures and heat waves, aquatic ecosystems will face new environmental conditions and thus most likely increasing pressures, also through still unknown interactions with other already persisting stressors of anthropogenic origin (i.e. chemicals). Most of the multiple stressor literature that considered GCC-related and chemical stressors report on single species responses and constant elevated temperature regimes rather than on population or community-level approaches and temperature variability, respectively. However, it is crucial to include intra- and interspecific interactions of species at the same or adjoining trophic level and thus ecosystem complexity as well as the variability factor in temperature if we want to study the effects of chemicals in the environment under GCC with respect to an environmentally relevant approach. The objective of this study was to investigate the single and joint effects of current and GCC scenarios in freshwater systems under chemical stress. With the aim to enable temperature variability in lab and field experiments, we present for the first time a newly developed ***T**ransportable temperature a**n**d hea**t**wav**e** control **e**ngine* (TENTACLE). The microcosm experiment applied 3 different temperature scenarios, representing current and GCC conditions with the presence or absence of the fungicide carbendazim. Each microcosm contained various environmental components of a natural shallow freshwater ecosystem. We considered 10 different endpoints in total which describe ecosystem processes and functioning as well as zooplankton species dynamics and community structure. First results of the temperature treatments indicate a successful experimental design with TENTACLE. Constantly recorded environmental parameters point out reasonable effects of the distinct temperature treatments on the dissolved oxygen concentrations. A significant presence of *L. stagnalis* and absence of *G. pulex* was noted at the end of the experiment. Further upcoming results will support the identification of mechanisms by disentangling effects and will point out important mechanistic effect chains.

2.05P.11

Dissolved Organic Matter and pH control toxic response, tolerance acquisition and trade-offs to micropollutants exposure

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The widespread of bioactive micropollutants classified as Pharmaceutical and Personal Care Products (PPCPs) in freshwater ecosystems is of concern. Non-target organisms such as freshwater phytoplankton can adapt to chemical stress, but acquisition of tolerance may trade-offs with fitness impairing their growth efficiency non-selective environments (i.e. in absence of PPCPs). Hence, the net benefit of tolerance acquisition cannot be easily forecast. Furthermore, key environmental factors such as dissolved organic matter (DOM) and water pH may mitigate the toxic effects and the selective pressure pose by contaminants through complexation and speciation processes, respectively. While the effects of DOM and pH on the toxicity of PPCPs are relatively well-documented, their influence on the process of tolerance acquisition and the subsequent emergence of fitness trade-offs is still unexplored. Hence, we hypothesized here that by mitigating the toxic response of microalgae exposed to PPCPs, DOM and water pH can modulate both acquisition of tolerance to the contaminants as well as the deriving fitness trade-offs. Using a 2-phase experimental approach, we tested the influence of different levels of DOM (0, 5, 15 mg/L dissolved organic carbon - DOC) and pH (6.5, 8) on the regulation of the toxic effect of sub-lethal concentrations of a mix of 12 PPCPs on the growth of a freshwater microalgae (Chlamydomonas reinhardtii). Then, we investigated whether different levels of DOM during a multi-generational exposure to PPCPs may influence the process of tolerance acquisition by the microalgae and the occurrence of trade-offs in absence of contaminants. Our results showed that PPCPs effect on algae growth rate was reduced by the lower level of DOM (5 mg/L DOC) at pH 8. The adaptation period with the PPCPs increased the tolerance of C. reinhardtii to the same stressors, but the strains that acquired tolerance had significant lower growth in absence of the contaminants compared to un-exposed control populations. The presence of DOM and pH 8 during the adaptation period influenced both the effect of tolerance and the resulting trade-off, confirming our hypothesis.

2.05P.12

The influence of environmental conditions on effect of insecticide chlorpyrifos

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Ecological risk assessment of chemicals is mainly based on the results of indoor or outdoor experimental tests from microcosms via macrocosms to macrocosms. All the these type of experiments often have different environmental conditions (e.g. initial N/P ratio and water volume). Until now it is still unknown whether environmental conditions could change the effect of insecticides on communities and whether environmental conditions are matters for ecological risk assessment. We combined 18 independent experimental data sets, across different levels of environmental conditions (e.g. initial N/P ratio and water volume) and dose of insecticide chlorpyrifos. We found that the abundance of insensitive predators and herbivores was larger when dose of insecticide increased, but was smaller when water volume and initial N/P ratio increased. In contrast, the abundance of sensitive herbivores was smaller when dose of insecticide increased, but was larger when water volume and initial N/P ratio increased. Similarly, the abundance of sensitive predators was larger when water volume and initial N/P ratio increased, while the dose had no significant effect on abundance of sensitive predators. The photosynthesis capability (i.e. chlorophyll a concentration) was smaller when dose, water volume and initial N/P ratio increased. Overall, the environmental conditions had negative effect on insensitive predators and herbivores, but positive effect on sensitive predators and herbivores, and photosynthesis. Our results indicated that the environmental conditions (initial N/P ratio and water volume) could change the effect of insecticide on aquatic communities and that environmental conditions are matters for ecological risk assessment. Given that natural systems widely varied environmental conditions, we call for more studies eco-toxicological studies considering environmental conditions.

2.05P.13

Multiple stressors in agricultural freshwater ecosystems: interactions between two widely used pesticides (chlorpyrifos and diuron) and nutrients under Mediterranean conditions

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Water bodies located on the edge of agricultural fields are impacted by a wide array of chemical and non-chemical stressors. Among these stressors, a crucial role is played by chemical compounds such as pesticides, which may reach water bodies via runoff, leaching or spay drift. Indeed, co-occurring pesticides with different mode of action can impair different biological components of the ecosystem, resulting in yet unpredictable ecological consequences. However, few studies dealing with multiple stressors in freshwater ecosystems have considered mixture of chemicals with different modes of action in combination with a non-chemical stressor (e.g. nutrients. Here, we present one of the first studies assessing the single and combined effects of two widely used pesticides with different modes of action under Mediterranean conditions tested in two different trophic situations. We performed a mesocosm experiment in which a full factorial design (n=3) of chlorpyrifos (two levels: present and absent), diuron (two levels: present and absent) and nutrients (two levels: added, not added) was applied in a randomized fashion, resulting in eight different treatments. We tested the effects of single and multiple stressors on the macroinvertebrates, zooplankton and phytoplankton communities during fifty days after the pesticide application along with some relevant water quality and ecosystem functioning parameters. We found that each single stressor had a significant effect on the different aquatic communities' composition on at least one sampling date Our findings show that nutrients addiction often resulted in a positive antagonistic interaction with the pesticides (single or in a mixture), leading to a less impacted community and a faster recovery for the macroinvertebrates, but in contrast promote the development of an unbalanced ecosystem and increase the risk of harmful events such as cyanobateria blooms and anoxic conditions. On the other hand, the mixture of insecticide and herbicide often interact as a negative synergism at the population level. The synergistic effects between pesticides highlighted the importance of mechanistic undestanding of stressors interactions to understand ecosystem conequences and to avoid underestimations of the effects in the ecological risk assessement context.

From Lab to Field: Relevance of Effects Observed in Lab Studies for Non-target Local Populations and Communities and their Habitat Selection (P)

2.06P.1

Environmental concentrations of cocaine induced sub-individual and behavioural effects in Daphnia magna

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Illicit drugs represent a growing environmental concern. Several monitoring studies have shown the presence of illicit drug residues in the aquatic ecosystems worldwide. After human use illicit drugs are excreted through faeces or urine and, considering the low efficiency of Wastewater Treatment Plant (WWTPs) in their removal, they enter the aquatic ecosystems. Among illicit drugs, cocaine (COC) is the most used stimulant drug worldwide. To date the concentrations of COC in aquatic ecosystems are quite low (ng/L range). However, considering its high pharmacological activity the potential negative effect on non-target aquatic organisms cannot be excluded. To date, only few studies have investigated the potential toxicity of COC towards aquatic non-target organisms, mainly focusing on the effects at low levels of the biological organization. The aim of this work was to investigate the possible negative effects induced by 21-day exposure to two environmentally relevant concentrations (50 ng/L and 500 ng/ L) of COC on the cladoceran *Daphnia magna* at different levels of the biological hierarchy. We relied on a multi-level approach to assess the COC-induced effects at sub-individual and individual levels. A suite of oxidative stress biomarkers, in terms of the amount of pro-oxidant molecules (ROS) and the activity of the main antioxidant (SOD, CAT, GPx) and detoxifying (GST) enzymes was used to investigate effects at sub-individual level. The effects at individual level were evaluated as changes in the swimming (i.e., distance moved and swimming speed) and reproductive behaviour (i.e., number of offspring and reproductive events). Our results showed that the exposure to environmentally relevant concentrations of COC caused an increase in ROS levels mostly at the end of the exposure at both concentrations, as well as the modulation of the antioxidant enzyme activity (SOD and CAT). Furthermore, COC exposure boosted distance moved and swimming speed after exposure to 50 ng/L while the highest tested concentration negatively affected both endpoints. Lastly, we found a reduction in the mean number of offspring of *D. magna* exposed to both 50 ng/L and 500 ng/L. These results highlighted the toxicity of environmental concentrations of COC at different biological levels of *D. magna*.

2.06P.2

Can copepod diapause be induced in the lab?

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Marine copepods in the genus *Calanus* are keystone species in North Atlantic and Arctic marine ecosystems. Most calanoid copepods spend several months of the year at deep waters in a dormant state called diapause, a phase that has to our knowledge yet to be induced under laboratory conditions. During diapause, the copepods do not feed, have low metabolism and rely on endogenously stored lipids for energy while slowly developing towards adulthood. In nature, copepods may be exposed to various forms of anthropogenic pollution during their life cycle, including during diapause. Copepods in diapause may react differently to pollutant exposure than active copepods, though this has yet to be investigated under controlled circumstances. By inducing diapause in the lab, disturbances from the processes of collection, transportation and sorting can be avoided, as well as contamination by other species. In the present study, we hypothesized that diapause might be triggered in *Calanus finmarchicus* by changes in food availability and predation pressure. Specifically, we assessed diapause entry by analyzing expression of molecular markers of diapause and development in cultured *C. finmarchicus* exposed to chemical cues from a predator in combination with varying food availability. If diapause is successfully induced in the laboratory, we have lain the foundation for future studies to investigate effects of anthropogenic stressors occurring during this elusive life phase. For example, studying effects of petrogenic oil exposure during diapause in cultured populations can contribute to a better understanding of such events occurring in nature, which is essential in order to understand how anthropogenic pollution can affect marine ecosystem dynamics.

2.06P.3

Sublethal effects of pirimicarb, azoxystrobin and isoproturon on the snail Lymnaea stagnalis

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Agricultural pesticides in surface waters may pose serious risks to non-target organisms. As pesticides are bioactive and commonly occur in mixtures, it is important to investigate their long-term mixture effects on biota. We carried out a pilot study to determine the pesticide levels that would induce sublethal effects in the snail *Lymnaea stagnalis,* to be used in a follow-up mixture test. Adult snails were collected in the field and acclimatised to laboratory conditions for three weeks prior to the experiment. We selected pesticides commonly found in freshwater monitoring programs: the herbicide isoproturon, the fungicide azoxystrobin and the insecticide pirimicarb, and tested these individually at four levels, with a geometric series starting with environmentally relevant concentrations and amplifying these up to 1000 times. The snails were placed individually in glass jars with 200 mL of test media and exposed for 21 days. Snails were fed lettuce and media was renewed every 3-4 days. The endpoints were feeding rate, fecundity, embryo alterations, growth and pesticide bioconcentration in snails. The results showed generally lower feeding rates at high pesticide concentrations, but no difference for the snails' growth. Feeding rate was negatively correlated with the water concentration of isoproturon, but not for the other two pesticides. Exposed snails also showed a larger variability in feeding rates than control snails. Cumulative fecundity increased with increasing concentrations of azoxystrobin and isoproturon up to the highest concentration level, at which it dropped substantially (by up to 96%). At environmental levels of pirimicarb, no egg masses were laid until the third week of the experiment and thus these snails had the lowest, and most delayed fecundity. The largest fraction of altered embryos (13%) was registered in the two highest isoproturon treatments. Pesticide bioconcentration differed from that expected based on the compounds' chemical properties, with BCFs averaging 2, 23 and 2088 for isoproturon, pirimicarb and azoxystrobin, respectively. We conclude that, although effects on life-history traits were generally most pronounced at high concentrations of isoproturon and azoxystrobin, exposure to environmentally-relevant pesticide concentrations is also of serious concern, as shown by the reduction in fecundity due to pirimicarb and the high BCF found for azoxystrobin.

2.06P.4

Interactive effects of NaCl and Cu on spatial avoidance in multispecific non-forced exposure assays

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To appraise how salinization influences spatial migration and distribution of a freshwater lenthic community in the presence of copper contamination was what

this study aimed at. A community composed by *Daphnia magna* neonates, *Chironomus riparius* larvae and *Xenopus laevis* tadpoles was used to evaluate spatial avoidance. *Lemna minor* and *Chlorella vulgaris* were also used to allow a more ecologically relevant representation of a natural environment. In a multi-compartmented (3x6) system, two different contamination gradient scenarios were used: i) three Cu concentrations and six NaCl concentrations (Cu gradient of 0, 25 and 50 µg/L and NaCl gradient of 0, 0.25, 0.50, 1, 2, and 4 g/L) and ii) three NaCl concentrations and six Cu concentrations (NaCl gradient of 0, 1 and 4 g/L and Cu gradient of 0, 10, 20, 30, 40, and 50 µg/L). A control experiment using an artificial water (with neither NaCl, nor Cu) in all the compartments was also run to confirm the lack of preference for any system extremity. Before starting the experiments, the passages between compartments of the system were closed and added to each one: 50 g of sediment, 300 mL of solution, 1.5×10^5 cells/mL of *C. vulgaris*, two *L. minor* fronds, ten *D. magna* neonates, ten *C. riparius* larvae and one *X. laevis* tadpole. After this procedure, the passages between compartments were opened. Experiments ran for 8 h, in dark, at 20ºC and two replicates were used. Mortality was found neither in the controls, nor in the treatments. The three animal species strongly avoided both contaminants, corroborating previous findings in the literature for similar or close species exposed to each of the contaminants. At the highest concentrations of both NaCl and Cu, avoidance was less intense than at intermediate contamination levels, which was probably due to moribundity dictating a weaker motility. Results of the present study re-emphasize the need to further address contaminant-driven active migration alongside standard forced exposure assays, since extinction of local animal populations can occur at concentrations well below lethal ones. Moreover, they contributed to fill the knowledge gap on interactions among species and among chemicals regarding avoidance.**Key-Words:** Multi-specific scenario; multi-compartment non-forced exposure; spatial avoidance; contaminant misxture.

2.06P.5

Contamination-driven behavioral plasticity: When the habitat selection is based on a cost-benefits balance among contamination, refuges and predation.

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Factors like the presence of predators and refuges and levels of contamination might determine at some extent if an area is preferred or avoided by organisms. We hypothesized that when shrimps are exposed to a situation in which they have to decide among those three factors, they will avoid the situation that supposes higher cost for their survival (cost-benefits balance). Thus, the current study aimed to assess the behavioral plasticity of the freshwater shrimp in the following situations: moving to a clean and unprotected (no refuge and with a risk of predation) area, thus avoiding exposure to contamination, or moving to a contaminated and protected (with refuges) area, thereby avoiding potential predators. Shrimps were experimentally exposed in a multi-compartmented system, in which heterogeneous environments with a contaminant (copper), refuges and a predator signal (kairomones of *Salmo trutta*) were simulated. Avoidance to areas contaminated with copper was observed in the absence of, or presence of, refuges. When confronted with a choice between a cleaner zone with no refuge and a contaminated zone with refuges, they moved to the cleanest area. However, when the uncontaminated area contained a predator signal, the shrimps made a balance between the risk of predation and the damage due to the exposure to contamination by selecting a moderately contaminated area relatively further away from the predator signals. In conclusion, contamination seems to force behavioral plasticity of shrimps regarding habitat selection, which might be based on the cost-benefit balance.ACKNOWLEDGEMENTSC.V.M. Araújo is grateful to the Spanish Ministry of Science, Innovation and Universities for a Ramón y Cajal contract (RYC-2017-22324). K.C. Pereira thanks the Erasmus Mundus Programme (PhD in Marine and Coastal Management) for the doctoral fellowship. This study was funded by the Spanish Ministry of Science, Innovation and Universities (Explora Project: #CGL2017-92160-EXP).

2.06P.6

Detection of Biodiversity Loss in a Large Boreal Wetland Complex to Support Ecological Risk Assessment of Multiple Stressors

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The Peace-Athabasca Delta (PAD) is the world's largest inland delta wetland complex covering an area of 6000 sq. km. It currently supports a range of unique biodiversity including many migratory bird species, including the critically-endangered whooping crane, and this is reflected in its status as a UNESCO World Heritage and Ramsar Site. Located within the 4.5 million hectare Wood Buffalo National Park, PAD wetlands are currently threatened by encroaching resource development associated with upstream oil sands mining development and related infrastructure development which is approaching within 30 km of the Park boundary. As part of the Oil Sands Monitoring program, a long-term (2012-2019) focused study was carried out at a range of PAD wetland sites to identify current background (pre-impact) levels of contamination, and to establish baseline conditions for future monitoring. Objectives of the study were: (i) to develop and test a new freshwater macroinvertebrate biomonitoring tool capable of detecting a 5% regional biodiversity loss against a background of strong local environmental variability and (ii) to develop indicators suitable for separating the effects of climate drivers (temperature and hydrology) from potential impacts of contaminants associated with oil sands mining emissions, which included a diverse mixture of metals and polycyclic aromatic compounds, many of which do not have current risk-associated guidelines. This was achieved using a combination of new ecosystem observation technologies including geospatial observation systems and environmental DNA analysis to generate consistently-observed data at appropriate scales.

2.06P.7

Toxicity Translation: An emerging theme for modeling to understand adverse effects of chemical exposures on wildlife populations

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To understand the potential for adverse effects of chemical exposure on wildlife, researchers and risk assessors must synthesize chemical exposure potential, the resulting acute and chronic effects to individuals, and what those individual-level effects mean for population-level impacts. Toxicity translators are tools developed to facilitate the application of laboratory-derived endpoints (e.g., EC20, LC50) as ecologically-relevant representations of how population demography may be affected. EPA work on toxicity translators originated with the avian MCnest model and is now being expanded to other taxa of interest for regulatory risk assessment. This research defines the field of toxicity translation by highlighting the common challenges and approaches used in the interpretation of test endpoints within different taxa. Here, we discuss aspects of laboratory test data and modeling approaches and evaluate the potential for computational linkages between available data sources, exposure models, effect models, and population/community models. We introduce a suite of toxicity translators in various stages of development that represent birds, fish, amphibians, and invertebrates, emphasizing how toxicity translators leverage existing datasets, models, and theoretical approaches. Establishing toxicity translation as a well-defined research area will help determine synergies among research efforts and identify tools and techniques useful to those involved in toxicity translation.

Impact and Trophic Transfer of Chemical Pollutants in Food Webs within and across Ecosystem Boundaries (P)

2.07P.1

Impacts of Roundup® UltraMax and the active ingredient glyphosate on the growth and biochemical profile of two freshwater macrophytes

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Glyphosate pollution in freshwater ecosystems is an issue recognized for nearly five decades and has been mainly attributed to its widespread use as a broad-spectrum herbicide in the agricultural and urban settings. It is widely applied as a part of commercial formulations such as Roundup, which increases its herbicidal activity by facilitating the penetration of the active ingredient into the plant tissues. In the context of risk assessment, most approaches are based on the evaluation of mortality and classical sublethal endpoints, usually concerning growth measurements, which represents a cost-effective methodology to address single or combined effects of a compound. Nevertheless, the assessment of biochemical endpoints may provide a better insight on the effects of certain pollutants, which may not significantly influence the plant's growth but may alter its biochemical composition and thus its quality as food. In the present work, we assessed the effect of glyphosate and its commercial formulation Roundup® UltraMax on two duckweed species, *Lemna minor* and *L. gibba,* subjected to a 7-

day exposure to each pollutant. Growth rate and net increase in biomass, frond number, frond area, and root length were assessed. Fatty acid and polysaccharides profiles of plants exposed to both pollutants were also determined. Experiments were concluded in early November with the first results demonstrating a higher sensitivity of *L. minor* for all growth endpoints assessed. Furthermore, Roundup proved to be more toxic to *L. minor* than its active ingredient alone for the same endpoints, with less pronounced differences in the case of *L. gibba*.
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2.07P.2

Cyanobacterial bloom associated with mass fish mortality in the dam Béni-Haroun, Algeria

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Mass fish mortality in freshwater aquatic ecosystems is a widespread visible event, considered in many countries of the world as a major source of concern for water quality and ecosystem health. Investigations to date have linked fish mortality to multiple factors, such as hypoxic conditions resulting from the respiration of cyanobacterial proliferation, senescence and release of cyanotoxins. During July 2017, a substantial proliferation of potentially toxic cyanobacteria, Planktothrix sp. has been observed in the Béni-Haroun dam, the largest freshwater reservoir in Algeria, with a capacity of about 1 billion m3, accompanied three months later (October 2017) by a massive mortality of fish. Tissue sections (viscera and muscle) from moribund common carp (Cyprinus carpio) and crude water were sampled during this period. Environmental monitoring of the dam water during the fish mortality revealed dissolved oxygen saturation at 123.6% subsurface, water pH was 8.62, water temperature was 20.8°C, chlorophyll-a concentration was 85 µg/l, and cyanotoxins type microcystin concentration was 132.3 ng equivalent microcystin-LR/L. The high level of microcystins detected using the protein phosphatase PP2A assay in the viscera tissue (880.7ng microcystin-LR equivalent/g dw) and in the muscle tissue (396.6ng microcystin-LR equivalent/g dw), and complete lysis of hepatopancreas observed after dissection and examination of the fresh carcass of common carp individuals suggest that cyanobacterial microcystins contribute to fish mortality in this dam.Key Words: cyanobacteria; cyanotoxins; microcystins; fish mortality

2.07P.3

Are low mercury levels in commercial mid-trophic level fishes detrimental? Evidences from oxidative damage assessment

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Sardines (*Sardina pilchardus*) and mackerels (*Scomber* spp. and *Trachurus* spp.) are amongst the most common fish caught along the Iberian coast for human consumption and, as mid-trophic level organisms, they constitute essential prey in the diet of several marine predators. Thus, the assessment of trophic ecology, contamination exposure, and stress responses in such organisms provides valuable information on the environmental health in the mid-trophic chain and the potential risks for consumers. This study aims to contribute for an integrative mercury assessment in five commercial fish species by measuring mercury levels, stable isotopes and oxidative damage from different geographic areas and seasons in the northeast Atlantic. Samples of muscle and liver were collected from a total of 116 individuals in two oceanographic surveys (Spring and Autumn) along the whole Portuguese coast. Mercury concentrations and stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) were performed in the muscle, while oxidative damage parameters (lipid peroxidation and DNA damage) was measured in both muscle and liver. Mercury concentrations were found to be considerably lower than values recorded in previous studies and the limits set by the European Union law, which confirms the non-toxic status of these species along the Portuguese coast, concerning this harmful element. Interestingly, no differences were found between areas and among the three genera, despite clear differences in trophic niches. Still, mercury concentrations were significantly higher during Autumn than Spring, possibly due to upwelling events during the summer. The fact that no relation was found between the mercury concentrations and effects on the measured oxidative damage parameters, further corroborates the healthy status of these species considering this harmful element. Overall, this work provided an integrative assessment on the mercury levels of commercial fishes from the mid-trophic chain, particularly from pelagic (and neritic) regions in the northeast Atlantic, showing that mercury contamination in these species is negligible. However, it is important to note that this study focused on small- and medium-sized specimens of these species (overall ranging from 13-25 cm in length), and thus higher concentrations of mercury may be present in larger fish due to bioaccumulation.

2.07P.4

Combined impacts of pollutants and temperature on the biochemical profile of the freshwater primary consumer Daphnia magna

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The broad use of pesticides for pest control produces hazardous impacts on non-target species, which has been widely documented. Run-off of such pollutants into water bodies threatens their community and structure. Primary consumers are essential for the integrity of food chains, linking the energy flow from primary producers to higher consumer levels. The single effect of $CuSO_4$ and Tebuconazole was chronically assessed for *Daphnia magna* under distinct temperatures (20ºC and 25ºC), assessing potential impacts in fatty acid (FA) profiles. Combination of the highest temperature (25ºC) and the highest concentration of CuSO4 (1.20mg/l), resulted in the lowest FA content compared to organisms exposed to lower pollutant concentrations and the control, at both temperatures. Lower concentrations of $CuSO_4$ at 20ºC and 25ºC did not present statistically significant differences in terms of FA content, however, at 20ºC, a decrease in FA content was observed at higher contaminant concentrations when compared to the control. Tebuconazole produced a more evident effect on the FA profile of *D. magna*: at both temperatures, the FA content decreased with an increase of concentration, reaching a minimum FA content at the highest pollutant concentration of 0.20mg/l, regardless the exposure temperature. The present study highlights the hazardous effects of pesticides on primary consumers, underlining the need to better regulate and control chemical usage, as so to prevent damages along trophic webs, as well as the need to adapt pest control to ecosystem-sound practices. The study was supported by FCT through the strategic projects UID/AMB/50017/2013 granted to CESAM and UID/MAR/04292/2013 granted to MARE. FCT funded C.P. Rocha and M.O. Rodrigues through Doctoral Grants SFRH/BD/140922/2018 and SFRH/BD/136931/2018, respectively. T.Vidal and C.Nunes are funded by national funds (OE), through FCT-Fundação para a Ciência e Tecnologia, I.P., in the scope of the framework contract foreseen in the numbers 4,5 and 6 of the article 23, of the Decree-Law 57/2016,of August 29,changed by Law 57/2017,of July 19.The research was partially supported through the project Ref.POCI-01-0145-FEDER-022127. N. Abrantes is recipient of a research contract from FCT(CEECIND/01653/2017). A.M.M. Gonçalves acknowledges University of Coimbra for the contract IT057-18-7253.

2.07P.5

Improving Status of Imposex in Dogwhelk (Nucella lapillus) in Irish and UK Marine Waters.

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The growth of barnacles and other organisms on ship hulls, a process known as biofouling, has always been a problem for mariners. Historically antifoulant paints containing organotins and in particular Tributyltin (TBT) were used to prevent such organism growth. By the 1980s it was realised that female dogwhelks (Nucella lapillus L.) were particularly sensitive, with reproductive impairment via the condition of imposex, (i.e. the superimposition of male genitalia in female dogwhelks) being unequivocally associated with TBT contamination. A global ban on the use of TBT paints followed in 2008. This collaborative study presents temporal trends and a current status update of imposex based on over 25 years of monitoring completed in UK and Irish coastal waters. Overall, there has been a dramatic reduction of TBT contamination and while a number of locations still exhibit some problem levels, it is clear that improvement is also evident at these locations. This demonstrates that measures taken nationally and internationally to phase out known toxic substances can be very effective in reducing marine pollution.

2.07P.6

Environmental microplastics affected oyster (Crassostrea gigas) survival, reproduction and larval stages behaviour

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To better understand the potential effect of microplastics (MPs) on the marine environment, we are interested in this work on the impact of environmental microplastics in the shellfish industry on the oyster (*Crassostrea gigas*) model. In this study, we exposed for two months adult oysters to a cocktail of environmental microplastics (polyethylene, polypropylene and PVC). These microplastics were obtained by crushing macro plastics used in shellfish farming on the Pertuis Charentais coastline. During the period of gonadal maturation in adult oysters and exposures at two different concentrations of environmental MPs (0.1 and 10 mg MP.L^{-1}), various tissue samples were taken. These tissues were used to assay toxicity markers (SOD, GST and MDA). Secondly, the sampled tissues will be digested with KOH in order to potentially retrieve MPs ingested. In addition, regular sampling (once a week) has been set up to collect faeces in aquariums and to analyse the MPs egress process. After two months of exposure, a laboratory fertilization was conducted to observe the effects of MP on the F1 generation of the D-larvae obtained.Swimming behaviour was first observed (swimming speed and trajectories) as well as development (malformations and developmental arrest) and larval growth at different development time steps: 24 h, 48 h and 72 h after fertilization. The first results observed show an increase in mortality in adults exposed to the two concentrations of MP (0.1 and 10 mg MP.L^{-1}). With respect to larval generation, behaviour appears impaired in the early stages of development with reduced locomotor activity in larvae of parents exposed to high levels of MP (10 mg MP.L^{-1}). D-larvae appear to show swimming trajectories rather circular and non-rectilinear as can be seen in control larvae. As for developmental abnormalities, more than normal numbers can be quantified for larvae from parents exposed to MP at 10 mg MP.L^{-1}.In order to better complete this study, a GC-MS pyrolysis analysis made it possible to determine the various persistent organic pollutants contiguous to the environmental MPs as well as the numerous additives (phthalates) that could generate additional toxicity.

2.07P.7

A pilot study to determine the potential impacts of plastics on Aotearoa-New Zealand's marine environment

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Once in the ocean, plastics are rapidly colonised by complex microbial communities. Due to the buoyant and resilient nature of plastics, ocean plastics pose a significant risk to ecosystems and fishery-based economies through their role in the translocation of invasive species and pathogens or changes in ecosystem function. Factors affecting the development and composition of these communities are still poorly understood, and there is currently no information on the biofilms that form on marine plastics in the southern hemisphere or their potential risks to the environment. This study aims to address this knowledge gap. To do this, two chemically and structurally distinct polymers, which are also common in marine plastic litter, nylon 6 (PA) and polyethylene (PE), were deployed for 3-months in the Port of Lyttelton, Christchurch, New Zealand. Biofilm formation was evident after 2 weeks, with visual differences seen by scanning electron microscopy after 6 weeks. PE communities were dominated by diatoms and unicellular prokaryotes, in contrast to PA communities which were dominated by cyanobacteria. Metagenomic community composition analysis corresponded to this, showing that the plastisphere was polymer-specific, and distinct from those of glass control surfaces and the surrounding ambient water. Physical modification to the surface of the plastics was observed, with the most pronounced change in the PA, with pitting conforming to the shape of microbial cells. It is, however, not possible to determine whether these changes are due to chemical oxidation or microbial enzyme-mediated biodegradation of the polymer, but suggests that the presence of a young biofilm is involved in the alteration of the polymer surface. Greater changes in the surface structure of PA may also be a result of the water-absorbent nature of nylon due to the presence of hydrophilic amide groups. This is the first study to examine the microbial communities associated with marine plastics in New Zealand waters, allowing the improvement of the understanding of the potential risk they pose. A year-long study is now underway to examine the fate of 5 plastic types in New Zealand coastal waters and the plastisphere that develops, including changes to surface and mechanical properties, changes to inherent and acquired chemicals, presence of invasive species and potential pathogens, and plastic degrading microbes which may present bioremediation potential.

2.07P.8

QUALIPERTUIS PROJECT - ENVIRONMENT AND PORT AREAS: MONITORING THE QUALITY OF THE ENVIRONMENT FOR A BETTER UNDERSTANDING OF THE EFFECT OF ENVIRONMENTAL VARIABLES AT A SPATIAL-TEMPORAL SCALE.

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The QUALIPERTUIS project aims to bring a new look at the causes of mortality of bivalve and help to understand more broadly the impact of environmental factors (biotic & abiotic) influencing the health status of these in port areas and coastal waters. A monitoring of the health status of the black scallop (*Mimachlamys varia*), the blue mussel (*Mytilus edulis*) and the oyster (*Crassostrea gigas*) has been carried out since October 2019 on several sites distributed between the Port de Plaisance de La Rochelle, Port Atlantique La Rochelle and Pertuis Breton and Antioche using the technique of "caging". This technique allows, using a system composed of Australian oyster cages, mooring and floats, to keep the individuals in immersion for 30 days (duration of an experiment) with a sampling of 7 individuals on D0 (day 0), D07, D21 and D30. Before the experiment, the individuals (native from Pertuis) are placed on the reference site (Marais (Houmeau - CNRS site)) for 15 days (acclimation). During the experiment, they are taken and placed in liquid nitrogen before being stored at -80 ° C. Dissections and analyzes are then carried out in the laboratory. The experiment is repeated 3 times a year (January, April, October) until 2021. The laboratory analyzes aim at evaluating the health status of bivalves using biological indicators (biomarkers) as well as the possible presence of chemical contaminants. Thus, several biomarkers are analyzed by spectrophotometry: defense biomarkers (Glutathione-S-transferase, Superoxide Dismutase, Laccase) and damage (MalonDiAdehyde, acetylcholinesterase). The various chemical and inorganic contaminants observed are metallic trace elements, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides and TBT. The results obtained are used in statistical analysis by correlation matrix with principal component analyzes. These experiments are part of a series of those already implemented for the Marina on the black scallop during a previous thesis conducted by Marine BREITWIESER at the University of La Rochelle (2015-2018). The "caging" method is used for monitoring La Rochelle Atlantic Port as part of the Port Horizon 2025 development project with the oyster and the blue mussel. The aim is to evaluate the impact of dredging and removal work on shellfish farms in Pertuis.

2.07P.9

Food chain transfer and toxicity of engineered Zinc Oxide nanoparticles: effect of particle size

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The tremendous application of engineered zinc oxide nanoparticles (ZnO NPs) in various fields of science and medicine has brought its production to 550 tons/year. However, there is always a great concern about the release of these ZnO NPs and their plausible toxic and trophic transfer potential in both aquatic and terrestrial organisms. This study explores the dietary transfer and toxicity of ZnO particles of different sizes (50 nm, 100 nm and bulk particles), as well as the contribution of Zn_{ions} (effect of dissolved Zn ions from NPs) to the overall toxicity ZnO_{total} (contains both dissolved Zn ions and suspended ZnO particles) in aquatic food chain from algae (*Scenedesmus obliquus*) to daphnia (*Ceriodaphnia dubia)*. The toxicity and accumulation of ZnO particles on both algae and daphnia were observed to be dependent on both the concentration and size of particles. Feeding of ZnO particle accumulated algae (354-571 µg/g dry wt.) post-exposure to 61 µM of ZnO_{total} of 50 nm, and 100 nm particles caused a significant decrease in the survival of daphnia by 20%. After 48 h of the depuration period, there was still a significant amount of Zn accumulation was found in daphnids. Bio-magnification (BMF) factor of ZnO particles was nearly 1 for all the sizes tested, indicating that ZnO particles irrespective of its size can transfer from lower (algae) to a higher level (daphnia) aquatic organisms through diet. Further, the ultra-structural changes in daphnids fed with Zn accumulated algal cells were evident through electron microscopy, causing damages to microvilli, degradation of the nuclear envelope, mitochondria, the occurrence of small vacuolar structures and presence of ZnO particles in the peritrophic membrane. These observations would bring awareness to the society that the dietary transfer is one of the important routes of nanoparticles transfer in food chain.

2.07P.10

Predicting effects of contaminants on food webs: Aquatic pollution, adult aquatic insects, and terrestrial insectivores

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Organisms that move across ecosystem boundaries connect food webs in apparently disparate locations. As part of their life cycle, aquatic insects transition from aquatic larvae to terrestrial adults, thereby linking freshwater ecosystem processes and terrestrial insectivore dynamics. These linkages are strongly affected by contamination of freshwater ecosystems, which can reduce production of adult aquatic insects (i.e., emergence), increase contaminant concentrations in adult insect tissues, and alter contaminant flux to terrestrial ecosystems. Despite the potential impact of contaminants on adult aquatic insects, little is known about predicting these effects. Here, I develop a heuristic model based on contaminant properties and ecotoxicological principles to predict the effects of various classes of aquatic contaminants on adult aquatic insects and discuss implications for terrestrial insectivores living near contaminated freshwaters. The main finding is that contaminant classes vary greatly in their effects on aquatic insects and food-web mediated implications for terrestrial insectivores. Highly bioaccumulative contaminants that are well retained during metamorphosis, like polychlorinated biphenyls (PCBs), are often non-toxic to aquatic insect larvae at concentrations commonly found in the environment. Such contaminants flux from aquatic ecosystems in large quantities in the bodies of emerging adult aquatic insects and expose terrestrial insectivores to toxic levels of pollution. On the other hand, contaminants that are less bioaccumulative, excreted during metamorphosis, and more toxic to insects, like trace metals, tend to affect terrestrial insectivores by reducing production of adult aquatic insects on which they prey. Management applications of this model illustrate type and severity of risk of aquatic contaminants to consumers of adult aquatic insects.

Microbial Community Ecotoxicology under Multiple Stressors Scenarios (P)

2.08P.1

The effect of wastewater treatment plant effluents on the gut microbiome of aquatic and riparian organisms

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The microbiome is a community of bacteria integral in maintaining host health. At present, little is known about the gut microbiome of aquatic organisms, but preliminary research suggests that it is influenced by host species, diet, and environment. This study evaluated whether exposure to wastewater treatment plant (WWTP) effluent in the central Grand River, Ontario, Canada, affected the diversity and composition of the gut microbiome of rainbow darter fish (*Etheostoma caeruleum),* flutedshell mussels (*Lasmigona costata*), several species of insect larvae, and riparian long-jawed orb weaver spiders (Tetragnathidae). In October 2018, samples were collected from sites located at varying distances upstream and downstream of two major WWTPs and stored in buffer solution. Genomic DNA was extracted, PCR amplification of the 16S rRNA gene V3-V4 region was conducted, and Illumina sequencing were performed. Amplicon sequence variants were filtered using the DADA2 pipeline and mapped back to bacterial species using the SILVA database. Fish and mussel gut content or whole spider and macroinvertebrate microbiomes were dominated by the bacterial phylum Proteobacteria; in addition, spider microbiomes were dominant in Firmicutes and Bacteriodetes, both Heptageniidae insect and mussel microbiomes were dominant in Bacteriodetes and Cyanophyta; and rainbow darter microbiomes were dominant in Firmicutes and Cyanophyta. The gut content community of rainbow darter showed increased Shannon alpha diversity downstream of both WWTP outfalls (one-way ANOVA, $p< 0.001$) when compared to upstream samples. Fish microbiomes at the downstream sites also had significantly different Bray Curtis beta-diversity when compared to those of upstream fish (PERMANOVA, $p< 0.001$), and increased relative abundances of Proteobacteria and Cyanophyta. Shannon diversity decreased for mussel (Tukey HSD: $p=0.0008$) and increased for spider ($p=0.007$) microbiomes downstream of one WWTP. All invertebrate taxa had significantly dissimilar Bray-Curtis beta diversity among locations (PERMANOVA; mussels $p=0.0001$; spiders $p=0.0006$; Heptageniidae $p=0.0001$). Overall, results indicated that the gut microbiome of downstream organisms differed from those observed upstream of the WWTPs, and such differences may indicate altered health of the host.

2.08P.2

Holobiont: interaction between terrestrial isopods and their hepatopancreas' microbiome.

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Terrestrial isopods can inhabit heavily metal-contaminated areas and achieve tolerance/resistance by accumulative immobilization. Their hepatopancreas is comprised of two very different metal-sequestering cell types ('*B*' and '*S*' cells). '*B*' cells discharge their contents diurnally (i.e. metals, lipids and sugars) in a feeding cycle. A previous study presenting a draft genome of an isopod symbiont has showed to have truncated metabolic pathways. This fact leads to the hypothesis that such symbiont could survive due to the cycle of '*B*' cells and the content expelled. This could also imply they should have an important role altering the redox state of metals excreted together with the energy reserves. Terrestrial isopods and the microbiome present in their hepatopancreas can then be considered a single biological unit and called an holobiont. The present work was developed by amplifying and sequencing bacterial 16S. The different profiles of hepatopancreas' microbiomes studied, belong to different species of terrestrial isopods inhabiting different niches within different countries. The comparative analysis allowed the identification of bacterial groups that are common/unique between the species. These results provide the first evidence of physiological interactions between isopods and microbiome and can now be used for a deep characterization and study of their role in metal tolerance/resistance.This project was supported by a MSC COFUND Fellowship (H2020-COFUND-SIRCIW>MINT-512202) through Cardiff University, Welsh Government and the EU and by FCT/UEFISCDI/FORMAS through REWATER project (ERA-NET Cofund WaterWorks2015), and partially supported by Strategic Funding UID/Multi/04423/2019 (FCT, ERDF).

2.08P.3

Investigating the potential of probiotic to mitigate BPA toxicity in zebrafish (Danio rerio): A metagenomic perspective

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The technological development we have witnessed in the last couple of decades has made possible the study of the microbiota by allowing the identification of unculturable microorganisms. As a consequence, it is now possible to investigate microbial communities' composition and functionality providing new interesting insights on how the microbiota is able to modulate the organism's physiology. In the environmental toxicology field, interest has grown on the ability of chemical compounds to cause dysbiosis because pathophysiological changes in the gastrointestinal tissue caused by ingested chemicals and microbial metabolites can lead to systemic adverse effects. The probiotic has been widely used, especially in the aquaculture field, to improve the immune system of harvested fish given its ability to modulate the gut microbiota. Despite its many benefits, none of the studies so far has attempted the investigation of its ability to potentially mitigate chemical toxicity.

In this context, we employed the power of 16S amplicon sequencing to investigate the microbiota of adult zebrafish (Danio rerio) undergoing exposure to BPA, administration of probiotic, a combination of BPA and probiotic and control. Alpha and Beta diversity analyses suggest that the combination of BPA and probiotic is able to shape the microbial communities in a way which is different from either control, probiotic administration or BPA exposure. This finding was supported by the differential abundance analysis which revealed the significance difference already at the phylum level and down to the genus level. In addition, we predicted the functional capability of the microbial communities in the different condition and found out that differences in abundances and composition were reflected at the functional level. These results suggest that probiotic has the potential to interfere with the toxicity elicited by the BPA by modulating microbiota composition and, despite additional studies are needed, pose the basis for the inclusion of the microbial component into risk assessment strategies.

2.08P.4

Effects of metal mining effluents from abandoned mines on freshwater ecosystems. Frongoch mine as a case study.

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Effluents from abandoned mines represent a source of chronic uncontrolled pollution because of the lack of any regulation about these wastewaters treatment. The specific hazards posed by chemical stressors harboured in mine effluents to the aquatic environment have been barely investigated. Biofilms are complex microbial communities with a relevant role in aquatic ecosystem functioning that are considered as effective bioindicator of ecological impacts and are recognized as necessary target within the Water Framework Directive. This study aims to evaluate the ecological impact in freshwaters of a metal effluent from an abandoned mine (Frongoch) in Wales, United Kingdom. For that, aquatic biofilms have been used as biological indicators. Specifically we compared the behavior of biofilms in a pristine stream (Nant cell) with the mining effluent (SITE 1) and different impacted reaches (SITE 2 to 6) along the Frongoch river, downstream the abandoned mine. Mining effluent significanlty affected river biofilms by reducing its photosynthetic efficiency and changing its phototrophic community composition, which shifted from diatom to green-algae dominated community. In addition, diatoms found in the most impacted site presented deformities and a loss of biodiversity. By constrast, as distance to the mining area increased, the ecological impact decreased. Sites located more downstream presented a gradual dilution of the metal concentration in water and both, the phtosynthetic efficiency and the photothrophic community composition of the biofilm, were recovered. The main conclusions are: i) the mining effluent severely affect the biofilm communities with potential consequences at ecosystem level, ii) the sampling points downstream are affected by the metal content in water and iii) along the river, with the dilution factor, the river are able to recover the biofilm community composition but with a loss and deformities in the diatom species.

2.08P.5

Understanding the link between micropollutants in wastewater effluents and community tolerance of river periphyton

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Wastewater treatment plant (WWTP) effluents, which contain complex mixtures of micropollutants (MPs), are of major concern regarding their impacts on the quality of the receiving water bodies. Establishing a link between exposure to MPs from WWTP effluents and their effects is challenging due to the presence of other environmental factors. The aim of this study is to examine the effects resulting from microbial community-level exposure to different dilutions of WWTP effluents. To achieve this goal, we combined time-integrative passive sampling of organic MPs in WWTP effluents with the assessment of the sampler extract toxicity via bioassays, using stream periphyton. Periphyton was grown in flow-through channels continuously alimented with stream water and different percentages of urban WWTP effluent (i.e., 0%, 10%, 30% and 80%). After 4 weeks, periphyton was sampled and exposed to increasing dilutions of extract from passive samplers that were immersed in the WW effluent. Tolerance of periphyton communities to passive sampler extracts was determined according to the pollution-induced community tolerance concept by measuring the inhibition of algal primary production and photosynthetic efficiency. Periphyton was also characterized functionally and structurally by a set of biological endpoints, including 16S and 18S sequencing. Moreover, 54 MPs, including artificial sweeteners, pesticides and pharmaceuticals, were monitored in composite and grab water samples and biofilms from all treatments and in the passive sampler extracts. Among the 54 substances analysed, 34 were quantified in the grab water samples, reflecting the different dilutions of WWTP effluent. Microbial communities exposed to 30 and 80% WW were more tolerant to the MP extracts than the stream water control. This might be due to physiological adaptations and/or the selection of tolerant species to the MPs. Moreover, the analyses of microbial diversity and other biological descriptors showed a change in bacterial and algal community composition and function in response to wastewater. Overall, our study shows a link between exposure and community-level effects under controlled conditions that mimic the input of WWTP effluents in streams. The comprehensive integration of functional and structural responses of periphyton communities exposed to effluents, along with a detailed chemical characterization, is therefore crucial to link exposure to community-level effects in multi-contaminated ecosystems.

2.08P.6

Does history really matter? Aquatic microbial communities' functioning under multiple stress

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Microbial decomposition of terrestrially-derived leaf litter, mainly driven by aquatic fungi and bacteria, is a fundamental ecosystem process making nutrients available for aquatic food webs. Due to agricultural intensification and expansion into pristine areas around the globe, these microorganisms are increasingly exposed to pesticides and fertilizers. A previous study showed that *"History matters"*, meaning that communities with a history of pesticide exposure are adapted to these conditions and are more tolerant relative to communities with minor disturbance history. In order to validate these insights against a larger sample size, we assessed the potential differences in the stress tolerance of aquatic microbial communities sampled from streams considered as pristine (P) or receiving either wastewater treatment plant effluents (WWTP) or run-off from vineyards (VYRO), with two streams per category. We used a fully-crossed design exposing the six distinct communities to increasing concentrations of a fungicide mixture and nutrients over 21 days ($n=5$). The microbial leaf decomposition rate (*kmicrobial*) was measured as a functional variable, along with microbial enzyme activities. *kmicrobial* did not show a significant variation ($p = .691$) among the assessed communities with increasing fungicide levels, but the direction of effects was site specific. Moreover, the effects of increasing fungicide concentrations were mitigated with nutrient enrichment for all studied communities. The activity of β-glucosidase, β-xylosidase and cellobiohydrolase, enzymes responsible for the degradation of cellobiose, cellulose and hemicellulose polymers, showed a significant decrease ($p < .5$) in their activity with increasing fungicide concentration. On the other hand, the increase in nutrient content did not consistently lead to an increase of enzyme activity. All in all, the six studied microbial communities showed highly variable responses to the gradients of fungicide and nutrient exposure. In a next step, the interpretation of these functional variables should be supported by the study of the leaf-associated microbial community structure, which might ultimately be linked to the communities' trait composition. Irrespective of this, the present study was not able to support the conclusions drawn from an earlier work using a similar experimental approach. Therefore, this work highlights the complexity in natural communities and their strategies to cope with stress.

2.08P.7

Removal efficiency of Water Accommodated Fraction (WAF) in cold environments and its effects on Microbial communities' dynamics

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Oil extraction in the arctic environment is increasing due to the melting of the ice caps. Therefore, arctic and subarctic environments have become more vulnerable toward oil spills and accidental oil discharges. One of the major risks during oil spill is the introduction of low molecular oil fractions, called Water Accommodated Fraction (WAF), into the water column. With weathering processes and wave dynamics, WAF can be soluble and thus more bioavailable to marine organisms. The objective of the study is to investigate the microbial biodegradation of WAF at low temperature and its effects on the microbial communities structure and dynamics. A laboratory microcosm experiment was conducted for 21 days at 4 degrees using water and sediments obtained from polluted or clean sites in Tromso, Norway. Microcosm water was prepared with WAF from the Arctic oil Troll B with or without the addition of dispersant (Finasol 52). RNA and DNA were extracted from water and sediment, and then analyzed by 16S rRNA gene barcoding using Miseq illumina sequencing in order to follow the microbial community dynamics. Hydrocarbon degradation or evaporation rates from water and sediment samples were evaluated using GC/MS. Finally, toxicity assays were conducted on water samples from the microcosms using the copepod Acartia tonsa for determining LC50 and gene expression profiles of selected genes after the 96 hours exposure. The removal efficiency of WAF at cold temperature with or without the inclusion of dispersant will be related with the toxic effect of the metabolites produced by combining all the data sets. In addition, correlation analyzes will allow to determine the major microbial populations playing a crucial role in maintaining microbial community functioning under WAF biodegradation conditions. Such information is of paramount importance for understanding the effect of hydrocarbon contamination in cold areas, providing new insights for the implementation of counter measures to mitigate its effects.

2.08P.8

Climate change and nanoparticle toxicity effects on ecosystem functioning

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Aquatic ecosystems are threatened by multiple environmental stressors. Release of toxicants such as engineered nanoparticles (ENPs) into aquatic systems and their effects on these ecosystems remain poorly understood. This, combined with the acceleration of global climate warming (ENP + ΔTºC), could have significant consequences for aquatic life. This study focus on responses of fluvial biofilms, as key points of ENP entry into aquatic food webs, and examines the effects of combined stressors at community and ecosystem levels using an extended set of end-points as an integrated approach. To achieve this aim, an outdoor microcosm study using different temperatures (18ºC and 25ºC) was performed. After 4 weeks of biofilm colonization at both temperatures, ENP were added obtaining 4 treatments (x5 replicates each): (1) control , (2) 50 µg/L of PVP-coated AgNPs (50 nm), (3) 25 µg/L of Ag_2S NP (30 nm) aged from pristine AgNP as a stable form of silver, known to have lower dissolution potential, and (4) 50 µg/L of

AgNO3 as a positive (Ag+) control. Sampling was at the beginning of ENP exposition (0h), and after 1, 3, 15 and 30 days. Functional and structural changes were assessed using different end-points: enzyme activities, analysis of extracellular polymeric substances (EPS), metabolism, photosynthetic activity, algal biomass, ENP bioaccumulation and confocal observations of biofilm community. Metabolism increased with rising temperatures but reduced in the presence of AgNP and Ag+. Surprisingly, under warm conditions Ag2S decreased the photosynthetic activity and algal biomass like the Ag+ control, while AgNP increased both. Increased water temperature also caused homogenisation of biofilm composition and structure, and combined with AgNP or Ag+, an increase of dead cells and a decrease of biofilm EPS. Finally, enzyme activities (β-glucosidase, leucine aminopeptidase and phosphatase) measured in water and in biofilm showed a clear enhance under warm conditions and only β-glucosidase and phosphatase in biofilm were affected by ENP. These results point out that warmer water can enhance AgNP and Ag+ potential toxicity as well as modifying the behaviour of "non-toxic" compound like Ag2S. Rising temperatures, ENP and the combination of both stressors, may seriously affect the freshwater ecosystem functioning, like nutrient recycling, affecting essential freshwater ecosystem services such as water quality.

2.08P.9
Soil bacterial community responses to AgNPs
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Silver nanoparticles (AgNPs) are broadly used due to their antimicrobial properties. Consequently, AgNPs can be released into the terrestrial environment, leading to effects on soil bacterial community (SBC), essential to regulate nutrient cycles. This work aimed to study the impact of AgNPs and the ionic control ($AgNO_3$) on SBC, (1) at community structural level and, (2) in terms of SBC susceptibility and tolerance, which is the foundation of the Polluted induced community tolerance (PICT) approach (expressed as variation of 50% of inhibitory concentration-IC50). First, a microcosm was performed using Lufa 2.2 soil, spiked with AgNPs, or $AgNO_3$, at 5 µg of Ag.Kg^{-1} of soil (100xPEC) during 56 days (selection phase-SP). From this exposure, the SBC structure was analysed by denaturing gradient gel electrophoresis (DGGE) of the 16S rRNA gene V3-V4 region. Then, SBC sample was extracted and used in the susceptibility test, using the disc diffusion method (detection phase-DP). For that, the SBC extract was swabbed on Müller-Hinton Agar and discs impregnated with different amounts (0.1 µg, 1 µg or 10 µg) of each silver form (AgNPs or $AgNO_3$) were placed onto the agar surface. Plates were incubated for 24h at 25ºC. A three-way ANOVA tested differences in the susceptibility of the SBC considering: 1) the soil exposure and SBC selection-SP, and 2) the detection of the SBC response to the disc exposure-DP. Comparing the SBC from both SP and DP, enabled the assessment of a possible induced tolerance (PICT calculation) by measuring the growth inhibition zone (ZoI). After 56 days, structure of SBC was affected by Ag exposure, disregarding the exposure form. Regarding susceptibility of the SBC, significant differences were observed: (1) within SP, for the different silver forms; (2) within DP, with higher ZoI at $AgNO_3$ exposures, also increasing with the amount of Ag present on the discs (0.1 µg< 1 µg< 10 µg). Regarding PICT analysis, it was not detected any induced tolerance by AgNPs on SBC. A decrease in tolerance was only detected when the SBC was exposed to $AgNO_3$ both in SP and DP. The IC50 was lower for $AgNO_3$ than for AgNPs, for both SP and DP, meaning that a lower amount of $AgNO_3$ is needed to cause a change in SBC tolerance. Overall, AgNPs changes the SBC in terms of structure and susceptibility but not in tolerance while $AgNO_3$ showed stronger effects. Still, microbial-mediated processes can be affected by AgNPs exposure and may cause imbalances in this ecosystem.

2.08P.11
Biodegradation of mulch films in soil: Isolation and identification of degrading microbes
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Plastic residues in the environment are a topic of emerging concern. Those residues can not only be found in waterbodies and oceans, but also in soil. One application where plastics are intentionally introduced into the terrestrial environment are mulch films which are used in agriculture to enhance yields and provide stable soil conditions. Conventionally, mulch films consist of non-biodegradable polymer polyethylene (PE). However, when removed from fields after crop season, some plastic particles remain in the soil. One solution to overcome this problem are so-called biodegradable mulch films consisting of materials as polybutylene adipate terephthalate-polylactide blends (PBAT-PLA) or of microbially produced polyhydroxy butyrate (PHB) as alternative product. Manufacturers advertise those mulch films to simply be plowed under after growing season where they are to be biodegraded. Yet there is a need to investigate how quickly the residual mulch film actually is biodegraded and which microbes play a major role in the process. In the present study, pieces of PBAT-PLA mulch film from two different manufacturers, PHB mulch film and PE mulch film from two different manufacturers are incubated in petri-dishes filled with a field soil with no known history of mulch application at 30°C for two months. At different times, film pieces are sampled and from the adhering soil, potentially degrading bacteria and fungi are isolated by growth on selective media. The growth capabilities of the isolated strains on the mulch film monomers in liquid culture are quantified via optical density. Monomer dissipation in liquid medium is quantified with high-pressure liquid chromatography and gas chromatography–mass spectrometry, respectively. Further, metabolization of mulch film microparticles is quantified using carbon dioxide-sensors. Best performing strains will be identified via 16S rRNA amplicon sequencing and compared to the initially determined soil microbiome. With best performing microbes, further degradation tests will be conducted under differing conditions. With the results, strategies on a more sustainable use of mulch film and possibilities for upcycling will be discussed. The research is part of the iMulch project funded and supported by the European Fonds for Regional Development (EFRE).

2.08P.12
Effect of the antibiotic oxytetracycline on the growth of soil bacterial communities
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Tetracyclines are a group of antibiotics used in veterinary medicine to treat diseases (and as growth promoters in certain countries). This group of antibiotics represents 33.4% of the whole veterinary antibiotics used in the European Union. Among them, one of the most used worldwide is oxytetracycline (OTC). Like other antibiotics, an important proportion of the OTC supplied to livestock is not absorbed by the animals, and therefore, important amounts of it are excreted. Then, when livestock manures and/or slurries are employed as soil amendments, OTC may reach the environment as a pollutant. Once in the soil, OTC may cause negative effects on bacterial communities that participate in different processes in the soil. In this work, the toxicity of OTC on soil bacterial community growth was studied in 22 agricultural soils spiked with this antibiotic, under laboratory conditions during 42 incubation days. Results showed that OTC may cause toxicity for bacterial community growth when added in high concentrations. Also, results showed that OTC toxicity on soil bacterial communities was persistent during the incubation period, i.e it may be considered as a pollutant with a relatively high persistence for toxicity in soils. Moreover, the toxicity caused by OTC on bacterial community growth was highly dependent on soil properties. Thus, in soils with high clay and low silt contents, OTC toxicity was lower than in soils with high silt and low clay contents. In addition, OTC toxicity was higher in soils with low organic carbon contents than in those having high organic carbon contents. Finally, increases in soil pH resulted in higher toxicity due to OTC on soil bacterial communities. Therefore, and adequate soil management may lead to decrease the risk of bacterial communities damages in soils polluted with OTC.

2.08P.13
Sediment microbiome as a tool for assessing metal contamination in a multi-species setup experiment
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The ocean and coastal areas are subjected to many drivers and pressures (e.g. pollution inputs). Metals released from anthropogenic activities (mining, industry or urban sewage) can alter the structure and function of the ecosystems. Microbial communities are essential in natural ecosystems, since they play a significant role in the global biogeochemical cycles, and they can be modified by metal pollution. In this work, the effect of Cd and Ag, at 5 and 50 ug/L was assessed using metagenomic and metaproteomic approaches in a multispecies setup system. Microorganisms´ identification, by DNA sequencing of the 16S rRNA, showed a significant decreasing in the number and diversity of bacteria regarding to control at the lower concentration (5 ug/L) of both metals, Ag (21.6-fold) and Cd (1.6-fold). Proteobacteria were always the most abundant group (60-65%). The metaproteomic analysis identified 1170 proteins, and revealed expression changes (> 2-fold) in 179 and 59 proteins for Ag and Cd, respectively. Main biological processes affected were transport and biosynthetic pathways. Ag exposure had a greater effect than Cd on the microbiome, with a higher decreasing in the amount/diversity of microorganisms at the lowest dose, and greater protein expression changes at the high dose. In summary, sediment microbiome is key to assess the impact of metal pollution. **Acknowledgments:** Project (MINECO,

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Soil Ecotoxicology: New Methods and Novel Applications in Environmental Risk Assessment (P)

2.09P.1

Development of a more practical, cost effective and ecologically relevant multi-chamber circular ISO 17512-1 earthworm avoidance test.

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Avoidance is known to provide a detectable response more rapidly and at lower levels of contamination than more traditional endpoints such as survival or reproduction. The ISO 17512-1 standard details both 2 and 6 chamber tests. The 2 chamber option is widely employed but has limited comparative relevance to heterogenous field conditions. The 6 chamber design provides greater relevance but is not widely used partly due to the bespoke nature of design and associated costs of metal construction. This study sought to develop and evaluate a more practical and reduced cost circular avoidance test design (made from plastic and with removable dividers) using the soil dwelling earthworm Aporrectodea caliginosa and in direct comparison with the linear avoidance test developed by Lowe et al (2016). The test material used was waste ash from incineration of the energy crop Miscanthus x giganteus (considered a potential agricultural fertilizer). The linear gradient and circular chamber (diam : 31 cm, depth : 12.5 cm) designs were established (5 replicates), with Kettering Loam spiked with 5 concentrations of Miscanthus ash (0, 1, 2.5, 5 and 10 t ha-1). Five individuals were placed on the surface of each treatment and dividers between each treatment removed. After 14 days incubated at 10 °C, dividers were re-inserted and earthworm location recorded. In both devices, A. caliginosa were retrieved from all treatments suggesting that at the concentrations studied, individuals did not avoid Miscanthus ash. The circular chamber provided comparable results with the linear gradient and the plastic construction was shown to be robust, practical and re-useable with chambers constructed commercially at significantly lower costs than metal equivalents. The circular construction also provides a continuum of contaminant concentrations negating the potential effect of "ends" on earthworm movement experienced in the linear gradient. Further studies are required to confirm the effectiveness of this adapted circular chamber utilising other contaminants and experimental time periods.

2.09P.2

Toxicological effects of chlorpyrifos and glyphosate on soil organisms, Achatina fulica and Eisenia fetida

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Pesticides are chemical inputs that farmers invest in to ensure high quality yields of their crops. Insecticides like chlorpyrifos and herbicides such as glyphosate are extensively used to minimize the effects of insect pests and weeds on crops. However, literature shows that of the pesticides sprayed by farmers in agricultural fields, only a small fraction of these chemicals reach the targeted pests, the major portion of the sprayed pesticides end up on non-target organisms affecting their well-being. We investigated the effects of glyphosate and chlorpyrifos on the biochemical markers of two terrestrial organisms *Achatina fulica* and *Eisenia fetida*. Groups of land snails and earthworms were exposed to individual and mixtures of glyphosate and chlorpyrifos for up to 28 days. Sub sampling was done on days 14, 21 and 28. Post mitochondrial fractions were prepared and used to measure activities of selected enzyme systems. Time dependent increases in antioxidant enzyme activities (up to 3 fold) were observed while inhibitions of acetylcholinesterase (9-50)% and succinate dehydrogenase (3-60)% activities were observed. For both organisms the highest alterations in enzyme activities were caused by pesticide mixtures. Alternations of biochemical markers indicate that pesticides affect the normal wellbeing of soil organisms. There is need therefore, for routine monitoring of pesticides in soil to safeguard the health of soil organisms.

2.09P.3

Evaluation of an analytical method to determine pesticide residues in earthworms Eisenia andrei

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Earthworms constitute a major part of the biomass in soil and provide a significant beneficial influence on soil properties. They have been an indicator species in ecotoxicological effect studies for years e.g. in OECD Test Guideline (TG) 222 (Earthworm Reproduction Test *Eisenia fetida/Eisenia andrei*). However, there is a lack of knowledge concerning the actual concentration residues of the compounds within the earthworms themselves. In our study, we developed a method for the detection of pesticide residues in earthworms utilizing natural soil. Studies according to OECD TG 222 were conducted using seven different concentration levels (0.5, 1.0, 2.0, 4.0, 16, 32 and 64 mg test item/kg dry mass soil). To study the uptake, the earthworms were starved for 5 days and their excrements discarded. Earthworms exposed to the same concentration (7 in total) were selected for two sequential extractions (1 x 20 mL acetonitrile, 1x 20 ml methanol). In a first step, the 7 earthworms were transferred into a 40 mL polypropylene copolymer centrifuge flask and 20 mL acetonitrile was added. Afterwards, the samples were homogenized using a high speed ultra-turrax (25 000 rpm, 2min, Polytron PT 6100 D) and centrifuged (8000 rpm, 14°C, 10 min, Avanti J-26 XP Centrifuge Beckman Coulter). The supernatant was transferred into a volumetric flask. In the second extraction step, 20mL of methanol were used, before the residues were subjected to the high speed ultra-turrax and centrifuged again. The supernatant was combined with the supernatant of the first extraction step. In the next step, the combined extracts were filled up with acetonitrile to 50 mL final volume. Finally, 1.5mL of this solution were subjected to centrifugation to remove any precipitates before analysis by GC-MS/MS. In a test case we showed the presence of a herbicide within the earthworms. These first experimental results indicate that the methodology developed can provide a suitable technique for the determination of pesticides in earthworms. Therefore our results contribute to a better mechanistic understanding and may be a contribution to develop an earthworms modeling tool. Keywords: Earthworms *Eisenia andrei*, OECD Tests No 222

2.09P.5

Biomarkers responses in the earthworm Eisenia fetida (Savigny, 1826) exposed to commercial fungicides: laboratory and field toxicity assessment

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The use of pesticides in agriculture is a common practice to protect crops all over the world. However, pesticides potentially affect soil macro-and microorganism because only 0.1% of an applied pesticides reaches the target organism. Earthworm is a model organism in ecotoxicological research and it is a useful bioindicator of chemical toxicity in terrestrial ecosystem. The aim of this study was to evaluate the potential toxic effects of commercial fungicides (two foliar and two ear diseases with double and single active substance) on the earthworm Eisenia fetida (Savigny, 1826). E. fetida was exposed to sub lethal concentration of fungicides in laboratory and field condition and a set of biomarkers were investigated. In laboratory experiments, animals were exposed in a filter paper test to increasing concentration of the four fungicides. Field investigations were conducted transplanting E. fetida in cages in the soil of wheat and durum wheat fields during treatment with different combinations of the four fungicides. All the E. fetida were analysed to evaluate vitality, inhibition of acetylcholinesterase activity, glutathione S-transferase, lipid peroxidation, catalase activity, total glutathione, Comet assay and lysozyme activity. Laboratory studies with the fungicide with double active substances showed alterations in organism's vitality which increased with increasing treatment doses. Significant alteration of GST enzymes with respect to controls were detected at environmentally relevant doses of ear deseases fungicide. The laboratory treatment with foliar fungicides caused an alteration of LPO which increased with increasing treatment doses, and an increase inhibition of lysozime activity. All the fungicides showed a total glutathione levels decrease probably due to a cellular degeneration caused by oxidative stress. The Comet assay showed the highest DNA fragmentation values in the experiment with fungicides with double active principle. In the field studies, the test proved that leaf fungicides have greater toxicity compared to those applied to the ears. Foliar fungicide with double active substances revealed to have the most toxic effects, inhibiting the lysozyme activity and determining high levels of GST and LPO. This study represents a first step towards a better understanding of commercial fungicides toxicological potential to non-target organisms and a wide set of biomarkers allowed to investigate the main toxicological effect on these organism.

2.09P.6

ECPA cross industry working group on Soil Organism Ecotoxicology, Regulatory Testing and Risk Assessment

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The European Crop Protection Association (ECPA) represents the crop protection industry in Europe and is funded by industrial partners. ECPA brings together scientists, such as ecotoxicologists, from across industry, who discuss the key

topics and provide input on the practicality of new approaches to the regulatory and scientific community from an industry perspective. Topics include lower tier laboratory and higher tier test systems, and test guidelines and risk assessment guidance. Together, we advocate for changes to the soil organism risk assessment paradigm that benefit soil biodiversity and sustainable agriculture; we present the industry perspective and promote scientific process. We conduct research to ensure that the risk assessment paradigm is appropriately protective for non-target soil organisms and encourage discussion on the role of Ecosystem Services in the context of setting specific protection goals for Plant Protection Product Registration. Over the past 10 years the group has worked with independent consultants and academics to bring together the wealth of data, knowledge and expertise from different companies to develop the science to ensure the safety to soil organisms whilst providing plant protection product solutions to farmers. The ECPA group has established many collaborative projects that have been made available through posters and platform presentations at SETAC conferences and beyond, in addition to peer reviewed articles. Some examples of projects to date include: Broader use of historical control data e.g. from earthworm field studies: Occurrence and distribution of earthworms in agricultural landscapes across Europe (Dinter et al, 2013)The role of functional vs structural endpoints in risk assessment (SESSS Conference, 2019)Recalibration of the Earthworm Tier 1 Risk assessment of plant protection products (Christl et al, 2016)Review on Litterbag test (Dinter et al, 2008) Some of these papers have been discussed in the recent EFSA Opinion (2017) 'addressing the state of the science on risk assessment of plant protection products for in-soil organisms'. **This poster aims** to introduce ECPA Soil Organism group and showcase its collaborative work to date, and to highlight current projects and serve as a talking point for encouraging discussion and identification of hot topics in Soil Organism Ecotoxicology to support future collaborative projects to address risk assessment of Plant Protection Products in Europe.

2.09P.7

Plant ecotoxicity test using the tree species Mimosa scabrella Benth.: biomonitoring soil ecotoxicity and ecosystem recover potential

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Soil pollution is a theme of concern around the world. Mining activities and soil disposal of industrial activities can cause severe impacts on soil biodiversity and functioning. Ecotoxicity tests have been applied to environmental monitoring of contaminated sites, where the results obtained through the tests can indicate the recovery potential of an environment or the need for remedial actions. The tree species Mimosa scabrella Benth. (Fabaceae), popularly called "bracatinga" in Brazil, is a native species of the Atlantic Forest. This species is a pioneer tree, presenting a high degree of ecological interactions, and because that it has being recommended by many authors as a suitable species for the recovery of degraded areas in this biome. This work aimed to evaluate the use of bracatinga as a test organism in plant ecotoxicity tests, as well as its sensitivity to boric acid (BA) as reference substance, and its possible use to evaluate contaminated areas with residues originated from mining activities and from paperboard industry. The tests followed the recommendations of the ISO guideline 11269-2, adapted for this species in temperature (23ºC), test environment (greenhouse) and exposure period (20 and 35 days after germination). The first step was to evaluate the percentage of germination in Tropical Artificial Soil (TAS), used as control. The second step was to evaluate the species sensitivity to BA (0, 125, 250, 500, 750 and 1000 mg.kg-1), and the third step was to evaluate its sensitivity to contaminated soils from mining activities. The evaluated endpoints were: germination (%), root and shoot growth (cm), fresh and dry biomass (mg). Results showed that bracatinga presented a satisfactory germination percentage (96%) and survival in controls. Furthermore, this species was sensitivity to BA, showing a dose-response curve. The most sensitive parameter was shoot dry biomass after 35 days of exposure. This species also showed sensitivity to contaminated soils from mining activities and from paperboard industry. As the tested species fulfilled the validity criteria required to plant tests (germination, survival, coefficient of variation), M. scabrella has potential to be used as test species in a future adaptation of the ISO guideline for tree species.

2.09P.8

epicPCR - a Novel Culture-Independent Method to Study the Host Range of Antimicrobial Resistance Genes in the Environment

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Natural environments, such as aquatic systems and different types of soils are the original sources of antimicrobial resistance (AMR) genes. However, anthropogenic activity, including the discharge of waste water effluents from the pharmaceutical industry or the spread of dried sludge for crop fertilization, can disrupt this natural gene pool with AMR gene pollution. It is essential to find the origins of this genetic pollution, as well as to characterize the bacteria harbouring them to assess the possible threat to human, and animal health.The vast majority of microbial organisms are uncultivable: it is estimated that only 1 % of all bacteria can be cultivated in vitro. The remaining 99 % is called the microbial dark matter. Culture-independent, molecular methods are needed to characterize microbial dark matter as well as to define 'who does what' in the microbial world. EpicPCR, short for Emulsion, Paired Isolation and Concatenation PCR, is a novel emulsion-based method, which can be used to answer the question of 'who does what' in a microbial community. In epicPCR a culture-free, emulsion-based fusion PCR -technique is used to link phylogenetical markers, such as 16S ribosomal RNA gene, to functional target genes, such as antimicrobial resistance genes. EpicPCR has been successfully used to characterize the hosts of different antimicrobial resistance genes in human impacted environments, for example waste water treatment plants and agricultural soils. However, there is still room for optimization and new applications. My aim is to present an overview of different areas of research epicPCR can be used in, focusing in soils. I will also cover the advances made in the optimization of the technique in the last few years and present the future waypoints and possible applications of the technique.

2.09P.9

Impact of manure application on the enrichment of ARGs in agricultural soils: longitudinal study over two vegetation periods and natural resilience of the soil

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Antibiotic resistance (AR) represents one of the major threat of the 21st century, with an estimated of 10 million deaths each year followed by a damage economical loss by 2050. The rapid dissemination of AR in natural environments is directly related with the antibiotics consumption in both human and veterinary medicine. In the past years, veterinary medicine gained attention due to the worldwide increasing trend of AR in animal feedlots. Animal manure contains antibiotic resistant bacteria, extracellular DNA that may carry different antibiotic resistance genes (ARGs) as well as antibiotic residues, heavy metals that may pose a selection pressure. Long-term application of manure, a common practice for soil amendment, represents a particular concern as potential cause of AR spread in natural environments. The aim of this study is to determine the impact of animal manure application on the resilience capacity of the agricultural soil and its influence on the soil microbial community. A longitudinal study over two vegetation periods was carried out in an experimental field site. The agricultural catchment has never been irrigated (it is a rain fed area) and the amended agricultural soil receives animal manure from a small local farm situated in the catchment. The total DNA extraction was performed from the collected soil samples as well as from the animal manure samples. A total of seven ARGs belonging to 6 different classes of antibiotics and 16S rRNA gene were analysed using the TaqMan qPCR technique. Next generation sequencing was performed in order to identify how different agricultural practices affect the soil bacterial community. Our study highlightes that manure application boosted the absolute abundance of different ARGs in agricultural soil, but it emphasizes the natural soil resilience capacity that plays a key role in the dissemination of AR in manured agricultural fields. Among the 7 ARGs tested, tetracycline and sulfamethoxazole resistance genes were the most abundant in all soil samples, as well as in the animal manure samples. The gradual decreased of the ARGs concentrations and its capacity to reach back the baseline level in a few weeks, suggests a natural good resilience of the agricultural soil. Our findings provides evidence that the naturally occurring background concentrations of different ARGs remain stable over the study period, but in contrast the bacterial community is shaped by different agricultural management practices.

2.09P.10

Innovative solutions to extend selectivity and sensitivity of VOC analysis in soils.

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The ability for an agricultural soil to function and sustainably provide an increasing food supply for a soaring global population has become of vital worldwide importance. Several methods have been suggested to determine "Soil Health"; soil physio-chemical measurements, biological methods and metabolomic approaches. To date, none of these methods adequality indicated soil health. One method under investigation is a volatile organic compound (VOC) profile or fingerprint. VOCs in soils stem from a large variety of different sources;

microbial, fungal, animal- and plant-derived. These volatilomes are vital to plant/fungi-microbe and animal/human-microbe interactions and therefore offer a reactive diagnostic tool to determine soil health by investigating the interspecies interactions. The standard methodology for VOC determination has been solid phase microextraction (SPME). This automated extraction method allows the monitoring of the community structure, physiological state, and activity of any microbial community in a soil without the need of manual extraction or cultivation procedures. Other common techniques that could be used to monitor the VOC fingerprints from soils; static headspace, high capacity sorptive extraction (either headspace or immersive sampling) or thermal desorption using sorbent-packed tubes for active or passive, in situ sampling of soil gas. Combining each of these techniques with an innovative cryogen-free focussing and pre-concentration trap has two main advantages: All extraction techniques can run on a single platform with the need to change the hardware.Single and multiple extractions to be carried out automatically on a single sample to increase the analytical sensitivity, thus achieving a comprehensive VOC profile. To illustrate the enhanced capabilities of the platform we investigate the fingerprints of VOCs for a soil from a loamy and clayey floodplain soils with naturally high groundwater close to Cardiff, UK using a variety of different VOC sample preparation methods showing the benefits of adding high-performance trapping technology to the VOC analysis of soils.

2.09P.12

Identification of copper transport pathways in the isopod Oniscus asellus and their response to copper exposure.

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Oniscus asellus is a species of terrestrial isopod that use the oxygen-carrying cupro-protein haemocyanin. The organ responsible for synthesis of hemocyanin, the hepatopancreas, has been observed to contain the highest soft tissue copper concentration of any recorded terrestrial organism. It has been reported these organisms sequester and store large quantities of copper, greatly surplus to their respiratory demands. However, the mechanisms behind copper transport by this isopod species have been so far uncharacterised. We aim to provide the first insight into these organisms' copper importers, exporters and chaperones. Known eukaryotic copper transport genes were aligned to a previously generated transcriptome to identify possible *O. asellus* orthologous genes. The sequences identified were then analysed based on their phylogenetic and encoded domains. Taken together these results predict the presence of a functional Steap membranous copper reductase and two functional Cu import proteins Nramp and COPT1. A gene for the Ccs Cu chaperone was also identified and demonstrated to be actively transcribed in isopod hepatopancreatic cells. However, the identified Ccs does not contain the heavy metal associated domain needed to bind and transport copper. Thus, is likely a pseudogene or only involved in activation of superoxide dismutase proteins to aid protection from reactive oxygen species. Finally, RT-qPCR analysis on hepatopancreatic tissue was performed to assess transcriptional response of the identified genes to copper exposure. This suggested a possible but statistically insignificant ($p>0.05$) downregulation of Cu uptake genes to protect cells from copper toxicity. Overall, this is the first study to characterise copper uptake and transport genes in the species *O. asellus*, predicting proteins involved in their copper transport system. However, the mechanism behind this species Cu transport gene regulation and protection from copper toxicity remains unclear. This project was supported by a MSC COFUND Fellowship (H2020-COFUND-SIRCIW>MINT-512202) through Cardiff University, Welsh Government and the EU.

2.09P.13

Sustainable food production: how can we bring circularity to agriculture?

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Agricultural practices determine the level of food production, which are becoming more intensive and unsustainable due to current global food demands. Promoting sustainable soil management is of utmost importance for improving its quality and increasing food production while avoiding environmental degradation. Within the framework of sustainable development goals, where planet protection and social, economic, and environmental sustainability are required, new technologies are being explored and applied to the development of novel agroproducts, alternative food sources, and enhancing efficiency in resource use. Insects have a high content of nutrients and protein, and their use as feed and food has valuable environmental advantages over conventional protein sources, producing newly nutritional sources of food with low environmental impact. If coupled with innovative waste management strategies, this novel food source could be a solution for simultaneously addressing the increasing demand for food while promoting circularity in agriculture and economy, where all the products and sub-products are re-used with the aim of a zero-waste policy. Within the concept of circular agriculture and economy, nutritional waste is re-use as fed for insect larvae (e.g., black soldier fly) to convert organic waste, by digesting vegetable by-products, into organic fertilizers; before hatch, larvae are then used for animal nutrition. Therefore, the main goals of this study were to assess this "by-product" fertilizer efficacy in plant's productivity and also to understand the possible transfer/accumulation of metals, veterinary drugs, mycotoxins, and polycyclic aromatic compounds in edible insect species through bioaccumulation studies. Food safety and environmental sustainability of food production are two key issues that must be committed, and as every novel food source/technology, the risks associated with food security need to be studied. The obtained data from this study would be then used to help the urgent need to establish new legislation for larvae farming, as advised by the European Food Safety Authority and contribute to the optimization of these "by-products".

2.09P.15

A combined field and laboratory approach to assess pesticide effects on soil invertebrates

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A recent inventory by Silva et al. (2019) showed the presence of residues of several pesticides in agricultural soils from all over Europe. A similar inventory in France showed that soils from conventional agriculture contained more and also higher levels of pesticide residues than soils from organic farming. Although in general residue levels were fairly low, with few exceptions, in all soils residues were present of several pesticides and/or their metabolites. This triggered the question of the potential ecotoxicological hazard of the mixture of pesticide residues. To answer this question, soils were sampled from a conventional agricultural farm and a corresponding organic farm in two regions (Mons, Le Cage) in France. The soils were analyzed for pesticide residue levels and tested for their toxicity to springtails (*Folsomia candida*), potworms (*Enchytraeus crypticus*), and earthworms (*Eisenia andrei*, *Lumbricus rubellus* and *Aporrectodea caliginosa*). Lufa 2.2 soil was included as an additional control to test for the health of the test organisms. Tests followed existing or modified OECD test guidelines and focused on effects on survival and reproduction after 3-4 weeks of exposure. For both regions, the soil from conventional agriculture was toxic to all test organisms. A strong reduction of springtail survival and reproduction was seen on the soils from agricultural farming compared to organic farming or Lufa 2.2 soils. A similar but slightly less severe response was seen for the enchytraeids. The earthworm *L. rubellus* showed high mortality on the soils from conventional agriculture and large body mass loss on all field soils. The earthworm *E. andrei* did survive exposure in all soils but showed considerable mass loss and strongly reduced reproduction on the soils from organic agriculture. The tests with *A. aporrectodea* are still running. The high toxicity of one of the soils could partly be explained from a high level of imidacloprid (60 ug/kg), but toxicity of the other soil could not. These findings suggest that toxicity of the soils is due to the mixture of pesticide residues, and also that the quality of these agricultural soils is threatened. Further research into the cause of toxicity is needed.

Soil Function and Biodiversity: Impacts and Resilience under Stressed Environments (P)

2.10P.1

Synergistic interaction between effects of phenanthrene and dynamic heat cycles on Folsomia candida

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The frequency and intensity of extreme heat events are going to increase in the next century and they should be taken into account in risk assessment procedures. In order to understand the combined effects of PAHs and heat events to soil arthropods, we exposed *Folsomia candida* to combined phenanthrene (PHE) and dynamic heat cycles (20-36 °C in 4 h) in a full factorial experiment. In the present study, we studied the population growth of *F. candida* under combined a range of sub-lethal concentrations of PHE and a vary number of dynamic heat cycles (0-5 cycles). We found that heat cycles had a negatively significant effect on adult survival but had no significant interaction between PHE and dynamic heat cycles on adult survival. Furthermore, the content of PHE in adult tissue increased significantly with increasing nominal concentration in the soil and no significant effect of dynamic heat cycles was found. In addition, a synergetic interaction existed between the effects of PHE and dynamic heat cycles on both adult body mass and juvenile production of *F. candida*. These results showed the negative effects of PHE were intensified when animals were heat stressed, and/or vice versa. We discussed the physical damages of *F. candida* under PHE contamination and/or heat stress. This study highlighted that including realistic heat events in ecotoxicological studies can improve the current risk assessment of chemicals in the environment.

2.10P.2

Effects of different climate factors on target genes expression and enzymatic biomarkers in Enchytraeus crypticus exposed to metal soil contamination

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The Intergovernmental Panel on Climate Change (IPCC) predicts major changes in climate conditions throughout the 21st century (e.g., rising atmospheric CO2 concentration and air temperature, alterations in precipitation patterns, higher exposure to ultraviolet radiation). These climate alterations may intensify the hazard of metal contamination to soil invertebrates and, thus, affect the functionality and sustainability of contaminated terrestrial ecosystems. Previous studies have demonstrated that water stress situations may compromise the capacity of soil invertebrates to avoid metal contamination, and that increasing air temperature and soil dryness may affect the survival and reproduction of soil invertebrates in metal-contaminated soils. To better understand these changes at invertebrates' population level, it is necessary to look at the possible effects at sub-organism level (i.e., genetic and biochemical alterations). In this sense, the aim of the present study was to evaluate the alterations at genetic and biochemical level of different climate factors (atmospheric CO2 concentration, air temperature, soil moisture content, and ultraviolet radiation –UVR–) on invertebrates exposed to metal soil contamination. The soil invertebrate species Enchytraeus crypticus (phylum Annelida, class Oligochaeta, family Enchytraeidae) was exposed for 48 hours to a metal-contaminated soil from central-northern Portugal under different climate conditions, based on the IPCC predictions for the year 2100 for southern Europe: 1) 20 ºC + 50% soil water holding capacity –WHC– (climate conditions recommended by standardized ISO and OECD guidelines); 2) 20 ºC + 50% soil WHC + 600 ppm atm. CO2; 3) 20 ºC + 50% soil WHC + 800 ppm atm. CO2; 4) 20 ºC + 50% soil WHC + 1000 ppm atm. CO2; 5) 15–25 ºC + 50% soil WHC; 6) 20–30 ºC + 50% soil WHC; 7) 20 ºC + 75% soil WHC; 8) 20 ºC + 25% soil WHC; 9) 20 ºC + 50% soil WHC + UVR exposure. Lufa 2.2 soil was used as control soil. Then, a set of molecular (expression of target genes related to stress conditions: heat shock protein Hsp70, metallothionein MT1, and catalase cat1) and biochemical (enzymatic biomarkers related to stress conditions: activity of the enzymes catalase, glutathione-S-transferase and acetylcholinesterase and of the electron transport system, and levels of lipid peroxidation) analyses were performed in the surviving organisms.

2.10P.3

Does habitat quality influence bioenergetics and reproduction of Oppia nitens in response to cadmium-induced stress?

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Soil invertebrates interact with their habitat to provide services that are vital for the soil ecosystem. In this study, we evaluated the influence of habitat quality on the biological (reproduction) and physiological (bioenergetics) fitness of the oribatid mite, *Oppia nitens* in response to cadmium-induced toxicity. Cadmium reduced the carbohydrate reserve of mites without a change in lipid and protein reserve. Cadmium reduces energy production at high Cd concentration (700 mg Cd kg^{-1}) by disrupting the activities of glucose metabolism enzymes, glucose-6-phosphate dehydrogenase (G6PDH) and pyruvate kinase (PK) with estimated EC50 of 50.7 and 21.7 mg Cd kg^{-1} for PK and G6PDH respectively. Habitat quality (good and poor habitat) influences mites' reproduction (i.e., juvenile production), but did not directly influence bioenergetics. Our results suggest that cadmium reduces energy production in *O. nitens*, and habitat quality could modulate energy metabolic activities that affects the biological fitness of *O. nitens*. In addition, the effect of habitat quality is significant than metal concentration on the biological fitness of *O. nitens*. Hence, habitat characterization of contaminated sites improves the relevance of ecological risk assessment, since the quality of the habitat can affect the biological fitness of soil invertebrates, which ultimately is the cause of population decline.

2.10P.4

Investigating the impact of insecticides on soil mesofauna abundance and biodiversity and its impacts on organic matter break down in arable fields

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During a six-month field experiment we investigated the impact of two insecticides (Lindane and Methamidophos) on soil mesofauna (Acari and Collembola) abundance, biodiversity and their impact on organic matter degradation in arable fields. Our results show that both insecticides had strong effects on Collembola and Acari abundances over the entire study duration by reducing their populations by up to 80%. In comparison the effects of insecticide exposure on mesofauna biodiversity were less pronounced and more complex. We document insecticide dependent temporal fluctuations (both reductions and increase) of different estimates (indices) of biodiversity over time and with the majority of treatments having no lasting impact after six months. When looking at the effects of both mesofauna abundance and biodiversity on three different measurements of organic matter breakdown (minicontainer with lucerne and straw and bait lamina strips) we find no evidence that either of them had a measurable impact. These results suggest that organic matter breakdown is likely driven by other trophic levels (e g. microorganisms or earthworms) with only limited contributions of the mesofauna community. These findings should be discussed in the context of the current understanding of soil food web function and its implications for future European soil risk assessments, in particular the definition of specific in-field protection goals. **This poster aims** to present a in depth field study linking mesofauna abundance and diversity to functional endpoints in order to inform the ongoing discussion about soil protection goals.

2.10P.5

Gut and faecal microbiota of the terrestrial isopod Porcellionides pruinosus: potential use for monitoring exposure scenarios

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This study aimed to characterize the gut and faecal microbiota of *Porcellionides pruinosus* using high-throughput sequencing. A similar experimental design to those of laboratorial tests for exposure scenarios (e.g. ecotoxicological tests) was used to serve as basis for microbial community analysis in a multi-level approach. Faeces and purged guts of isopods (n= 3 x 30) were analysed by pyrosequencing the V3-V4 region of 16S rRNA encoding gene. Results showed that gut and faecal microbial communities were dominated by Proteobacteria, particularly by an OTU (Operational Taxonomic Unit) affiliated to genus *Coxiella*. Diversity and richness values were higher for faecal microbiota, mainly due to the occurrence of several low-abundance phylotypes. Although differences were not statistically significant, these results may reflect faecal carriage of bacterial groups that cannot settle in the gut. Microbiota associated to *P. pruinosus* comprised: (1) common members of the soil microbiota, (2) bacterial symbionts, (3) bacteria related to host metabolic/ecological features, and (4) bacterial etiological agents. Comparison to other invertebrates, revealed common bacterial groups across taxa. The information provided by the analysis of *P. pruinosus* microbiota is of value as an additional indicator in future ecotoxicological assays but also as biomonitoring tool for several exposure scenarios (e.g. detection of cattle diseases).

2.10P.6

Development Of A Tiered Approach For Ecotoxicological Risk Assessment Of Abandoned Landfills And Dump Sites

L. Jansen, M. Vangheluwe, Arche consulting; T. Behets, OVAM; B. Meyns, Sertius

Licensed landfills in Flanders are highly regulated and monitored. Nevertheless, older and often unofficial dump sites or abandoned landfills exist which were, after use, only covered with a layer of soil and vegetation. As these sites are often located in ecologically vulnerable areas, it is important to characterize the contamination and identify any risks related to the ecosystem. As part of a global evaluation, which includes also toxicological risk assessment and risk for spreading of the contamination, there is a requirement to assess the potential ecotoxicological impact of these sites to evaluate whether or not remediation is required in order to protect or restore the ecosystem. A tiered approach has been developed as the basis for a standardized and unambiguous procedure to identify the need for soil remediation, which can be applied by policy-makers to come to an objective conclusion. This approach, which is focused on the soil compartment, is to be applied to landfills in which the concentration of one or more contaminants exceeds a pre-defined, compound-specific threshold value, pointing to a potential ecotoxicological risk.In successive tiers, the ecotoxicological risk is evaluated, starting with general properties of the site such as the size of the contaminated area and the current land-use, to more advanced assessment methods in higher tiers. These imply the calculation of the potentially affected fraction (PAF), taking into account bio-availability of contaminants, or ecotoxicological testing of site-specific samples and inventory of the local biota (TRIADE). The conclusion of each step can either be that no unacceptable risk is present, in which

case no further action is required, or that an unacceptable risk to the ecosystem cannot be excluded and that higher tier analysis is required. At each step, the accuracy and level of detail of the evaluation increases, while on the other hand the complexity and cost for further analysis can increase. In a last step, the urgency for remediation based on soil contamination is estimated.This tiered approach, which was developed on request and with the support of OVAM, can serve as a solid backbone for the evaluation of the ecotoxicological risk of historic landfills, but can also be extended to contaminated sites in general. The proposed approach has been tested on some case studies for which results will also be presented.

2.10P.7

Biological activity of metal mine tailing soils under different scenarios of climate change

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Current climate change predictions estimate that atmospheric CO_2 concentrations will continue to rise throughout the 21st century as well as global air temperature. Soils will be among the most affected components of terrestrial ecosystems, especially in the Mediterranean region. Their response against such changes is crucial for assessing how terrestrial ecosystems may adapt to global warming. This adaptation capacity (resilience) depends on the soil status, the soils from degraded areas being less resilient. Among them are the soils affected by metal mining wastes that normally show hostile conditions for soil biota (e.g. extreme pH, salinity, high metal levels, organic matter and nutrients deficiency). This study aimed to assess the effects of different climate scenarios on the biological activity of metal mine tailing soils related to different stages of colonization by native vegetation under Mediterranean climate. Four environments were selected in an abandoned metal mining area (SE Spain): bare soils (S); small groups of pine trees (2-5) ≤≈2.5 m high growing scattered (P); dense patches with several pine trees (>5) >≈4 m high and shrubs and herbs under the canopy (DP+MS); non-contaminated control forest with pine trees >5 m high and shrubs and herbs under the canopy (CF). Soil cores were taken from each environment in spring 2019 and subjected for 17 days to different climate scenarios based on the IPCC projections for the year 2100: 400 ppm atm. CO_2 + 15-20 °C (climate scenario representing current spring field conditions); 400 ppm atm. CO_2 + 20-25 °C; 1000 ppm atm. CO_2 + 15-20 °C; 1000 ppm atm. CO_2 + 20-25 °C (worst case scenario). Soil physico-chemical (pH, salinity, dissolved organic C and N, soluble metals) and microbiological (microbial biomass carbon –MBC–, β-glucosidase activity) parameters and mesofauna feeding activity (bait lamina sticks) were evaluated at the end of the exposure period. The soils from the contaminated environments with plant cover (P and DP+MS) showed better response to rising atmospheric CO_2 concentration and air temperature than the contaminated bare soils (S) in terms of MBC and β-glucosidase activity. This was more evident for the environment with higher vegetation complexity (DP+MS) that showed closer or even higher values than the soil from the control environment (CF). Soil mesofauna feeding activity was only detected in the CF environment, with lower values after exposure to high atmospheric CO_2 concentration.

Advancing Use of Behavioural Studies in Regulatory Assessment of Contaminants (P)

2.11P.1

Are behavioural effects of a synthetic oestrogen mediated by brain size in female fish?

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Contamination of the environment with pharmaceutical residues is cause for major concern. This is because pharmaceuticals are specifically designed to have biological effects at low doses, can be highly persistent in the environment, can act on drug targets and physiological pathways that are highly evolutionarily conserved across diverse taxa, and can bioaccumulate in organisms and biomagnify in food chains. What is more, various pharmaceuticals have particularly high rates of bioaccumulation in the brain tissue of exposed wildlife. However, despite the immediate relevance and possible widespread implications for wildlife inhabiting polluted environments, no research to date has considered the potential for one key trait to mediate impacts of pharmaceutical contaminant exposure: brain size. This is surprising given that brain size is associated with a wide array of traits and behaviours that are directly linked with fitness, including mate choice and reproductive behaviour. To remedy this, we tested the hypothesis that relative brain size mediates impacts of exposure to the potent synthetic oestrogen and pervasive pharmaceutical contaminant 17α-ethinyloestradiol (EE2) on reproductive behaviour in female fish. We utilised long-term (seventh generation) selection lines for ‘large’ and ‘small’ brain size in a live-bearing fish, the guppy (*Poecilia reticulata*), where lines differ by 15% in brain size relative to body size. Female guppies from each brain size class were exposed to one of two environmentally relevant concentrations of EE2 (nominal levels: 1 and 5 ng/L) or a freshwater control for 14 days before being tested for mating behaviour and reproductive vigour using a dichotomous choice test, where focal females were allowed to associate with either a stimulus male or a stimulus female (both unexposed). We measured the duration and frequency of reproductive behaviours performed by the focal female, with each individual being tested three times over one week to also investigate potential impacts of brain size and/or EE2 exposure on within- and among-individual behavioural variation. The results of these reproductive behaviour trials will be presented with reference to the broader ecological and evolutionary consequences of pharmaceutical pollution.

2.11P.3

Analysis of inter-individual variation in locomotor activity of larval zebrafish to explore toxicological response pathways

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The increasing occurrence of chemical contaminants in the aquatic ecosystem have become a major environmental concern, but many of the mechanisms and molecular targets of these chemicals are still unknown. This adds complications when it comes to predicting the impact of such contaminants on the aquatic biota and ecosystem. The aim of this study is to establish a new approach to elucidate molecular mechanisms of toxicity by taking advantage of inter-individual differences in sensitivity towards a given contaminant. In our project, behavioural measures are used to sort chemically exposed individual zebrafish larvae based on their sensitivity. Zebrafish locomotor behaviour is widely used as a read-out for the assessment of external challenges to the nervous system, mainly because of its use as a non-invasive, cost- and time-effective high throughput testing method. However, larval zebrafish behaviour is highly variable and difficult to predict, specifically at the individual level. Therefore, initially it was essential to determine whether inter-individual differences in unexposed larvae are stable and whether intra-individual consistency of locomotor behaviours emerges during the development. So we analyzed individual locomotor activity from 5 to 7 days post fertilization (dpf) in a standard well-plate to determine the most stable and predictable periods of individual locomotor behaviour. Our results show that variability in locomotor activity is lowest when fish are exposed to dark phases and that this behaviour of an individual, although still under maturation, becomes stable over 6 to 7dpf. Using this information, we carried out chemical exposures, where larvae have been exposed to non-toxic concentrations of chemicals which cause effects to their behavior. These larvae have been sorted into tolerant and susceptible populations based on the changes in their behaviour. Individuals of the different sensitivity categories will then be subjected to transcriptome analysis to explore in more detail the molecular mechanisms that underpin their differences in sensitivity to the specific chemical. In addition, differentially regulated genes might hint to molecular targets involved in the chemical response pathways. Overall, this project will help to elucidate toxicological response pathways at the molecular level, providing essential basis for proper risk assessment of contaminants in the aquatic environment.

Impacts on Agroecosystems Resulting from Chemical Exposure in the Anthropocene (P)

2.12P.1

Breaking the Cycle of Pesticide Use in Agriculture: A Conceptual Framework Focused on Risk Management

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Concerns over the real or perceived environmental impacts of neonicotinoid insecticides have spurred intense public and scientific debate on the management of pesticides in agricultural landscapes. Banning efficacious pesticides like the neonicotinoid insecticides, an important tool for farmers to ensure high quality agricultural products, is not a viable solution, but the current practice of prophylactic application is not environmentally sustainable and has led to routine detections in the environment, occasionally at concentrations that exceed risk thresholds for aquatic and terrestrial non-target organisms. Many are quick to blame pesticides but teasing apart direct effects attributable to them from other factors (e.g., habitat loss; landscape homogenization; climate change) known to explain some of the observed impacts is difficult. Banning will inevitably lead to the introduction of new compounds or a resurrection of old compounds which, in the absence of a more structured approach to pesticide management, will only lead to a perpetuation of the "pesticide cycle" following introduction to the market: Ramping up use, greater frequency of occurrence in the environment, possible exceedances of risk thresholds, and the inevitable public outcry for bans. In this presentation, I outline a conceptual framework focused on risk management rather than risk assessment of pesticides, utilizing a number of approaches, some of which are already practiced, that I believe can be used to develop a more structured and rationale approach to the use and management of pesticides in agriculture. Aspects considered include: Adoption of "use as needed" programs (e.g., the Ontario model) to reduce pesticide use; greater application of universal beneficial management and technology practices (BM/TPs); greater incorporation of IPM principles; promoting ecologically diverse landscapes (e.g., edge-of-field habitat; landscape design); and targeted monitoring programs.

2.12P.2
Cumulative Hazard and Risk Assessment of Pesticides from agricultural activities
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Pesticides from agricultural activities inevitably end up in the aquatic environment, where assemblies of these pest-combating compounds may affect non-target species. Although the Mode of Action (MoA) and toxicity of these compounds are often well documented due to the rigorous regulation of pesticides, the knowledge of how multi-component mixtures of such biologically active (toxic) compounds cause combined toxicity and characterisation of the total risk these mixtures represent under ecologically relevant exposure scenarios are still limited. Efforts to develop computational approaches for cumulative hazard (CHA) and risk (CRA) assessment and implementing such approaches for spatio-temporal studies of relevant agroecosystems are thus highly warranted. The present approach demonstrates how a combination of exposure information from monitoring studies and effect data from various sources can be used to characterise risk hot spots, identify susceptible species groups (taxa), identify site-specific risk drivers, and provide mechanistic information to inform further assessment of complex multi-compound exposures. It uses data from five years of data from pesticide monitoring in small streams in agriculturally dominated catchments in Norway, and takes advantage of recent developments in computational toxicology. The approach provides a standardised way to perform CRA and characterise the hazard potential of complex mixtures of pesticides (and other chemicals) to target the most relevant combinations of MoAs and adversity. The approach is anticipated to assist environmental risk assessment and management processes, aid biological monitoring efforts and improve the linkage between laboratory and field-based initiatives. *Acknowledgements:* Financing - Norwegian Agricultural and Environmental Monitoring Program, National Action plan for reduced risks from pesticide use (2010-2014), RCN 268294 "Cumulative hazard and risk assessment of complex mixtures and multiple stressors (MixRisk)" and NCTP: NIVA's Computational Toxicology Program, NCTP (www.niva.no/nctp).

2.12P.3
Influence of soil organic amendments on carbon based metabolites in rosemary (Rosmarinus officinalis L.) plants
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Rosemary (*Rosmarinus officinalis* L., Lamiaceae) is an aromatic shrub native from Mediterranean regions, grown as a common herb around the world. This species constitutes an exceptional source of different bioactive compounds, among them, terpenoids, with proved antimicrobial and antioxidant properties. Furthermore, different studies have shown the potential and important role that this shrub can also have in the rehabilitation of degraded soils, contributing to the reduction of erosion by improving soil quality. Because the quality of rosemary leaves depends on the amount and type of chemical elements taken up from soil, different soil fertilization strategies, used to rehabilitate degraded soils in the Mediterranean area, may affect the nutritional status of rosemary leaves, in terms of metabolites. Indeed, different studies have shown the effect of fertilization on the metabolism of terpenoids in plants. Also, plant nutrient balance in the soil could influence the production of primary metabolites, such fatty acids and polysaccharides as cellulose and hemicellulose. In this study, two composts (CM, mainly composed by cattle manure anaerobic digestate and CS, mainly composed by pig slurry anaerobic digestate), at two different rates (30 t/ha and 60 t/ha respectively) were incorporated into a semiarid soil. These organic amendments were compared with the soil without amendment (control, B) and an inorganic treatment (I). Subsequently, rosemary plants were planted on these soils. The aim of this study was, to evaluate not only the efficiency of the treatments as well as to evaluate the nutritional status of rosemary leaves, in terms of primary and secondary metabolites. The results obtained have shown that the incorporation of the organic amendments into the semiarid soil did not alter the concentrations of terpenoid compounds in rosemary plants. However, we observed a decrease in leaf fatty acid contents and an increase in leaf saccharides (from cellulose and hemicellulose hydrolysis) in plants grown on CM-amended soils at the high dose and on CS-amended soils at both doses with regard to plants grown on control and inorganic-fertilized soils. These results may indicate that organic fertilisation increases growth, including synthesis of structural carbohydrates but also leads to decreased concentrations of other carbon-based metabolites, primary as fatty acids and secondary as terpenoids.

2.12P.4
Nickel toxicity to Brasica napus cultivars under optimal and deficiency of sulphur availability
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Increased levels of heavy metals are an important problem of environmental pollution. Ni is one of the metals essential for normal plant development but elevated levels usually cause deleterious effects on plant growth. The aim of the study was to evaluate effects of Ni toxicity on growth, biochemical parameters and accumulation under optimal concentration or under deficiency of sulphur as an available nutrient. Two different winter oilseed rape (Brassica napus L.) cultivars – Hammer and Compass were chosen for the experiment. Plants were grown under sulphur (S) deficiency and under optimal S availability. Plants were exposed to 0.1, 0.3 and 0.5 mM Ni concentrations for 3 weeks. Under the S deficiency conditions biomass was lower than rapes grown under optimal sulphur conditions. Statistically significant lower chlorophylls content was found in Hammer rape grown in S deficiency condition, compared with those grown in optimal S conditions. Significantly higher malondialhedyhe (MDA) concentrations were found in both cultivars grown under sulphur deficiency conditions under the Ni treatment. Compared with optimal S conditions, significantly higher Ni concentrations were determined under sulphur deficiency conditions in Hammer roots, Compass shoots in all case of Ni exposure.

2.12P.5
Transfer of contaminants of emerging concern (from irrigation water to crops in Saudi Arabia)
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Contaminants of emerging concern (CECs) presents in irrigation water can be uptaken and translocated by crops. However, preliminar studies under controlled field conditions were performed at concentrations higher than that reported in the environment [1]. The objective of this study was aimed to assess the presence of emerging pollutants in soils and crops irrigated with treated wastewater in Saudi Arabia to evaluate CECs plant uptake under environmental conditions [2,3]. A triple quadrupole time-of-flight mass spectrometer (QqTOF), the Triple-TOF (ABSciex, Darmstadt, Germany) was used. This instrument provides precursor ion fragmentation with high sensitivity and selectivity. In this 'suspect screening', compounds of interest are not previously selected. Instead, a database containing information of a high number of them is used to identify potential substances present in the sample. The MS/MS spectra of every compound is analysed to discriminate the compounds detected. The study included suspected screening analysis of water sample for irrigation taken in four different sites after the South Riyadh wastewater treatment plant. In addition, soil and crops growing in the surroundings were also analysed. Numerous emerging contaminants were found in water, soil and crop samples. These were for instance phenols, antioxidants, and plasticizers of the phthalate-type. Also a fibrates compound (Gemfibrozil, CAS: 25812-30-0) was frequently found in all sample types and in high concentrations, Gemfibrozil is a drug used to lower lipid levels. This compound increases the activity of extrahepatic lipoprotein lipase, by activating Peroxisome that is involved in metabolism of carbohydrates and fats. To our best knowledge it has not been detected before in plant samples. The detection of this emerging pollutants provides global information about the possible risk for the environment in this area of Saudi Arabia and others with similar conditions that are using treated waste water for irrigation. Acknowledgements The financial support from the Distinguished Scientist Fellowship Program (DSFP) from King Saud University, Saudi Arabia is gratefully acknowledged. R. Álvarez also acknowledges the Spanish Ministry of Economy, Competitiveness and Innovation

for his FPI grant BES-2016-078612. References [1] Y. Picó, A. Alfarham, D. Barceló. TrAC Trends Anal. Chem., 94 (2017) 173-189. [2] M.J. Redondo, V. Andreu, Y. Picó. TrAC Trends Anal. Chem., 94 (2017) 21-38 [3] Y. Picó, R. Alvarez-Ruiz, L. Wijaya, A.H. Alfarhan, M. Alyemeni, D. Barceló. Anal. Bioanal. Chem. 410 (2018) 1163–1176

A Risky Life History: Contaminant Threats to Scavenging Wildlife (P)

2.13P.1

Derivation of indicators for assessing the quality of biota samples and their suitability for environmental monitoring studies

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The aim of the EU-funded LIFE Apex project is to foster and improve the systematic use of chemical monitoring data from apex predators and prey (AP&P) samples for regulatory purposes. In the context of chemicals' management, monitoring data from appropriate biota samples could prove the presence of chemicals in the environment and support the selection of most relevant substances for further hazard assessment. AP&P samples for such investigations are already available in Environmental Specimen Banks (ESBs), Natural History Museums (NHMs) and Research Collections (RCs). These organisations apply different sampling approaches (systematic or opportunistic) and sample storage conditions. However, for a regulatory usage of monitoring data it is important that certain important specimen data are available and basic quality assurance aspects have been considered. To this end a survey among ESBs, NHMs and RCs on the application of guidance documents and quality assurance measures for sampling, processing and archiving of AP&P samples was conducted in 2019. The survey evaluation revealed great differences regarding applied quality assurance measures between participating organisations. Basic information on archived specimens are mostly available (e.g., location, sampling date, biometric data). The temperature for archiving mostly is -20°C. Only one RC and two ESBs in the survey are using -150°C storage in the inert gas phase of evaporating liquid nitrogen. Some institutions collect samples specifically for contaminant monitoring purposes and sample treatment is conducted by trained experts for chemical monitoring, often following specific guidance documents. Organisations archiving samples for other purposes are sometimes less aware of considerations regarding contamination aspects. Based on the survey answers and discussions in the LIFE Apex project team, indicators were derived to allow an assessment of the suitability of biota samples for environmental monitoring investigations. Important indicators for suitable samples are the availability of, e.g., species name, date of sampling/finding, location where sampled/found, and sample descriptions (weight, size, sex, development stage). Further indicators cover information on handling procedures, archiving conditions and availability of supplementary sample information.Acknowledgement: We thank all organisations which participated in the survey. This project is funded by the EU LIFE programme (grant ENV/SK/000355).

2.13P.2

Mercury in feathers of the Andean condor (Vultur gryphus) from three areas of contrasting anthropogenic influence in Chile

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Mercury (Hg) is known to pose potential adverse health effects on both humans and wildlife. The bioaccumulation and biomagnification capability of Hg primarily impacts species located at the top of the food chain. Thus, foraging behavior influences the exposure and consequently the health status of individuals. The Andean condor (*Vultur gryphus*), as a long-lived scavenger, is expected to suffer high exposure to Hg in areas highly contaminated. Due to the lack of data on Andean condor, a study of mercury in feathers of scavengers from northern Patagonia (Argentina) proposed the use of other species, such as Turkey vultures (*Cathartes aura*), Black vultures (*Coragyps atratus*), and Southern crested caracaras (*Caracara plancus*), as surrogate species in distribution areas shared with endangered scavengers such as the Andean condor. In this study we report for the first time baseline concentrations of Hg in Andean condor feathers sampled at three locations of the Chilean territory, distributed across ~2,500km of latitude and characterized by different levels of anthropogenic pressure. Between winter 2017 and 2018, we collected molted flight feathers from 205 individuals of different age ranges. Hg was analyzed by atomic absorption spectrophotometry using a DMA-80 (Milestone, Italy). Feather Hg concentrations ranged from 0.02 to 9.06 mg/kg dry weight (dw). We found significant differences in feather Hg concentrations across zones, with highest concentrations in Andean condors from the Austral zone (0.4±1.2 mg/kg dw), intermediate values in the central zone (0.2±0.5 mg/kg dw) and the lowest values in the southern zone of Chile (0.07±0.05 mg/kg dw). Although the results were not significantly different, subadult had over twice as much Hg compared with adults and juveniles in the austral zone. We did not find high Hg concentrations in condors inhabiting the more heavily industrialized and urbanized areas of the country (central zone), but we did in condors inhabiting more austral and coastal environments (austral zone). The results showed that Hg values provide a good proxy for separating condor habitat use along a latitudinal gradient, which may correlate more with a gradient in Hg atmospheric transport and deposition than with local emissions. These results are the first findings of mercury in the Andean condor, and they are of great relevance in terms of population health and potential consequences for its conservation and management.

2.13P.3

Scavenging increases the risk of exposure to lead and other heavy metals in raptors.

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Among birds, raptors are especially suitable for monitoring persistent and bioaccumulative contaminants due to their position at the top of food chains; and they are able to integrate contaminant exposure both over time and relatively large spatial areas. On the other hand, the majority of the contaminants reach the bloodstream after their absorption in the digestive tract; and, therefore, the eating habits presumably will affect to the pollutant exposure. Finally, it is well known that blood lead level is able to offer information on recent exposure of interest in risk assessment. The objective of this study was to evaluate the influence of the scavenging in the exposure to lead and other heavy metals in raptors inhabiting a Mediterranean area of the Southern Europe (Spain). A total of 401 blood samples from 16 raptor species were analyzed. The samples were collected between 2003 and 2006 in Andalusia (Southern Spain). To interpret the results, the species were grouped according to their feeding habit in three groups: Obligate scavengers (Griffon vulture-*Gyps fulvus* n=236, Egyptian vulture-*Neophron percnopterus* n=26, Black vulture-*Aegypus monachus* n=21*,* Bearded vulture-*Gypaetus barbatus* n=3); Facultative scavengers (Black kite-*Milvus migrans* n=43, Spanish Imperial eagle-*Aquila adalberti* n=28, Golden eagle-*Aquila chrysaetos* n=4, Red kite-*Milvus milvus* n=2); and Non-scavengers (Bonelli's eagle-*Aquila fasciata* n=33, Eurasian sparrowhawk-*Accipiter nisus* n=15, Goshawk-*Accipiter gentilis* n=12, Montagu's harrier-*Circus pygargus* n=7, Eagle owl-*Bubo bubo* n=3, Booted eagle-*Aquila pennata* n=2*,* Barn owl-*Tyto alba* n=2*,* Eurasian buzzard-*Buteo buteo* n=1). Lead, cadmium, copper and zinc analyses on blood samples were performed by anodic striping voltammetry (ASV). Lead, cadmium and copper blood concentrations were higher in obligate scavengers; and excepting cadmium, the concentrations of the rest of the metals were similar among facultative scavengers and non-scavengers. Regarding lead 90% of the samples of non-scavengers had concentrations below the threshold related to significant subclinical effects (20 μg/dL). In the same sense, no raptors of the non-scavenger group had lead concentrations upper than intervention threshold (40 μg/dL). On the contrary, close to 13% of obligate scavengers had concentrations upper this critical threshold for intervention. These data suggest the need for greater monitoring and control to prevent lead effects on scavenger populations.

2.13P.4

Assessment of the exposure to environmental toxics in free-living European griffons (Gyps fulvus) and Cinereous vultures (Aegypius monachus) in the Balkans

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In some parts of Eastern Europe, vultures suffered a dramatic decline in their populations during the last century, fundamentally due to the intentional use of poisons to kill predators due to the conflicts with extensive livestock farming. Vulture species play a key ecosystem role and are valuable as biomonitoring sentinels for both human and environmental health. Currently, several reintroduction projects are being developed in the Balkan region, and it is necessary to know the potential exposure to some environmental contaminants that vultures may encounter in these reintroduction areas. The aim of this study is to determine organochlorine compounds, lead, cadmium, antibiotics and anti-inflammatory drug (NSAIDs) levels in blood of vultures from Bulgaria. Blood samples of European griffons (*Gyps fulvus*) (N=33) and cinereous vultures (*Aegypius monachus*) (N=45) were taken. The organochlorine compounds in blood samples were analysed by gas chromatography coupled to an electron capture detector. The analysis of heavy metals in blood samples was performed by atomic absorbance spectroscopy. The analysis of antibiotics and anti-inflammatory drugs were performed in blood samples by liquid chromatography coupled to a time-of-flight mass spectrometer. Organochlorine compounds were present in blood samples at very low levels. Antibiotics and NSAIDs were not detected in none of the analysed blood samples. Cadmium levels in blood were low. Lead levels were elevated in some individuals, although these levels were below those compatible with clinical poisoning in birds. It is remarkable that griffon vultures showed markedly higher levels or organochlorine compounds and lead in blood than the cinereous vultures. It must be also highlighted the absence of antibiotics and the relatively low exposure to lead compared with other monitoring studies performed in Spanish vultures.

2.13P.5
Lead exposure in Bearded Vultures (Gypaetus barbatus) : risk analysis for a reintroduced population in the French Alps
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The Bearded Vulture has been reintroduced in the Alps, thanks to national and European action plans since 1987. It is still in critical danger of extinction in the Alps and its population dynamics make it very vulnerable to lead exposure or poisoning. Most lead exposure cases are related to the ingestion of lead from alimentary sources (game species with embedded lead shots) in birds of prey around the world, but also in the Bearded vulture in the Alps (Margalida, 2015). Therefore, a survey was designed to estimate the risk of lead exposure from hunting ammunitions in GB in the pilot area of High-Savoy in the French Alps. The survey included information on the presence of GB (observations reported by bird watchers) and a semi-quantitative estimate of the observation frequency. A sensitivity analysis was also conducted to define the most likely areas of feeding of GB (altitude, open spaces, accessibility or not for hunters). A questionnaire was sent to all hunting groups and companies, available online on the web site of the hunting federation of High-Savoie (FDC74). A total of 112 forms were completed and analyzed. Means were computed with 95% confidence intervals.The questionnaire investigated the species hunted, the number of animals killed, in different ecosystems or altitudes. The period of the year for hunting was also examined, together with the type of hunting and predominant shooting place, the number of failed shots. The results show that large game species (deer, roe deer, chamois, wild boar) are commonly hunted in places where GB feed and live. The proportion of animals surviving shooting varies (10-35%), viscera are normally buried locally, but not systematically. Hunting is maximal when food is scarce, and 90% of hunters confirm using lead bullets, thereby increasing the risk of exposure for GB. The results clearly point out the risk of lead exposure through game species in GB in the French Alps and highlight key practices and hot spot areas where this risk should be reduced.[jp1] [jp1]

2.13P.6
Improving the systematic use of contaminant data from apex predators and their prey in chemicals management
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Screening and prioritization of chemicals for regulatory chemical management is challenging. Over 20.000 different chemicals are registered under REACH which regulates the production and use of chemical substances. For many of them there is a lack of data on their potential hazardous properties and in some cases risk assessment fails because standard tests do not grasp the whole picture of the fate and properties of chemicals (e.g. Perfluorinated compounds & highly hydrophobic substances). On the other hand there is a growing number of high-quality monitoring data available which are a valuable support for the assessment of chemicals. LIFE APEX is a demonstration project aiming to support and sustain regulatory applications of chemical monitoring data from apex predators and their prey for the purposes of REACH and to enhance quality and availability of monitoring data. Apex predators are particularly well suited to contaminant monitoring because (1) being at the top of the food chain and relatively long-lived, they strongly accumulate persistent, bioaccumulative and toxic (PBT) chemicals and (2) they integrate contaminant exposure over time and over relatively large areas. This poster demonstrates how chemical monitoring data can be used to facilitate prioritization for further hazard assessment, in particular for bioaccumulation assessment of a potential PBT substance. Chemicals detected in prey and apex predators will be ranked according to their frequency of appearance and properties for PBT. This exercise is conducted for emerging substances based on targeted monitoring and unambiguously and tentatively identified substances based on non target screening results. Acknowledgement: This project is funded by the EU LIFE programme (grant ENV/SK/000355).

Advances in Bioavailability Science and Application to Chemical Regulation (P)

3.01P.3
Biochar as a Tool forAmelioratingthe Effect of Antibiotic intheBiodigestion of Doxy-Gen Contaminated Poultry Dropping and Cassava Peel
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Anaerobic digestion of doxy-gen contaminated poultry dropping and cassava peel under a mesophilic temperature condition was investigated. Two blends of the wastes labelled as A (70:30 doxy-gen contaminated poultry dropping/cassava peel) and blend B (70:30 doxy-gen contaminated poultry dropping/cassava peel + 5% biochar) were used for this study. Biodigestion of the blends were carried out simultaneously under the same environmental and operational conditions of 40 days using two 32L capacity biodigesters of fixed dome prototype. The biogas cumulative yield result shows that blend B had the highest cumulative biogas yield of 41L while blend A had the cumulative biogas yield of 13.5L. Blends A and B had a lag period of 39 days and 33 days respectively. The composition of flammable biogas showed that blend A had a methane composition of 60.8% while blend B has a methane composition of 62.2%. The results therefore favour biochar as an effective tool in ameliorating the effect of antibiotic in a biodigester system.

3.01P.4
Impact of use of the ionizable pH buffer MOPS on bioavailability research: tests with the cyanobacteria Microcystis aeruginosa
Q. Zhang, GhEnToxLab (Ghent University) / Bioscience Engineering
Ionizable chemicals are able to transfer between states in response to changing environmental pH, and therefore, have their bioavailability partially regulated by pH. In order to better represent the toxicity of ionizable chemicals in natural environments, where a wide range of pH exists, risk assessment of ionizable chemicals needs to be increasingly performed at multiple pH levels. pH Buffers applied in toxicity tests to facilitate pH control in test mediums, however, are ionizable too, meaning that a buffer might interfere with each pH treatment to different degrees. Underestimating the effects caused by ionizable buffers on test outcomes can lead to inaccurate estimation of the toxicity of target chemicals. Here we investigate the toxicity of buffer 3-(N-morpholino) propanesulfonic acid (MOPS), a zwitterion with an isoelectric point of 7.2, on the cyanobacteria *Microcystis aeruginosa* PCC 7806. Experiments are carried out across the pH range in which MOPS is commonly applied (6.0~8.0), to evaluate the suitability of MOPS in future toxicity tests of ionizable chemicals with *M.aeruginosa.* Taking the impact of buffers, especially ionizable buffers, into consideration will contribute to more realistic use of experimental toxicity data in ecological risk assessment.

3.01P.5
Miniaturised passive dosing Daphnia magna biotest with Polycyclic Aromatic Hydrocarbons (PAHs) - Investigation of single PAH substances and PAH mixtures
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Polycyclic aromatic hydrocarbons (PAHs) are distributed worldwide and play a major role in environmental risk assessment. PAHs consist of a various number of

benzene rings with lipophilic characteristics, which results in low aqueous solubilities and biodegradability. Their environmental relevance is high as they may exhibit properties like persistence, bioaccumulation and toxicity, and are considered to be mutagenic, cancerogenic or genotoxic. In this study a subacute passive dosing biotest (seven days exposure) with *Daphnia magna* was developed based on DIN EN ISO 6341:2012 to investigate the immobilisation effects caused by PAHs. The performance of aquatic toxicity tests with hydrophobic organic compounds like PAHs is difficult due to poor water solubility, volatilisation and sorption losses. The passive dosing method was used to overcome these difficulties. Passive dosing is based on a constant distribution of the chemical, initiated by gradual diffusion from a biocompatible polydimethylsiloxane O-ring in the aqueous medium, which serves as a reservoir for PAHs. Consequently, passive dosing allows a stable and well-defined exposure concentration during biotests to the test organisms. The effective concentration to 50 % of a population of *D. magna* (EC_{50}) was determined for single PAH substances and PAH mixtures. The EC_{50} of PAHs in nominal biotests were lower than in passive dosing biotests. Therefore, concentration losses during nominal biotests were suggested. In comparison to PAH mixtures, single PAH substances provoked weaker effects to the immobilisation of *D. magna* than PAH mixtures. Consequently, combined or synergistic effects may have occurred. In order to investigate the effects of natural PAH mixtures to *D. magna*, PAH mixtures of native samples were recreated. Therefore, the freely dissolved concentration (C_{free}) of PAHs, which is bioavailable for organisms, in sediment porewater was determined by using solid phase microextraction. The recreated mixtures were prepared based on the natural PAH composition of C_{free}. In the present study, the first miniaturised passive dosing bioassay with *Daphnia magna* as test organism was developed and successfully performed for single PAH substances and PAH mixtures. Additionally, this approach is suitable for native PAH mixture samples as well. The combination of passive sampling and passive dosing will help to improve future risk assessment with regard to bioavailable fractions of sediment pollutants.

3.01P.6
Measuring of the bioavailable fraction of HOCs in North Sea sediment pore water: Recreation of realistic mixtures in passive dosing bioassays
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The bioavailability is a key factor responsible for ecotoxicological effects of contaminants and important for risk assessment approaches since total exhaustive extractions of contaminants do not take the importance of the bioavailable fraction into account. Hydrophobic organic contaminants including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are some of the most occurring 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 heavily polluted marine regions in the world. The intensive use of this marine area leads to a growing pollution of the ecosystem by chemicals such as HOCs. The mobility of HOCs, and consequently the bioavailability, depend on the porewater concentrations of these compounds. Currently, the freely dissolved concentration (C_{free}) will establish as an important endpoint for sediment quality and risk assessment Based on C_{free}, the chemical activity (a), describing the potential for ecologically relevant spontaneous processes, was determined. The sum of activities is an indicator of the baseline toxicity potential of a mixture. In this study, solid phase microextraction (SPME) was applied to examine the spatial and temporal distribution of C_{free} of PAHs and PCBs in sediment cores of the North Sea. The chemical activities (a) were calculated to predict the baseline toxic potential of pore water. Furthermore, the ecotoxicological effects of single PAHs and realistic PAH mixtures were examined in miniaturised passive dosing bioassays representing different trophic levels. Since nominal dosing often exhibit significant losses (i.e. sorption, volatilisation), the passive dosing method was used to overcome these difficulties and to enable stable exposure concentrations. The PAHS are released into the aqueous medium via passive diffusion from a biocompatible polydimethylsiloxane (PDMS) O-ring serving as a reservoir for PAHs. The chemicals distribute between the silicone (C_{PDMS}) and the medium (C_{free}) in the test vessels trough thermodynamic equilibrium. In addition, bioassays with individual PAHs were performed as well as recreated PAH mixtures of North Sea pore water (C_{free}) with respect to mixture toxicity.

Advances in Exposure Modelling: Bridging the Gap Between Research and Application (P)

3.02P.1
Bottom up exposure modelling of engineered nanoparticles in urban environments: tools for deciphering spatial and temporal trends
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The development and use of mathematical models in the field of emerging contaminants, such as engineered nanoparticles (ENPs), has grown exponentially over the last decades. These models provide a cost-effective solution to determine potential environmental concentrations when monitoring data is limited or unavailable. Models typically incorporate ENP specific fate and transport processes and exposure estimates can then be obtained for all the different environmental compartments . However, there is common agreement on the need for better spatial and temporal resolution when deriving exposure estimates [1, 2]. The main limiting factor that has been identified for generating high resolution exposure predictions is the lack of information regarding ENP usage and release rates [3]. This information is often confidential or subject to high degrees of uncertainty. Here were present a methodology that moves away from the use of national/regional scale assessments of ENP emissions and fate to model at the local scale in urban systems and which, therefore, provides emission and exposure estimates at high levels of spatial and temporal resolution. For the first time a spatially and temporally resolved ENP emission model has been integrated with a spatially resolved dynamic fate model, providing a high level of detail about the sources, pathways and main drivers of emissions and associated exposure of ENPs in cities. The presented modelling framework has the potential to be adapted to other urban contaminants such as particles from tyre wear, becoming a potential enabler of more refined ERAs that are able to account for the spatial and temporal variations of pollutant exposure. [1] Nowack, B. 2017. Evaluation of environmental exposure models for engineered nanomaterials in a regulatory context. NanoImpact, 8, 38-47. [2] Dale, A. L., Casman, E. A., Lowry, G. V., Lead, J. R., Viparelli, E., & Baalousha, M. 2015. Modeling nanomaterial environmental fate in aquatic systems. Environ. Sci. Technol, 52, 4, 1704-1724. [3] Williams, Richard J., Samuel Harrison, Virginie Keller, Jeroen Kuenen, Stephen Lofts, Antonia Praetorius, Claus Svendsen, Lucie C. Vermeulen, and Jikke van Wijnen. 2019. Models for assessing engineered nanomaterial fate and behaviour in the aquatic environment. *Current opinion in environmental sustainability* 36 (2019): 105-115.

3.02P.2
How to make one's (spatiotemporal multimedia nanomaterial) exposure model as extensible, usable and useful as possible
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@page size: 21cm 29.7cm; margin: 2cm p margin-bottom: 0.25cm; direction: ltr; line-height: 115%; text-align: left; orphans: 2; widows: 2; background: transparent Recent years have seen significant advances in exposure modelling, partly driven by the emergence of new chemicals. Engineered nanomaterials (ENMs) are an example of such an emergent chemical with unique physicochemical properties, necessitating the creation of nano-specific environmental fate and exposure models. Accordingly, over the last decade the latest science has informed the development of numerous nanomaterial fate models, and most recently, the Horizon 2020 project NanoFASE has led to the development of a spatiotemporal multimedia model of nanomaterial fate, speciation and bio-uptake – the so-called NanoFASE model. Fully spatiotemporal and multimedia models maximise realism, giving insights into likely spatial and temporal distribution of ENMs. However, such complex models mean larger data demands, difficult to interpret outputs and potentially reduced usability. Here, we present considerations relating to these difficulties in the context of the NanoFASE model, detailing best-practice modelling strategies to aid in the creation of exposure models that are as *extensible*, *usable* and *useful* as possible. For example, making a model geographically *extensible* can be aided by: sourcing data with geographical extension in mind (e.g. using global datasets); creating data parsing pipelines to automate common tasks, such as spatial resampling and interpolation; aiming for model algorithms to be as parameterisation-free as possible, and; performing sensitivity analyses to identify critical ranges of key parameters. These practices improve *usability* by making it easier for non-developers (e.g. industry, regulators) to create their own scenarios. Other recommendations for usability include: making models and the data they require accessible, ensuring key model outputs are reproducible; use standardised model interfaces to aid model coupling; make models cross-platform, and; produce good documentation. We will also consider the *usefulness* of the NanoFASE model and similar complex models from a regulatory perspective, be it in using model outputs to scope changes or validate simpler models, or as part of a tiered assessment approach in which complex models can be used where outputs from simpler models are not sufficiently detailed to inform regulatory decisions.

3.02P.3
Fate and transport models for nano- and microplastics
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Potential environmental risks associated with plastic particles in the nano- and micrometer size range have given rise to concern, but current exposure data are insufficient for a sound risk assessment. In this context, models are powerful tools to increase our understanding of nano- and microplastic fate at different environmental scales and generate much-needed exposure estimates. Nano- and micrometer size plastic particles are particulate contaminants, whose behaviour is not only dictated by their chemical properties, but also by their, often heterogeneous, physical characteristics, such as size, shape and density. Therefore, established fate and transport models, developed for and applied to mainly organic chemicals cannot be directly applied to study their environmental behaviour. Particle-specific processes, such as for example aggregation and sedimentation, need to be described in models for nano- and microplastics. Additionally, models need to be able to represent heterogeneity in particle properties, either by allowing for several "property bins" or by representing certain properties as distributions. In this work we present a recently developed flexible framework for modelling the fate and transport of micro- and nanoplastics in aquatic environments. This model is based on existing models for the fate and transport of nanoparticles, that already describe colloidal behaviour via aggregation and sedimentation processes. To be fit for nano- and microplastics, these model have been extended (i) to span both the nano- and microrange, (ii) to include fragmentation processes, (iii) to account for the effect of particle shape, (iv) to describe effects of biofouling on particle density and (v) to include a surface water compartment important for representing the behaviour of buoyant particles. Following a multimedia box modelling approach our plastic particle fate and transport framework can be readily adapted to different scales, spanning from local, to regional, to global. Here we present a first application of the model framework to a local scale in form of a lake model. The model was parameterised to represent Lake Geneva in Switzerland. In a case study, a wide range of possible plastic particles and fibres were modelled and predicted exposure concentrations were compared to available field data as a first step towards model validation. *Acknowledgement* – This work received funding from CEFIC LRI under projet ECO48: Nano2Plast.

3.02P.4

Sensitivity analysis of the aquatic pesticide fate models in SYNOPS and their parametrization for Switzerland

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Most European countries have initiated national action plans to reduce risks of pesticides for human health and the environment. To evaluate risk reduction targets for surface waters in Switzerland, a national aquatic risk indicator is currently being developed based on the model "SYNOPS", which is also applied to evaluate risk development in Germany. This indicator aims at evaluating long-term trends in aquatic risks of pesticides based on changing pesticide usage and risk mitigation measures. To consider the effects of risk mitigation measures in risk indicators, a realistic representation of the contribution of different routes of entry (run-off, erosion, drainage, drift) is essential. To apply SYNOPS in the Swiss geographical context, parameter values of the aquatic fate models of SYNOPS have to be selected. This requires a clear understanding on how parameter selection influences the predicted environmental concentration (PEC) of pesticides in surface waters and the contribution of different routes of entry to total pesticide concentration. Therefore, a sensitivity analysis of the aquatic fate models of SYNOPS was conducted, using realistic ranges of Swiss-specific parameters and their combinations. The most important parameters were identified, which were then used to select a set of environmental parameters for Switzerland. The sensitivity analysis revealed that slope and K_{OC} were the parameters with the highest impact on pesticide concentration. Run-off was the dominant route of entry in most tested scenarios, followed by drift. From over 40,000 tested environmental scenarios (i.e., full set), a reduced set of 75–113 environmental scenarios was finally selected, which predicted similar total pesticide concentrations and comparable contributions of different routes of entry compared with the full set. With the parametrization and reduction of used environmental scenarios, aquatic fate calculations became more efficient and realistic for Swiss conditions. The thoroughly parametrized fate models provide a solid basis for developing an aquatic risk indicator for Switzerland in a next step.

3.02P.5

Multidimensional Modelling of Reactive Transport of Plant Protection Products

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In order to minimize the undesired transport of Plant Protection Products (PPPs) from agricultural fields to neighboring water bodies via run-off, vegetated filter strips (VFS) can be used as buffer and retention zones. It is therefore of high relevance to understand the water and solute dynamics in and beneath VFS, to be able to make robust predictions of their retention efficiency and capacity. Different scenarios were simulated with several example compounds that cover a broad range of environmental fate properties. These compounds were assumed to be applied in the field under realistic worst case run-off conditions. VFSmod is often used to predict for regulatory purposes the surface run-off and PPPs reduction efficiency of the VFS. VFSmod simulates the water flow and transport processes at the surface of the VFS during rainfall-runoff events. For a more detailed understanding of the processes involved, HydroGeoSphere model is used to simulate the behavior of the VFSmod estimated infiltrated mass into the soil beneath the VFS. A 2D rectangular flow using Richards'equation for single phase flow with the single porosity model of van Genuchten-Mualem and no hysteresis were considered. The soil hydraulic parameters were estimated using the Rosetta pedotransfer functions. With the help of the simulation results, we estimated residence and travel time of the PPPs underneath the VFS. The increased retention time of PPPs in the soil underneath a VFS reduces the exposure of the active ingredients to adjacent water bodies as it allows for degradation and results in much lower exposure peaks in the water body. The model could potentially be used for refined soil risk/ run-off and leaching assessments in future.

3.02P.7

Advances in exposure and risk assessment of fertilizers under REACH: updates of the fertilizers environmental exposure (FEE) tool

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Under the new EU Fertilizing Product Regulation (EU) 2019/1009 all substances incorporated into fertilizing products, on their own or in a mixture, shall be registered under the REACH Regulation (EC) 1907/2006, with a dossier and chemical safety report covering the use as fertilizer. For many substances in fertilizers, the exposure and risk assessment of fertilizer uses were described in a qualitative way in the corresponding REACH dossiers by lack of proper emission estimates and exposure models for fertilizers. Under the umbrella of Fertilizers Europe, specific environmental release categories (SPERCs) for fertilizer uses and the Fertilizers Environmental Exposure (FEE) tool (v1.1, 2017) were developed. Proper emission estimates and the exposure tool were made publicly available online for quantitative environmental exposure assessment of fertilizer substances. The FEE tool allows for calculations of realistic worst-case local Predicted Environmental Concentrations (PEC local) for fertilizer constituents in the various environmental compartments (soil, water and sediment). A new version of the FEE tool is now available. The main focus of the updated FEE tool was on micronutrients such as manganese, copper and zinc, which are identified as hazardous for the aquatic environment; but the tool also allows the assessment of other inorganic and organic substances in fertilizers. Conceptually, the tool has been based upon existing REACH exposure modelling and is adapted for fertilizer uses by including relevant environmental processes, such as runoff at local scale, crop interception of fertilizer sprays and crop uptake of nutrients based on information from other chemical legislations or Eurostat. Calculations in the FEE tool for the assessment of e.g. runoff and sediment exposure have been updated and refinement options for runoff reduction and soil incorporation have been included in the FEE tool v 1.2. Further information on the project, including downloads of the FEE tool and SPERC factsheets, can be found on https://www.reachfertilizers.com.

3.02P.8

Update of Input Parameters for the PEC Calculation of VMP - A Farm Survey of Manure Application

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Since coming into force of the guidelines on the environmental impact assessment of veterinary medicinal products (VMPs) (CVMP/VICH/592/1998, CVMP/VICH/790/2003) and the guidance document supporting the guidelines (EMEA/CVMP/ERA/418282/2005 Rev. 1 Corr.), the German Environment Agency (UBA) is tasked with environmental risk assessment in marketing authorisation procedures of VMP. Two factors that affect the value of the predicted environmental concentration (PECsoil) are the nitrogen content of manure and the depth of manure penetration into soil. Stricter regulations in the revised German Fertilizer Ordinance are likely to cause a change in common practice of storage and application of manure. This study aimed at gathering information about the status quo of current storage and application preferences for fertilizer of livestock origin. A questionnaire for farmers containing 18 questions in German was pubished online for 3 weeks in October 2019. The request to participate was distributed as a link by email, social media and livestock-associated press. In total, 182 people opened the link, 172 answered at least one question, and 111 finished all questions. Since 19 people only missed to close the last page, but 5 finished without any feasible answers, 125 data sets were included in the statistics. Participants were predominately male (81%). The majority farmed crop- and grassland (81%), whereby 16% had only cropland and 3% grassland. Livestock housed by participants were specified as mainly cattle, followed by swine. Slurry and solid manure from hoofed livestock originated largely from one´s own animal production, whereas fermentation residues from biogas plants and poultry litter were also purchased in parts. On croplands, fertilizer was mostly applied to the soil surface with grubber. Only solid manure from hoofed livestock was sometimes applied with plough. Therefore, slurry and poultry litter penetrated into soil by 3 to 8 cm, fermentation residues by 3 to 15 cm and manure from hoofed livestock by 8 to 15 cm. In very few cases, fertilizer was applied deeper than 15 cm. On grasslands organic fertilizer was applied predominantly on top (69%). In order to comply with the future regulations regarding the nitrogen and phosphorus balance, also N/P reduced feeds were used. Our results regarding penetration depth and nitrogen content of manure will provide information for future modifications of the PEC calculation of VMP. This project was funded by the UBA.

3.02P.9

Multimedia fugacity-based model for the exposure assessment of antiparasitic drugs used by salmon farms

F. Tucca, Universidad Andrés Bello / Department of Ecology and Biodiversity; F. Encina-Montoya, Universidad Católica de Temuco / Environmental Sciences; R.O. Barra, Universidad de concepcion / Aquatic systems

Recently, the Chilean salmon industry has been strongly affected by the occurrence and resistance of ectoparasitic diseases, mainly caused by sea lice *Caligus rogercresseyi*. Therefore, the industry relies on some chemotherapeutic alternatives (i.e., cypermethrin, deltamethrin, emamectin benzoate, diflubenzuron, hexaflumuron and azamethiphos) to contribute in the control and prevention of infections on salmons. However, currently there are few predictive tools to assess the exposure of chemicals used for the salmon treatments (e.g., DEPOMOD, AutoDEPOMOD and BathAuto v5). This study aims to propose a multimedia fugacity-based model (Level III), especially designed for salmon farming, to understand the dynamic and fate of antiparasitic drugs. For the modelling, environmental data collected in areas with active aquaculture (e.g., current speed, suspended particles, depth, etc.) and physical-chemical properties of the antiparasitic drugs (n= 6 total) were required. Temporal treatments were carried out to estimate the maximum predicted environmental concentration (PEC_{max}) of such drugs in the environmental. Predicted concentrations in water and sediment were consistent with the experimentally measured concentrations, in which the levels predicted by the model were in the order of magnitude of ng L^{-1} and ng g^{-1}, respectively. Emamectin benzoate reported the longer persistence in sediment, while diflubenzuron and hexaflumuron showed a low occurrence in the marine environment. Pyrethroids showed low concentrations in the water and sediments, however, these levels suggest toxic consequences for surrounding benthic invertebrates. On the other hand, azamethiphos reported to be a compound with the higher occurrence in seawater. In conclusion, the multimedia fugacity-based model developed in this study can be a valuable tool to assess the chemical concentration of the antiparasitic drugs released to the marine environment. Supported by CONICYT/FONDECYT/3180159, CONICYT/FONDECYT 1180063 and CONICYT/FONDAP/15130015.

3.02P.11

An improved method to calibrate emission estimations for pharmaceuticals from sewage treatment plants

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Micropollutants can be detected nearly everywhere in the aquatic environment. Especially human pharmaceuticals such as diclofenac and carbamazepine are ubiquitously measured in European rivers. For a better understanding of the entry and the fate of these chemicals in surface waters the geo-referenced exposure assessment tool for European Rivers (GREAT-ER) had been designed which predicts spatially explicit concentrations of 'down-the-drain' chemicals in whole river basins. Realistic parameterization of sewage treatment plants as the most important point sources of pharmaceuticals is a crucial point for the model output. GREAT-ER computes the emission loads from an average per-capita-consumption, human excretion rate and the removal efficiency in sewage treatment plants. In this study, a method for evaluating the quality of these substance parameters is presented that uses two intermediate variables, namely concentration ratios instead of single concentration values and a per-capita emission. The use of concentration ratios between the target compound and a conservative reference substance (benchmark) eliminates the impact of the variability of daily discharge that often aggravates comparison of simulations and monitoring. Here, carbamazepine was used as reference since it is only little eliminated during sewage treatment and is not removed significantly in surface waters. For input parameter calibration, we defined emission loads in terms of a per-capita-emission which is independent from the size of the treatment plant. Theoretical per-capita emissions from the model were compared with corresponding real values obtained from measured effluent concentrations, total flow and the number of connected inhabitants.The usefulness of the methodis exemplarily shown for diclofenac. Comparison of effluent concentration ratios of diclofenac and carbamazepine with GREAT-ER simulation results revealed the need for input parameter calibration considering natural variability and/or uncertainty. Using aprobabilistic Monte-Carlo approach the emission parameters for both substances were individually adjusted so that the theoretical per-capita emission matches the one derived from measured data. Effluent concentration ratios simulated with the adapted parameter sets were then in much better agreement with measured concentration ratios.

3.02P.13

A new tool for assessing the potential bioaccumulation of active substances and their metabolites in food webs: MOSAIC-bioacc

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Toxicokinetic/toxicodynamic (TKTD) models are used to describe and predict the toxicity and the effects of chemical substances on individual traits based on experimental data. The toxicokinetic (TK) part describes the relationship between the exposure medium and the organism, considering various processes such as ADME (accumulation, depuration, metabolization and excretion). Regulation laying down the data requirements for active substances for the placing of plant protection products on the market, a bioaccumulation study on fish is required for substances with a partition coefficient octanol / water greater than or equal to 3, following the OECD guideline 305. Such a TK test allows to define the bioconcentration factor (BCF), the biomagnification factor (BMF) or the bioaccumulation factor (BAF). These factors are decisive criteria for estimating the concentration of active substances present in food items of vertebrates and more particularly of piscivorous birds and mammals. In a recent study, a relevant inference framework (Bayesian inference) was proposed to estimate toxicokinetic model parameters accounting for their correlation, based on the experimental data from standard guidelines and leading to the calculation of BCF/BMF/BAF. Models underlying this framework can be directly integrated and used as TK parts of TKTD models. Achieved in agreement with EFSA's scientific opinion on good modeling practices, the objective of this project is to develop a ready-to-use on-line tool for easily estimating BCF/BMF/BAF in a regulatory framework, by taking into account bioaccumulation of a parent compound and its metabolites through biotransformation. We built a Shiny interface to make available the inference method and the BCF/BMF/BAF calculation from the MOSAIC platform, which may be used for research as well as by companies or regulatory agencies. This tool will be integrated more broadly into the development and widespread use of TKTD models within the risk assessment framework.

3.02P.14 - SorpKinAnalysis - Implementation of a two-site aged sorption model in accordance with EFSA PPR Panel (2018)

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SorpKinAnalysis is a user-friendly implementation of kinetic sorption assuming a two-site modelling approach. It is fully in line with the scientific opinion about the Guidance of the Chemical Regulation Directorate (UK) on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessment. The user has to enter the experimental aged sorption study data on concentration of substance in liquid phase and mass of substance in soil. After the successful optimisation the user obtains a report file containing the model input and output (e.g. statistical assessment, regulatory endpoints like kinetic rate constants or DegT50_{EQ} values). Furthermore, a chart showing the experimental and predicted residue in time, a predicted-measured chart and a residual chart are created by the tool. The results of SorpKinAnalysis are intented to be used as input parameters for groundwater exposure models like PELMO and PEARL (FOCUS GW Tier 2a as described in EFSA PPR Panel 2018).Results of the model are presented using different experimental aged sorption studies and compared with alternative tools (e.g., PEARL NEQ (Boesten and ter Horst 2012). Additionally, the impact of aged sorption data on the results of FOCUS groundwater simulations is discussed. SorpKinAnalysis was implemented in VB.NET as a user-friendly stand-alone program. The graphical user interface is intuitive. The user does not need programming skills. References: Boesten, J.J.T.I and M.M.S. ter Horst (2012). "Manual of PEARLNEQ v5". Working document 305. Wettelijke Onderzoekstaken Natuur & Milieu, Wageningen. EFSA PPR Panel (2018). "Scientific Opinion about the Guidance of the Chemical Regulation Directorate (UK) on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments". EFSA Journal 2018;16(8):5382, 86 pp. https://doi.org/10.2903/j.efsa.2018.5382 FOCUS (2009/2014): Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU. The Final Report of the FOCUS Groundwater Work Group of FOCUS. –Sanco/13144/2010, version 3, 10 October 2014 Klein, J. (2019): SorpKinAnalysis - Implementation of a two-site aged sorption model according to EFSA PPR Panel 2018 available at https://www.ime.fraunhofer.de/en/Research_Divisions/business_fields_AE_BR/Businessareas_AE/Software_E/SorpKinAnalysis.html

3.02P.15

Development of a prediction model for daily change of hydrogen peroxide in rivers all over Japan

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Hydrogen peroxide is used in many ways mainly as bleaching agent for both home and business use. It is now categorized as "Priority Assessment Chemical Substances" in Chemical Substances Control Law in Japan, which has ecotoxicity with concern for a considerable amount of it remaining in the environment. Although Japanese government will conduct the risk assessment of hydrogen peroxide, it is hard to do because of its dynamically changeable concentration due to high reactivity with other organic and inorganic matter. In addition, hydrogen peroxide could also be produced through solar photochemical processes in both the atmosphere and surface water. In this study, we were developing a simulation model for daily change of hydrogen peroxide concentration in rivers. Since hydrogen peroxide was produced through photochemical processes of dissolved organic matter (DOM) and oxygen, we were also developing a method for simulating concentrations of dissolved organic carbon (DOC) in river water. The model was developed based on an existing multimedia environmental fate model, G-CIEMS, constructed geographic information system data of sewage collection areas, and information about sewerage and sewage treatment plants. We selected chemical oxygen demand (COD) as DOC indicators, performed simulating COD concentrations in rivers all over Japan, and compared these simulated concentrations with measured ones which were performed by the Japanese government. The simulated concentrations were calculated from load units of COD for point sources categorized by sewage treatment (flushing toilet, combination septic tank, single septic tank), and non-point sources categorized by land use type (forest, paddy fields, agricultural fields, and urban). Then we simulated daily changes of hydrogen peroxide in rivers all over Japan under several scenarios. and compared these with measured concentrations which performed various rivers in Japan.

3.02P.16

Development of emission inventories and exposure modelling of emerging pollutants to define a "Watch List" for the lagoon of Venice

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The Environmental Quality Standards Directive 2013/39/EU requires the establishment of a periodically updated "Watch List" of *emerging pollutants* to be monitored in surface waters of Member States to determine the risk they pose to the aquatic environment and whether EU Environmental Quality Standards (EQS) should be set for them. The identification of chemicals included in the list that is in force since 2018 followed for most of the chemicals a risk-based prioritization procedure at the EU scale. However, integrated experimental and modelling efforts at the regional scale can better identify realistic patterns of use, emissions, and environmental concentrations of these pollutants in order to tailor future monitoring activities. The lagoon of Venice, an important coastal transition environment in the Mediterranean Sea characterized by complex hydro-morphological conditions and the presence of several valuable aquatic and terrestrial habitats, has been subjected since decades to chemical pollution due to the presence of several anthropogenic drivers increasing the pressures on its environment. The project "Venezia 2021" aims at the identification of a watch list of emerging pollutants specific to the lagoon of Venice, to be included in local monitoring programme in the future. Starting from a review of the existing literature, a set of target pollutants that could be included in a local watch list was first identified (including pharmaceuticals, pesticides, cosmetics and industrial chemicals). Several seasonal sampling campaigns are being performed to detect these chemicals in water, sediment and biota in the Venice lagoon. In parallel, modelling activities are addressing emission inventories (through the elaboration of geographical information and statistical data treatment) and environmental fate to estimate the load of each chemical in the case study area and estimate realistic Predicted Environmental Concentrations (PECs) through a tailored multimedia model. PECs will be then integrated with the results of hazard assessment (including also ecotoxicological testing on species relevant to the case study area), with the final goal of quantifying ecological risks and prioritize emerging chemicals accordingly. Preliminary modelling results will be presented, and main potentialities and issues of the proposed integrated experimental and modelling approach will be discussed.

3.02P.17

Evaluating the CiP-CAFE and RAIDAR models with monitoring and biomonitoring data from Europe

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Humans are exposed to a wide variety of chemicals that originate in indoor environments as a part of building materials, consumer goods and articles, and products used daily. Humans can also be exposed to these same chemicals through far-field pathways, i.e., diet. Mechanistic, mass-balance, multi-media models are tools to understand chemical fate and exposure pathways and to predict this information in the absence of measured data. The Risk Assessment IDentification And Ranking (RAIDAR) model simulates chemical fate in the natural environment and far-field human exposure. The RAIDAR-Indoor and Consumer Exposure (RAIDAR-ICE) model combines an indoor fate model with a toxicokinetic model to simulate chemical exposures from direct (e.g., personal care products) and indirect (e.g., vaporization of chemicals from building materials) sources of chemicals. It simulates human exposure from the near-field environment through inhalation of indoor air, dermal absorption, non-dietary object-hand-mouth contact. Collectively, the RAIDAR models simulate aggregate human exposures. There is a need to test (evaluate) aggregate exposure models. Chemical use and emission rate data are required to apply exposure models; however, these data are difficult to obtain. Some exposure model applications use total production volumes (TPV) to estimate emission rates. A method in which emission rates are derived by comparing model predictions to monitoring data, also known as "inverse modelling", can also be applied to estimate emissions. The Chemicals in Products - Comprehensive Anthropospheric Fate Estimation (CiP-CAFÉ) model is a new chemical life-cycle emissions tool. In this study we use TPV, inverse modelling and the CiP-CAFÉ model to parameterize RAIDAR and RAIDAR-ICE to predict indoor dust concentrations and human exposures for a set of approximately 45 chemicals. The model predictions are compared with monitoring and biomonitoring data collected in Europe (i.e., obtained in the Cefic-LRI SHINE project). The case study chemicals include flame retardants, plasticizers, and biocides comprising a broad range of use categories, partitioning properties, and degradation half-lives.

3.02P.18

Use of toxicokinetic (TK) model applications on freshwater invertebrates in regulation.

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Toxicokinetic (TK) models have been developed to describe the bioaccumulation of chemicals in organisms. They are used as the first step to the toxicity assessment of a contaminant. TK models provide a theorical framework for understanding the links between exposure and accumulation in biota, for testing hypotheses, and making predictions (e.g. predictions of the chemical concentration in organisms according to environmental concentration or inversely). In France, polycyclic aromatic hydrocarbons (PAH) have generally been analyzed in sediment as part of annual river monitoring. Current regulation specifies that for PAHs, the environmental quality standard (EQS) applies to biota, especially invertebrates. However, modifying the monitoring protocol used for several years would lead to a loss of data continuity. In this context, TK models could be used (*i*) either to predict concentrations in the sediment equivalent to the EQS_{biota}, or (*ii*) to predict concentrations in biota, from concentrations observed in field sediment, which could then be compared to EQS_{biota}. Our aim here is to explore the approach (i), i.e. determine a concentration in sediment equivalent to EQS_{biota}. To achieve this purpose, we first used laboratory data from a published TK study where *Hyallela azteca* and *Chironomus tentans* were exposed to benzo(a)pyrene (BaP) spiked sediments. The distributions of model parameters allowed to predict a concentration limit in sediment that would lead to a concentration in biota below the EQS_{biota}. For *C. tentans*, the limit value depends whether biotransformation (i.e. metabolites) is accounted for or not. We further explore the influence of sediment organic carbon content on the concentration limit in sediment, based on experimental data.

3.02P.19

Benzene in-vitro characterization and oral bioavailability assessment in 3-tier model of gastrointestinal epithelial cells

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The human health risk from chemicals in the natural built environments varies on the exposure route along which the chemical is absorbed by the body, the exposure pattern, the duration and frequency of the exposure, and the nature of the substance. A primary concern is human incidental ingestion of contaminants, such as hydrophobic pollutants like benzene and polycyclic aromatic hydrocarbons (PAHs) and heavy metals like arsenic and cadmium at downstream hydrocarbon sites, either from groundwater or from adhered soil. However, it is ethically difficult to justify animal use to estimate bioavailability at a contaminated site; hence, the present study aimed to develop 3-tier passive dosing of *in vitro* test systems for benzene transport across intestinal epithelial cells. This study establishes (1) methods to control *in vitro* exposure and (2) test systems that characterize a well-defined exposure level. A partition-controlled delivery for freely dissolved concentrations of benzene was maintained at constant levels of exposure to cells, and up to 210 min exposure period, following adsorption of benzene onto solid-phase silicone polydimethylsiloxane (PDMS) from aqueous solution, and subsequent desorption in cell media: Dulbecco's modified eagle medium (DMEM). Up to 80% benzene was adsorbed to silicone PDMS during the adsorption phase that was conducted under laboratory conditions with temperature control to 22 ± 0.52; hence and the desorption in 6-well plates during incubations at 37^0C. Finally, the study explored the use of advanced in-vitro digestors, 3-dimensional cell culture approaches and gene expression assays to investigate the interaction between pollutants, metals and the human intestine to help set remediation objectives. Keywords: Chemical risk assessment, Bioavailability, *In vitro* test systems, Epithelial cells

Advancing our Understanding of Contaminants of Emerging Concerns Associated with Plastic and Microplastics: Identification, Analysis, Occurrence and Effects (P)

3.03P.1

The global environmental footprint of indigo denim fibers

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Microfibers are a ubiquitous contaminant group that include natural, semi-synthetic and synthetic textile fibers. While most research has focused on synthetic fibers, natural and semi-synthetic fibers are sufficiently persistent to undergo long-range transport, accumulate in the environment, and potentially cause ecological effects. Because natural fibers maybe anthropogenically modified, we describe cellulosic fibers with evidence of anthropogenic modification (e.g., chemical additives, manufactured morphology) as anthropogenic cellulose (AC) fibers. Our goal was to document sources, pathways and sinks of AC fibers. We collected sediment samples from sites across the Canadian Arctic Archipelago, sediment from inland Canadian lakes, sediment and fish from the lower Laurentian Great Lakes, as well as WWTP effluent from plants in Ontario discharging to the Great Lakes. Material composition and surface morphology of fibers enumerated from all samples were analyzed using micro-Raman spectroscopy. An abundant AC fiber present in all samples was indigo denim fibers (6%). Inland lake and Canadian Arctic sediments contained the highest concentrations total fibers of all environmental compartments with 2520±1260 fibers kg dw^{-1} and 1957±1380 fibers kg dw^{-1}, respectively. The high levels in sediments may indicate that this compartment is a sink for AC fibers in the aquatic environment. The most abundant AC fiber in WWTP effluent was indigo denim (37%), as well as other textile fibers (e.g., polyester), suggesting laundering as a likely source of these fibers. To further investigate indigo denim as a source of these fibers, we washed individual pairs of new and used blue jeans, a garment that dominates the indigo denim market. In-lab washing trials showed that all fibers shed from blue jeans consisted of cotton, with most containing indigo dye (84%). New jeans (210±3 fibers g^{-1} of denim) shed significantly more fibers than used jeans (130±14.5 fibers g^{-1} of denim). Our results indicate a single pair of used blue jeans can release $5.6x10^5$±$4.1x10^3$ indigo denim fibers in one wash. Chemical composition and surface morphology of indigo denim fibers found in environmental compartments were consistent with those identified from blue jean washing experiments. We conclude that indigo denim garments (e.g., blue jeans), one of the world's most popular textile materials, is a source of AC fibers to the global aquatic environment.

3.03P.2

Biofouling on solid litter: what do they prefer?

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Solid litter affects coastal systems where increasing amounts of synthetic material are being deposited. Litter such as plastic persist in the environment. It can then interact with organisms as a surface to biofouling i.e. a colonization process in which organisms associate with hard substrates. Biofouling can cause fragmentation, degradation and/or sinking of items, besides transport of exotic fauna, increasing the impacts on the ecosystems. This thematic is yet understudied in salt marshes. In the Patos Lagoon Estuary (Brazil) most of salt marshes are contaminated by litter. This work evaluated the association of organisms with solid litter in different zones of a salt marsh in the Patos Lagoon Estuary. The Molhe Oeste salt marsh presents zones that differ flooding rates, which increases from the mudflat (MF) to the low marsh (LM), mid marsh (MM) and high marsh (HM). Solid litter was sampled in 30m-long transects covering all zones. A total of 227 items were analysed, with 13 groups of macroorganisms associated with these items, divided into 6 groups of vagile and 7 groups of fouling/sedentary organisms. There was no difference in biofouling (%) between zones, although total values were higher in the lower zones as items in constantly flooded areas are thus constantly being colonized. The % biofouling was more associated with fragmented, intermediate degraded and malleable items. A contribution index was calculated to evaluate preferences for zones, type of material, fragmentation and colour of items. Most of the groups (8/13) were recorded in the lower zone, which is expected due to the nearly constant water exposure in this area. However, secondary colonizers such as algae and fungi were more present in the higher zone, which can indicate that items did not stay exposed to water long enough or that they lost colonization due to desiccation. Also, this result might indicate that these groups are more resistant to air exposure, which also avoids competition with other groups. Most groups were recorded on plastic (5/13), which might be related to the high fragmentation of these items. Regarding colour, brown items were preferred (4/13). Algae preferred transparent items, which might be related to its sunlight need. Thus, plastic items are more colonized in a salt marsh environment, as well as brown, intermediate degraded and malleable items. The preference for zones remains to be further analysed.

3.03P.3

Low cost and high-throughput: Nile red a tool for microplastic monitoring and hotspot identification.

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Beyond simple identification of either presence or absence of microplastic particles in the environment, quantitative accuracy has been criticised as neither comparable nor reproducible. This is in part due to difficulties faced in the identification of synthetic particles amidst naturally occurring organic and inorganic components. The aim of this study was to test the efficacy of Nile red for microplastic detection by systematically investigating what drives variations in particle pixel brightness (PB). Accordingly, the results showed that PB varied between polymer type and staining procedure, but remained similar among different sized particles. This data informed the use of a lower PB threshold limit of 100 a.u. that improved the detection of 4 out of the 6 polymers tested, and reduced processing time compared to unstained samples. This practical and cost-effective tool will allow for high throughput sampling needed in monitoring and citizen science whereby results need to be disseminated rapidly. Additionally, a large volume of samples may be analysed with sites of particular interest (hotspots of microplastic accumulation) then investigated further with spectroscopic techniques, to chemically confirm the polymer type; allowing laboratories without spectroscopic equipment to outsource a handful of samples for further investigation without incurring a substantial cost.

3.03P.5

Microplastics analysis of Environmental Samples using FTIR and Raman Spectroscopy

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Microplastics are particulates, roughly 20-1000 microns in size, originating from various man-made materials. These enter aquifers where they can join the food chain when consumed by filter feeders. Microplastics are chemically and physically stable, making excretion or digestion very difficult. Sample collection is often done by filtration, and the filter becomes the sample holder for further analysis. FTIR and Raman microscopy are excellent for analyzing these materials non-destructively and without further sample preparation, through either point-by-point identification or through area mapping or imaging. Data collection and analysis using the complementary techniques of Raman and FTIR microscopy is straightforward, providing particle sizes, counts and identities. This is essential for the rapid assessment of the situation and remediation efforts. We will show data collected using the Thermo Scientific DXR3xi Raman and Thermo Scientific iN10 FTIR Microscopes on reference materials and real samples. The balance between simplicity of operation and completeness of the analysis will be discussed.

3.03P.6

Screening of Plastic Additives and Organic Contaminants in Microplastics exposed to the Marine Environment

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Microplastics, defined as plastic particles below 5 mm in size, have attracted great interest and concern in the last years for their prevalence and detection in marine environments, increasing concentration over time and potential for trophic transfer with biomagnication in food chains[1]. The presence of plastic debris in the marine environment, including anthropogenized coastal areas, is a consequence of their worldwide use in most human activities (fisheries, domestic, agriculture, industry, etc.) through direct discharges which can be transported from continental areas to the marine environment (rivers, air, etc.)[2]. Furthermore, microplastics can not only cause physical damage to marine organisms[4] due to absorption or ingestion, but also can provide a potential pathway of exposure to organic chemicals[3]. Microplastics can contain two types of chemicals: polymeric raw materials (monomers or oligomers) and additives added during plastic production to enhance the performance of plastic products; and other chemicals (mainly hydrophobic organic contaminants) sorbed from the surrounding seawater[1,2]. The aim of this work is to screen for additives and hydrophobic organic contaminants potentially sorbed to microplastics of low-density polyethylene exposed during 8 weeks in a portuary area in Mallorca (Andratx). Microplastic and spot water samples were weekly sampled and additives and contaminants were extracted by ultrasonic solvent extraction and liquid-liquid extraction, respectively. All samples were analysed by gas chromatography coupled to time-of-flight-mass spectrometry (GC-QTOF). Compounds identification was carried out with the Agilent MassHunter Unknowns Analysis B.10.00 using the SureMass deconvolution algorithm and matching against two high-resolution libraries (one in-house library of 363 compounds and the Agilent pesticides commercial library of 844 compounds) and one low-resolution library (NIST). *Acknowledgements:* The authors acknowledge the financial support provided by Galician Government (Consellería de Economía e Industria, Xunta de Galicia, ref. ED431C2017/36), by the Spanish Government (CTM2017-84763-C3-2-R, CTM2017-84763-C3-3-R, CTM2017-90890-REDT and CTM2017-88332-R) and FEDER funds *References:* [1] Hong, S.H., et al. Anal. Methods **9** (2017) 1361-1368. [2] Victor, M.L., et al. Environ. Pollut. **236** (2018) 442-453

3.03P.7

Determination of bisphenol A in sheep's urine and faeces

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There are a few published analytical methods for the determination of bisphenols in animal excreta. The objective of our work was therefore to introduce a sensitive and selective analytical methodology for determination of bisphenol A (BPA) and its main metabolite bisphenol A-glucuronide (BPA–GLUC) in sheep's urine and faeces by testing for the presence of both free (aglycone) and total (a sum of free and conjugated) BPA. Conjugated BPA was determined by an enzymatic deconjugation of the glucuronide bond using ß-glucuronidase, followed by subtraction of the free BPA from the total BPA. Optimizations of the BPA analysis were made mainly regarding the enzymatic deconjugation, extraction from the matrix, concentration of the extract and chromatographic separation. A clean−up of both urine and faeces samples was performed by solid phase extraction (SPE) using two SPE sorbents, namely Chromabond HR−X and molecularly imprinted polymer (MIP) SPE Bisphenols. Urine samples were applied on the cartridges after dilution, while faeces samples were extracted first by acetonitrile. Final sample extracts were analysed for the presence of BPA by gradient reversed-phase HPLC using water and acetonitrile and/or water and acetonitrile/methanol as a mobile phase, Hypersil Gold C18 (3 µm) analytical column, and fluorescence detection. The recovery values for determination of BPA in urine and faeces ranged from 52 to 67% and from 41 to 81%, respectively. The repeatability and within-laboratory reproducibility of the measurements, represented by the coefficient of variation (CV) values, ranged from 1.3 to 27.4% and from 8.8 to 32%, respectively. Regarding urine, the estimated limit of detection (LOD) values for determination of free and total BPA were 0.1 and 10 µg/l, respectively, while the limit of quantification (LOQ) values determined were 0.5 and 100 µg/l, respectively. With regard to the faeces analysis, LOD and LOQ values were 1 and 2 µg/kg for both free and total BPA, respectively. The results obtained show that the method could be applied in toxicokinetic and environmental studies of BPA, BPA–GLUC and total BPA in animal urine and faeces and could thus contribute to the risk assessment of environmental contamination.

3.03P.8

Degradation products from novel biodegradable polymers in marine settings

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There is increasing concern about plastic in the ocean due to its longevity, its potential toxicity and its ability to harm wildlife. The oceans are where much of the waste plastic we create accumulates but are not an ideal environment for degradation because of lower temperatures and fewer microbes than in an ideal environment like compost. It can take decades for large plastic pieces to degrade in the ocean but during this time they are not inert. As they break down they can release chemical additives many of which are toxic. Biodegradable plastics could be a solution since they are designed to break down into carbon dioxide and water, however information on this degradation process is scant as is knowledge of the released chemical by-products and their impact. Four different types of biodegradable plastics (polycaprolactone, polypentadecalactone, polylactic acid, and polyvalerolactone) were produced at the University of Hull. These plastics were made from monomers derived from plant material rather than hydrocarbons and are expected to degrade far better in the natural environment. The four plastics, a commercially produced biodegradable plastic (polylactic acid straw) and a conventionally produced plastic (polystyrene coffee cup lid) were aged in seawater for one month to discover if and how they degraded in a marine environment. Analysis by GC-MS showed some chemicals including the monomers of polycaprolactone and polyvalerolactone were released to the water from aging plastics. Biodegradable polymers released more chemicals than commercially produced plastics (polystyrene released the fewest), which could indicate faster degradation. Chemicals released included hydrocarbons, ketones and esters that would be expected to result from the degradation of the plastic molecules (such as stearic acid and triphenylmethane, a possible environmental contaminant). Some chemicals used in the creation of the polymers such as benzyl alcohol were also found. Other analysis by Fourier transform infrared (FTIR) spectroscopy suggested some breaking of bonds in the weathered samples compared to the unweathered samples but that the physical structures of the plastics did not change much over the weathering period. Further experiments involving a more long-term degradation and an analysis of plant based plasticizers and toxicity tests could provide more information on whether biodegradable plastics are better for the oceans than conventional plastics.

3.03P.9

Investigation of microplastic particles in rural karst groundwater systems and their links with on-site domestic wastewater effluent

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Groundwater from karst aquifers, through springs and wells, is a major source of drinking water for one quarter of the world's population. These aquifers are exceptionally vulnerable to pollution as a result of predominantly rapid recharge of water from the surface and strong aquifer heterogeneity. Groundwater pollution is a complex problem that can be associated with a variety of sources, but human wastewater effluent and diffuse agricultural sources are generally considered among the most significant threats to groundwater quality worldwide. This is particularly true in rural and suburban areas where the primary wastewater treatment options for communities without access to centralized wastewater treatment facilities are on-site domestic wastewater treatment systems (DWTSs). The domestic wastewater is primarily discharged from toilets, washing machines, showers and dishwashers, thus, a wide range of contaminants eventually reach the environment even after on-site wastewater treatment processes. Microplastic particles, as contaminants of emerging concern, are found with other solid materials in the wastewater effluent principally due to household washing and cleaning processes. While microplastics occurrences and related ecological concerns have been well studied in marine and other aquatic environments in the past, investigations in groundwater systems are extremely rare. Toxicological and health concerns with microplastics presence in groundwater used or potentially used for human water supply are a result of ability of microplastic particles to absorb persistent organic pollutants (POPs), while the wider ecological and environmental concerns are related to springs – points at which water flows from an aquifer to the land surface affecting surface water quality and ultimately contributing to microplastic pollution in the oceans. In this study, a number of karst springs in the west of Ireland have been sampled over time for quantification and identification of microplastic particles using Fourier-transform infrared spectroscopy (FTIR) and for the analysis of fluorescent whitening compounds (FWCs; well-known indicators of human contamination since their origin is mostly from laundry detergents). Our results show a significant correlation between microplastic particle counts and detected FWCs signals at different springs, which helps to understand the contribution of household-derived contaminants to this environmental problem.

3.03P.10

First record of the occurrence and composition of microplastics in sediments in Eyjafjordur, Iceland.

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There is a growing concern regarding plastic pollution, its distribution and effects on the ecosystem, both within the scientific community and among the public. Scientists have measured microplastic (plastic fragments less than 5 mm in size) in almost every corner of the world, including in the marine environment, both in the sediment and the water column. The accumulation of the microplastic in the food web is threatening the ecosystems health but microplastic has been reported in fish and other marine organisms as well as in birds, mammals and lately in human stool in number of publications. The sewage is an important source of microplastic pollution in the costal sea, depending on the sewage treatment, more or less of the microplastic in it ends up in the sea. This study is the one of first records of the occurrence and composition of microplastics in costal sediments in

Iceland. The sampling site Eyjafjörður, is the longest fjord in Iceland, located at the north coast. Several small towns are situated by the fjord, the far biggest being Akureyri with 18.000 inhabitants and none of them have any sewage treatment installed. The samples were prepared for analysis using a sequence of enzymatic driven degradation followed by a strong oxidizing reaction and a density separation based on a zinc chloride solution. Floating particles were filtered on 1 µm fiberglass filters. A fast and sensitive method based on a GCMS-pyrolysis was developed and adopted for the study. The results show considerable amount of microplastics in sediments in the vicinity of the sewage outlet of Akureyri where PA66, PVC and PET were the most accumulated polymer types.

3.03P.11

A temporal sediment record of microplastics in Haukadalsvatn, a lake in Iceland

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Research has found that plastic pollution is being deposited into the fossil record with contamination increasing dramatically since the 1940s. It has been suggested that the plastic layers could be used to identify the start of the Anthropocene, the proposed geological epoch in which human activities have come to dominate the planet. In this study a well-dated sediment core collected from lake Haukadalsvatn in west Iceland, covering the whole of the Holocene, was used to provide data on the historical accumulation trend of microplastic waste. Samples were analysed from the top section of the sediment core dated from 1958 to 2002 CE as 5 yearsЙ_ time series. Microplastics were extracted from sediments by a combined multistep enzyme and oxidizing purification steps followed by $ZnCl_2$ dense-liquid separation. The size class distribution and the chemical characterization were performed by µ-FTIR microscopy followed by GCMS-pyrolysis. Polyethylene, polypropylene, and polystyrene microplastic particles were among the most dominant identified polymers. A total of 12 polymers where identified. Proliferation of microplastics is evident in the core from the late 1960s to the present. Relatively low numbers of particles were found in older sediments, comparable to laboratory blanks. This study shows that robustly dated sediments can add an important temporal perspective to our global understanding of microplastics sources, transport pathways, distribution and temporal trends.

3.03P.12

Bis(4-chlorophenyl) sulfone (BCPS) found in Austrian fresh water fish and cormorants (Phalacrocorax carbo) indicating biomagnification - a screening study

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Bis(4-chlorophenyl) sulfone (BCPS, CAS No. 80-07-9) is manufactured and imported in the European Economic Area (EEA) in 10,000 to 100,000 tons per year. The substance is used as monomer for the production of several groups of polymers like polysulphones and polyethersulphones. Residual amounts of monomer remain in the polymer matrix. These polymers exhibit some special features at high temperatures (high strength, heat resistance and excellent dielectric properties) and belong to the group of thermoplastics, which are used e.g. for the production of microwave cookware, water and oil pumps, as electric components, membranes for food processing and wastewater treatment. BCPS is very persistent and had been previously found in biota in the Baltic region. In the present study, fresh water fish and fish-eating birds (cormorants) were examined and detection frequency of BCPS in biota samples was 100%. Healthy specimens of the following fish species (top predators) were collected by electrofishing at two Austrian locations: *Sander lucioperca*, *Silurus glanis*, and *Lota lota*. In total, whole fish samples were analyzed for BCPS and DDT (including metabolites). BCPS levels in fish ranged between 1.3 and 9.3 ng/g fat, DDT (incl. metabolites) was not detected. In addition, breast muscle and liver samples from six cormorants *(Phalacrocorax carbo sinensis)* were investigated. BCPS levels in breast muscle were in the range of 4.3 to 40 ng/g fat (mean: 16.3 ng/g fat, n=6) and 28 to 86 ng/g fat (mean: 53.5 ng/g fat, n=6) in the liver samples. BCPS concentration in liver was 3.3-fold higher than in muscle tissue. The pollutant in fish-eating birds was higher than in fish, indicating biomagnification. One of the cormorants had ingested fish with a BCPS level of 5.5 ng/g fat; BCPS levels in the cormorant were 23 ng/g fat (breast muscle) and 28 ng/g fat (liver), suggesting biomagnification values (BMF) of 4.2 and 5.1, respectively. A BMF value significantly higher than 1 can be considered as an indication for very high bioaccumulation.

3.03P.13

Safety of commercially available child-dedicated chocolate food products on the Polish market - PBDEs levels and children's exposure estimation

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The object of the research were child-dedicated chocolate food products containing the polymeric package with a toy inside. We used three types of these products available on the Polish market), with different price, the composition of chocolate and the type of toys. In the study we developed a new analytical procedure for polybrominated diphenyl ethers (PBDEs) determination in all parts of studied products. This procedure eliminates the costs associated with the use of liquid nitrogen when grinding polymers with a cryogenic mill. The results showed the PBDEs presence in all parts of the cheapest product. The highest PBDEs concentration ranged from 5.32 to 1768 ng/g, was noticed in the case of chocolate samples. Despite the detection of PBDEs in parts of the tested food product for children, no high risk associated with exposure of children to PBDEs has been reported, based on the hazard quotient (HQ) and cancer risk for BDE-209 ($CR_{BDE-209}$) assessment.

3.03P.14

Determination of the retention time of microfibers and their effects on behaviour in zebrafish Danio rerio

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Microfibers are emerging pollutants that are found ubiquitously in the environment. These fibers consist of various materials with different sizes and shapes and enter aquatic systems via washing of clothes and other textiles. Fibers may originate from synthetic polymers or natural materials, and they have been found in the gut contents of several organisms. However, there are very few studies describing their effects on aquatic organisms, and knowledge about their presence and effects in freshwater organisms is limited. Therefore, the present study aimed to 1) determine the retention time of microfibers in the gut when fish are exposed via contaminated water, and 2) observe effects of textile microfibers on behaviour in adult *Danio rerio* (zebrafish). For retention time analysis, zebrafish were exposed to standard black polyethylene terephthalate (PET) textile microfibers (length 500 µm, 25000 fiber/L) for 5 minutes before transfer to clean water. Fish were killed after 0h, 1h, 2h, 6h, 24h, and 48h and gill and gut tissue dissected. Microfibers were isolated from the gut tissues and results show the highest numbers of fibers at 0h, 5 min after the start of the exposure, and no fibers could be found after 24 hr. In the gill tissues, the highest numbers of fibers were found after 2 h, but some degree of retention was observed after 48h. The results of this study show that ingested microfibers are excreted rapidly demonstrated with low retention times as shown statistically ($p< 0.0001$). For behavioural analyses, zebrafish are exposed to cotton textile microfibers, PET textile microfibers, and black textile (46% polyamide, 25% acrylic, 14% alpaca, 12% wool and 3% elastane) microfibers with four size classes (< 500 µm, 500-1000 µm, 1000-2500 µm, 2500-9300 µm; 1000 fiber/L) The effects of these fibers on schooling behaviour, boldness, and locomotion in adult zebrafish are being assessed.

3.03P.15

The impact of plastic and car tire rubber leachates on the Mediterranean mussel Mytilus galloprovincialis

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Widespread ocean contamination by plastic materials is causing increased concern regarding their potential detrimental consequences on marine ecosystems. While many recent studies have addressed ingestion and effects of microplastics (MP) and nanoplastics (NP) on most marine taxa, the risks posed by leached chemical additives have so far been studied to a much lesser degree. The current study aimed to assess the impact of aqueous leachates from car tire rubber (CTR), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC) on the Mediterranean mussel Mytilus galloprovincialis. Endpoints encompassed early life stage parameters and cellular/physiological biomarkers measured following in vitro and in vivo assays. The exposure of mussel gametes to leachate concentrations ranging from 0.6 to 100% of all tested materials resulted in a dose-dependent and significant ($p < 0.05$ vs. control) decrease of the fertilization success, with the CTR leachate resulting in the lowest effective concentration (EC50) of 36.4%. Highly significant effects were also induced by all leachates on mussel embryo-larval development, with effects ranked as CTR > PVC > PP > PS > PET and EC50 values in the range of 2.2 (CTR) - 96.8% (PET). Among the tested leachates, only those from CTR and PVC were found to significantly affect the motility and survival of mussel D-shaped larvae, with EC50 values in the range of 18-25% and 55- 59%, respectively. Leachates also affected cellular endpoints of general stress, including the lysosomal membrane stability in adult mussel haemocytes, which decreased significantly following in vitro exposure to PS, PP, PVC and CTR leachates and (7-day) in vivo exposure to PP, PVC and CTR leachates. To screen for potential relationships between the leachates toxicity and chemical composition, all leachates were analyzed by GC-MS, which revealed the occurrence of plasticizers, antioxidants, antimicrobials, lubricants, vulcanizers and metals at concentrations spanning from hundreds of ng/L to thousands of µg/L depending on the tested polymers. This study provides clear evidence of plastic leachate toxicity on mussel ontogeny and cellular fitness, highlighting the need for a better understanding of their overall impact on marine ecosystems.

3.03P.16

Effects of microplastic-associated chemicals on European perch (Perca fluviatilis): an exposure through sediment and leachates

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Microplastic particles (MPs) are found globally in the aquatic environment. The chemicals associated with MPs can gradually leach into the water, leaving aquatic ecosystems potentially exposed to an altered chemical background. Studies on MPs have so far focused on the acute effects of ingestion, and the possible trophic transfer of the particles. Meanwhile, the projects examining the possible effects of chemicals associated with plastic particles are few. Thus, the aim of this study was to investigate acute and chronic effects of low-level exposures to MPs and their associated chemicals. European perch (*Perca fluviatilis*) were exposed to a realistic mixture of secondary MPs, originating from consumer products and car tires, mixed with sediment. After four respective seven months of exposure, the potential chemical effects were investigated by measuring various biomarkers (EROD, AChE, GR) and biomarker gene expression (CYP1A, VTG, DIO1, DIO2, DIO3, TRHA and TRHB). After four months, there was a significant increase in hepatic EROD activity in the exposed fish when compared to controls. After seven months, the differences in EROD activity between controls and exposed fish were lower, but still indicating a higher activity among the exposed fish. Regarding the other biomarkers, there were no significant differences between treatments. Thus, some effects of leaching chemicals could be detected after a prolonged and constant exposure to MPs in sediment. These results may be important in order to bring attention to the altered chemical background in aquatic environments caused by MPs, and their effect on aquatic organisms. During the acute study, perch were exposed to either methanol or water extracts of the same mixture of MPs for one week. The same biomarkers as above were measured. EROD activity was significantly higher in both exposed groups when compared to controls, while gene expression of the thyroid hormone metabolizing enzymes DIO1 and DIO3 was slightly upregulated in the water respective methanol extract exposures. This indicates that exposure to chemicals in MP leachates causes biological responses. Moreover, a chemical analysis showed that the methanol-extracted leachate contained known plastic additives such as phthalates, triphenyl phosphate and PAHs. Hence, this study suggests that they have the potential to leach from consumer products, thus becoming more bioavailable to aquatic organisms via interactions with organic or lipophilic matter.

3.03P.17

Cu affects worm bioturbation and the fate of microplastic particles in sediment

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The increasing production and use of plastic has led to widespread distribution of plastic (including microplastic; < 5 mm) in the environment. After release into the aquatic environment, these particles will be distributed in water and sediment. Infauna, such as the oligochaete Tubifex tubifex, living in the sediment, will greatly affect the nutrient and contaminant distribution and availability in sediment via their bioturbation activity. Contaminants on the other hand (e.g., the toxic metal Cu which is omnipresent in sediments) may affect bioturbation of infauna and thus in turn affect the distribution of microplastics in the environment. The aim of this study was to examine 1) the influence of T. tubifex bioturbation on the fate of microplastic in sediment, 2) the effects of sediment-associated Cu on T. tubifex bioturbation and feeding activity and thus 3) the influence of Cu on the fate of microplastic in sediment. T. tubifex worms were exposed for 7 day to clean or Cu-spiked sediment (125 µm) at 6 Cu concentrations (up to 626 µg Cu g-1 dw sediment). Effects of Cu on worm mortality, feeding rate (fecal layer thickness), bioturbation (particle diffusion and advection) and thus microparticle fate was assessed. A thin layer of green fluorescent polyethylene microspheres (90-106 µm) was added on top of the sediment and vertical particle transport was followed over 7 days using calipers. Worm mortality and feeding rate were affected by Cu exposure concentrations. In controls, microplastic particles became buried in sediment over the 7 days indicating active bioturbation by T. tubifex. There was a tendency for a decreasing bioturbation activity (both advection and diffusion) with increasing Cu concentration in the sediment, except for at the lowest exposure concentration (2 µg Cu g-1 dw sed.) where bioturbation activity increased. Consequently, presence of one contaminant, such as Cu, may affect bioturbation and thus indirectly the fate of microplastic particles in sediments: i.e., decreasing burial of microplastic particles at higher Cu concentrations.

3.03P.18

Increasing the environmental realism of laboratory exposures of Daphnia magna to polyethylene microplastics affects the degree of toxicity of the plastics and associated co-pollutants

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Microplastics are increasingly recognised as a contaminant of concern in the environment, however recent studies have shown that often the toxicity of pristine plastics under idealised laboratory conditions are neglibible. The potential of microplastics to act as vectors to chemicals (co-pollutants) commonly found in the environment, often termed the Trojan Horse effect, has been highlighted. This is when co-pollutants bind to the plastic surface and are thus carried into organisms in higher concentrations than they would otherwise achieve based on chemical potential and equilibrium processes. Our research focused on the need to increase the environmental relevance of the testing media in addition to these co-polluatant exposure scenarios, and highlights how this can influence the degree of toxicity observed using OECD tests on *Daphnia magna (OECD* 202 & 211). In this sudy we accessed the affect of 3 chemicals that are often found in waste water (Triclosan, Sodium Lauryl Sulphate (SLS) and Diclofenac) in additon to polyethylene (PE) microbeads. The aim was to understand if the variation in the media and mixture complexity of the chemical & microplastic exposures changed the degree of toxicity by combining three main pathways of analysis: 1) A mass balance using HP-LC-MS to quantity the chemical absorbance, release and transfer in the PE mixtures with daphnia;2)The daphnia excreted proteins, and the protein corona on the PE beads were quantified3)The acute (OECD 202) and chronic (OECD 211) toxicity tests were performed in the bare and conditioned MPs and the MP-cocontaminant mixtures to determine the effect that both realistic exposures and mixtures All exposed daphnia had a decrease in growth compared to the control population, however the extent of the growth reduction varied among exposures, with the diclofenac population having the largest reduction. Through the total protein concentrations, we can show that the SLS significantly reduced the amount of protein on the PE surface, which can affect the daphnia interaction with the particles. By combining these results with neonate production, growth and acute toxicity we can determine how various media and chemical co-pollutant mixtures impact the toxicity of polyethene microplastics, and how we can increase the environmental relevance of toxicity assays to include mixture effects, using media conditions that are more representative of the real-world environment.

3.03P.20

Marine Biodegradability and Toxicity of Commercially Available Biobased Plastics -A Sustainable Alternative To Petrochemical Plastics?

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The presence of conventional petroleum-based plastics in marine ecosystems is of increasing global concern. Slow biodegradation and ingestion of plastics and microplastics may result in adverse effects in marine organisms from different trophic levels. More easily biodegradable plastics produced from biobased sources have been proposed as alternatives. Global production of new bioplastics is therefore increasing but there is often limited knowledge about the fate and effects in marine environments. In this study, we studied the aerobic biodegradability of several new bioplastic polymers in seawater and compared with conventional petrochemical plastics (e.g., PE and PET). The study also included a comparison of degradability and toxicity of microplastics (< 100 µm) originating from conventional plastics and microplastics originating from biobased plastics ("microbioplastics"). Biodegradability of plastics in seawater was measured by RAMAN spectroscopy and as O_2 consumption and CO_2 production. Fiber-optic oxygen sensors were used to study aerobic biodegradation in sediments. Marine ecotoxicity was examined by measuring inhibition of the marine bacterium A*liivibrio fischeri* and the marine rotifer *Brachionus plicatilis*. The study showed significant differences in biodegradability and toxicological signature of commercially available biobased plastics. Hence, it is important to examine the fate of novel polymer innovations in different environments and under different environmental conditions to select the most eco-friendly alternatives and avoid large scale release of unsuitable products.

3.03P.21

Problems with electrostatic charge when measuring the mass of plastics; a practical, problem-solving approach

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Plastic polymers are used in a multitude of products with a wide range of practical applications. They are widely used in the food-packaging industry, being inexpensive, durable and light, while keeping the contents fresh and sterile. Unfortunately, their widespread use and subsequent inappropriate disposal (i.e. littering as well as inadequate waste and waste water management) in combination with the described intended chemical properties, has led to their global introduction into the environment. Being durable, they can remain in the environment for decades. Though plastic pollution is one of the most pressing environmental issues of our time, little is known about degradation processes under natural conditions, e.g. the transformation from macro- to microplastic. Within the scope of the BMBF (Federal Ministry of Education and Research, Germany) project ENSURE ("Development of new plastics for a clean environment by determining relevant entry paths), the degradation and fragmentation process in semi-terrestrial zones is currently being investigated in artificial wave mesocosm systems of the German Environment Agency´s field station, where semi-natural conditions can be simulated on a larger than laboratory scale. The fragmentation processes of macroplastics and rates of secondary microplastic formation in the environment are crucial and not sufficiently investigated to date. Suitable parameters and methods have to be found first. One suitable parameter for the degradation in our artificial wave mesocom systems is the loss of mass of different test objects, like drinking bottles and films due to mechanical abrasion. Plastics display very low electric conductivity, which can lead to electrostatic charge on the surface of the materials. Friction or electrostatic induction can in consequence lead to the accumulation of electric charges on the surface of the plastic material, making gravimetric measurements very difficult when using sensitive electronic equipment. The poster presents a practical approach to tackling the problems of electrostatic charge with test objects e.g. drinking bottles and films.

3.03P.22
Adsorption of polychlorinated biphenyls on microplastics under environmental conditions.
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One of the greatest problems that the world is facing today is environmental pollution. Micro (MPLs) and nano-plastics (NPLs) are recognised by the scientific community as an emerging risk for the environment and human health. Main problems are the lixiviate of contaminants used in their formulation, their small sizes, wide occurrence and long life in the natural environments. Moreover, due to their properties, MPLs and NPLs can also enhance the transport of many organic chemical contaminants by adsorption/desorption processes. These proceedings, where plastic contaminants act as a passive carrier of hydrophobic organic substances, produce a potential impact on living organisms for the possibility to transfer them other co-contaminants present in the same compartment. The polychlorinated biphenyls (PCBs), are a specific group of persistent organic pollutants (POPs), that are characterised for their resistance to degradations, toxicity, bioaccumulation, biomagnification and long-term environmental transport [1]. In this context, the goals of this study were to study the adsorption/desorption behaviour between Polystyrene (PS), Polyethylene (PE) and Polyethylene terephthalate (PET) MPLs and PCBs under controlled conditions experiments. Aged MPLs were characterised by scanning electron microscopy (SEM), and the sorption isotherms of these interactions were studied though experiment carried out in microcosms emulating relevant environmental conditions along 50 days. Measurements were carried out at time 0, 4, 7 and 50 days by high-resolution gas chromatography coupled to high-resolution mass spectrometry (HRGC-HRMS). Finally, the results showed that the MPLs based on PS, PE and PET could act as carriers for PCBs in the environment; and in the Freundlich model was shown as the most suitable to explain these kinds of interactions.

Analysis of Microplastics and Nanoplastics - From Harmonisable Protocols and Data Treatment to the Peculiarities of the Environmental Nanofraction (P)

3.04P.1
Characterization of chemical and morphological changes in microplastics during bacterial degradation at environmentally relevant conditions
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Microplastic (MP) residues in food items and environments have received increasing public concern in recent years. Biodegradable plastic alternatives such as polylactic acid (PLA) are seen as a mitigation strategy to combat MP loads in the environment. Characterization of MP in environmental samples is challenging, however, as reference particles are often not similar to those found in real samples. Using a novel technique to create MP reference particles by ultrasonic treatment in alkaline solution [1] we produced PLA particles, which are suspensible in water, have a broad size range, display a multitude of shapes and show modified particle surfaces with comparable properties to aged MP found in environmental samples. To enable studying bacterial growth and degradation, sterile and foreign particle-free PLA particles were produced, thus requiring adjustments to the established procedure by combining standard operating procedures for working in sterile and particle-free environments. We exposed the suspended particles to two bacterial species, Sphingomonas koreensis and Pseudomonas libanensis, both part of the natural microbiome present in water and soil. Growth of the bacteria and changes in particle morphology were tracked in aqueous solution over the course of 28 days at 25 °C. Growth of the bacteria was monitored by plating on agar plates. We observed an increase in colony forming units after an initial incubation period for both bacterial species. The increase in bacterial concentration was also visible in SEM images. Our observations show a change in Raman spectra of the PLA particles subjected to bacteria compared to PLA particles suspended in sterile water. Morphological changes were visible in SEM images and, for larger particles, also to the naked eye. This suggests that biodegradable plastic can be subject to changes of its surface, which leads to changes in the original polymer spectrum. Specifically, an elevated baseline and additional bands could be observed, which might derive from degradation of the polymer or the colonization of the polymer particles with a biofilm. This indicates that degradation is not only observable under standard degradation conditions (58 °C, composting plant) but also under conditions close to nature. References: [1] von der Esch E, Lanzinger M, Kohles AJ, Schwaferts C, Weisser J, Hofmann T, Glas K, Elsner M, Ivleva NP. Front Chem.; under review

3.04P.2
Suspected screening of nano- and microplastics composition in the Ebro Delta area
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Micro (MPLs) and nano plastics (NPLs) are recognised as an emerging risk for the environment and human health. MPLs and NPLs are difficult to degrade, can lixiviate biologically active compounds, adsorb and transport co-contaminants and be accumulated by biological interaction with fauna or due to their potential ingestion. Therefore, they can affect aquatic environments by physic-chemical interactions. In this regard, the EU have implemented a series of programs and measures to maintain and restore the good environmental status of the marine environment. However, their implementation requires first to solve the significant gaps in knowledge. Among them, one of the main limitations is the lack of analytical approaches for identification and quantification of smallest size MPLs and NPLs in complex samples. The main objectives of this work are to present a suspected screening approach to assess MPLs and NPLs (toluene soluble) and associated chemicals in complex environmental samples by size exclusion chromatography coupled to high-resolution mass spectrometry (SEC-HRMS) and, second to assess their occurrence in water, soils and biota from the Ebro Delta area (NE of Spain) in two sampling campaigns comparing summer and winter-spring periods. The major identified polymers contribution to NPL/MPLs contamination has been PP, followed by PS and PE, but also PI and PBD were detected. Furthermore, polymers with chains between 1000 – 2000 Da were the most frequently found. Sampling sites near the wastewater treatment work (WWTW) discharges and urban population presented the highest number of different polymers. Also, semi-quantitative measurements showed the highest concentrations, e.g. in the pg/L range in waters. Comparing the presence in marine bays and the river samples, a significant impact was found in the river samples, as expected due to the dilution. The authors detected that the strong contribution of MPLs in surface river waters is between 100 and 1000 µm. But in the present work comparing the results for the summer and the winter campaigns, a relevant contribution of the seasonal population is shown during summertime as well as the activities during this season. In addition, other contaminants frequently used in plastic formulations were as well identified including bisphenol A, phthalates, perfluorinated compounds and inorganic polymers as siloxanes.

3.04P.3
Plastic in our waters; microplastic analysis in The Netherlands
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Widespread occurrence of microplastic in marine and freshwater ecosystems is evident. Plastic in the environment is subjected to various degradation processes resulting in the formation of smaller fragments. Apart from high amounts of fibers, these fragments are the pre-dominantly found form. Recently, it was shown that fragmentation of polystyrene, polyethylene and polypropylene into nanoplastic occurs. This shows the need for more advanced analytical methodologies determining small micro- and nanoplastic. We present an overview of our studies addressing riverine transport, sediment analysis and underlining routes of exposure to organisms and humans. In the last years, sampling and identification protocols are getting more and more standardised and exchange of

results improve method development, but the comparability of results is yet quite limited. Studies that rely on visual sorting/counting of microplastic exists while our laboratory work has been focused on chemical-analytical techniques. We explored a range of techniques. This was aimed at material identification and attempt to detect very small particles (nanoplastics), not visible by the human eye. We tested the usability of several analytical techniques for the detection of microplastic, such as Fourier- transform infrared (FTIR)- microscopy to Pyrolysis- and TDS-GC-MS. Our most recent technique involves the application of Laser Direct Infrared (LDIR). The LDIR chemical imaging system provides a sophisticated new approach to chemical imaging and IR spectral analysis. With our studies, we study the plastics in many types of environments, but mainly in waters. Preliminary results may be presented at the conference, on the occurrence and applied to the assessment of solutions to reduce the outflow of plastics in the environment.

3.04P.4

Microplastic pollution in the Garonne catchment: seasonal variability and consequences of rainfall

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Microplastic (MP) pollution is considered as a global environmental crisis due to its potential adverse effects on biodiversity. Because they are surrounded by intensive anthropogenic activities, freshwater ecosystems represent a direct supply of pollution and play an important role in the transport of these debris. Therefore, a better understanding of its dynamics is crucial to risk assessment and management of this contamination. This study aims to understand the spatial and temporal variability of MP pollution in the Garonne catchment (main river and tributaries) and identify the potential drivers of this variability by providing a comprehensive monitoring of MP pollution in a freshwater ecosystem. For the purpose of this study, large MP (LMP) from 1-5mm were quantified and analysed to determine the polymer type, size, colour and mass. MP pollution was quantified by water filtration in a total of 14 sites: 6 located along the main Garonne River (upstream and downstream of a major city) and 8 for major tributaries flowing through various landscapes. Sampling was performed in four seasons of 2019 and replicated three times at each sampling, totalising 168 samples. Additionally, 3 sites (upstream, downstream and within the major of Toulouse) were monitored with a higher temporal resolution during 2 months to assess the effects of rainfall on MP pollution. A chemical digestion protocol was developed and applied to remove the organic matter content from the samples. Characterization of particles was performed under optical microscope, image treatment and Fourier transformed infrared (ATR-FTIR). A total of 2800 particles were detected. Preliminary results demonstrate an increase of LMP pollution in the mainstream of Garonne River after Toulouse agglomeration, resulting in > 0.3 particles/m3. Different levels of plastic pollution are present at the eight different tributaries, varying from 0.04 to 3.3 particles/m3, and are likely driven by different land use and levels of urbanisation. An inundation episode may represent more than ten times augmentation of LMP, from 0.09 to 1.17 particles/m3, in the mainstream. About 50% of LMP are polyethylene and the predominant colours are black and white. The sample preparation of the small MP (SMP) fraction, from 25-999 µm, is under development in order to a faster and sensible analytical identification by pyrolysis-gas chromatography-mass spectrometry. First attempts lead to a limit of detection in the nanogram range.

3.04P.5

One Pot Method for the Collection and Preparation of Natural Water for Microplastic Analyses: Microplastics in the Mississippi River System During and After Historic Flooding

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Microplastic (MP) pollution is a major concern in the aquatic environment. However, methods for sampling and preparation of natural water to quantify and characterize MPs therein are not harmonized, particularly for smaller plastic particles (< 333 µm) that not captured by many sampling nets. Further, most methods require multiple sieving and transfer steps, which increase the likelihood of both contamination and losses of MPs during processing. These issues lessen the quality and comparability of experimental data and limit conclusions that can be drawn. Here, we describe a one-pot method which minimizes sample contamination, avoids transfer losses, and simplifies sample preparation. The method prepares samples in the same vessel (Mason canning jars) that they are collected in right up to the point where they are transferred onto filters for analysis. Because it uses relatively inexpensive and easily obtained and assembled materials it is suitable for many MP surveys. We evaluate its effectiveness compared to traditional sieving, demonstrating that it lowers contamination, losses and carryover between samples and improves recoveries. The method is particularly useful for the analysis of MPs that can't be easily manipulated with tweezers or are too small to be seen with the naked eye. We applied the method to water collected from the Mississippi River and its major tributaries during and after historic flooding in 2019. Microplastics with sizes down to 30 µm were detected and characterized using both fluorescence microscopy and micro-Fourier transform infrared spectroscopy (FTIR) imaging. The concentrations of MPs were generally lower during decreased during the flooding likely due to dilution. Mean concentrations (counts/L) during the flooding ranged from 14 to 45 in the Tennessee and Ohio Rivers, respectively, and from 35 to 83 during summer (low-flow) conditions in those same rivers, respectively. Loads of MPs tended to increase down the Mississippi River, ranging from ~80-120 trillion particles per day in the lower Mississippi River depending on flows. Most of these particles (>70%) were in the lowest size fraction (30 – 90 µm) and consisted primarily of fragments, followed by fibers, and beads. Imaging by FTIR identified polyethylene, polyester, polypropylene, and nylon were the primary type of microplastic.

3.04P.6

Method development for microplastic analysis in atmospheric bulk-deposition and first evidences from a transect in Bavaria (GER)

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Microplastic (MP), synthetic particles and fibers < 5mm, became an ubiquitous contaminant in marine, limnological and terrestrial environments. MP is suspected to have negative effects on organisms due to ingestion or interaction with other pollutants. Up to now, only little attention is paid to the atmospheric compartment, although MP with its low density can easily be dispersed by wind. Only a handful of studies addressed the issue of atmospheric (long-range) transport and deposition of MP. This study proceeds over five months (October 2019 – February 2020) to investigate the atmospheric deposition of MP over a four-site transect in Bavaria (Germany). The characteristics of the sampling sites vary from remote alpine (environmental research station Schneefernerhaus/Zugspitze), alpine valley (Garmisch-Partenkirchen), peri-urban lowland (Augsburg) and rural lowland (Kulmbach). Samples are collected electronic bulk deposition samplers equipped with glass funnels (Ø 20 cm) and glass bottles. The sampling interval is 28 days. Validation of the pretreatment of samples was performed by recovery experiments with spiked samples. The sampling is validated by continuous duplicate sampling at one sampling site. Blank samples of both, the sampling and the sample processing, are included into analysis. All deposition samples were prefiltered and particles > 1mm were analysed separately. The prefiltered solution was filtered over a 10 µm-mesh stainless steel filter, since herein used µFT-IR spectroscopy is limited to particles > 11 µm. For a first discrimination of MP and natural particles, staining of the particles with a lipophilic fluorescent dye (Nile red) was carried out prior to investigation with a fluorescent microscope (Zeiss Microscopy, 20x objective, 475-555nm, ZEN 2.6). Samples are initially identified by visual observation to gain information about the ratio of microplastics and natural particles. Selected samples were investigated by µFT-IR spectroscopy (Hyperion 3000, equipped with 15x IR-objective and 64x64 FPA detector, Bruker, Germany) to identify polymer species of suspect particles. With this study, we give a first glance on the amount and composition of MP in atmospheric bulk-deposition over southern Germany.

3.04P.7

Separation of microplastics from aquatic systems using reused zinc chloride

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Microplastics (plastic particles with < 5 mm; MPs), considered as emerging aquatic pollutants, can derive from a variety of sources (primary and/or secondary), reach high densities and interact with abiotic and biotic environments, depending on their shape and polymer type/specific density. Their ubiquity, high persistence and toxicity has led to an increasing societal and scientific concern, however there is not yet a standardized method for separating MPs from aquatic systems resulting in inaccuracy data. The most adopted methodologies include sieving, organic matter degradation and density separation (D.S.) methods, vacuum filtration, quantification and identification of the polymers. Among D.S. methods, sodium chloride, zinc chloride ($ZnCl_2$) and sodium iodide (NaI) are the most used salt solutions. These high concentrated or saturated salt solutions are mixed with the samples, notwithstanding they are not equally efficient. Currently, $ZnCl_2$ is reported as the most efficient (density of 1.6 – 1.8 g/cm^3) and less expensive methodology for separating MPs of low and high density (comparing with NaI), however this salt solution is also very hazardous, corrosive and requires large amounts of product. In order to overcome these disadvantages, the present study aimed to present a methodology reusing the $ZnCl_2$ solution and ensuring the efficiency of the product. For this substance, there is an immense lack of information when compared with other substances (e.g. NaI). The methodology used in this study included MPs preparation (polymers: polyethylene terephthalate, polyvinyl chloride; sieving: 5 mm), preparation of artificial samples, D.S. methodology (with $ZnCl_2$), vacuum filtration and visual inspection (using a stereomicroscope). Results showed that the $ZnCl_2$ solution can be reused at least five times with an efficiency above 95%.

3.04P.8

Chemometric Classification and Quantification of Microplastic (< 100 µm) using µ-FTIR Imaging

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Microplastics are defined as microscopic plastic particles in the range of 1 µm - 5 mm. These small particles are classified as primary microplastic when they are manufactured in this size range, whereas secondary microplastics rise from the fragmentation of larger objects. Microplastics are a widespread emerging pollutant and investigations are underway to identify sources, environmental occurrence, fate and potential harmfulness to ecosystems and human health. However, progress is hindered by the lack of suitable analytical methods for rapid, routine and unbiased measurements. This work aims to develop an automated analytical method for the characterization and quantification of small microplastic (< 100 µm) using µ-FTIR imaging and multivariate classification tools. Partial least squares discriminant analysis (PLS-DA) and soft independent modelling of class analogy (SIMCA) models were evaluated, applying different data preprocessing strategies for classification the most common polymers produced worldwide (PE, PET, PMMA, PVC, PC, PP, PU, PA and PS). Statistic models assessment were realized using the misclassification error, specificity and sensibility for cross-validation and prediction steps. The hyperspectral images were also analyzed to automatically quantify particle abundance and size. PLS-DA presented better analytical performance in comparison with SIMCA models with greater specificity, sensibility and misclassification error. SIMCA models presented lower sensibility, but it did not show any multiples classification and low misclassification rate. However, SIMCA models could not classify pixels toward the edge of particles due to the deteriorating spectra quality in this region. PLS-DA however performed better, and was less sensitive to edge effects on spectra and poorly focused particles. The size distribution was efficiently estimated for plastic particles bigger than 20 µm. These particles are close to the diffraction limit of IR spectroscopy providing poor spectra and low signal-to-noise ratio that underestimate the classification and diameter determination. The approach could also classify particles tested on: individual samples of each plastic, samples with plastic mixtures with and without sediment addition. The proposed method demonstrated a fast and efficient automated approach for microplastic polymer characterization, abundance numeration and size distribution with substantial benefits for method improvements and standardization.

3.04P.9

Rapid identification and quantification of microplastics using quantum cascade laser (QCL) imaging

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The presence of microplastics (MP) was proven by scientist in all compartments of the environment. With the growing concern for sciences and societies for this type of pollutant, a wider understanding in sources, transport process and human health is moving into the focus of research. Still, the design of such studies is challenging, as a large number of highly detailed datasets are mandatory, while standardized protocols for the three steps of MP analysis need to be found:1) sampling, 2) sample extraction/purification and 3) chemical identification. The size of these particles creates challenges in each of these steps. Currently, FTIR imaging provides a relatively fast chemical identification via automated analysis of complete filter areas. Modern systems measure within 4 hours a field sizes of 12 × 12 mm² (pixel resolution = 11 µm). Monitoring or large scale studies face the challenge of low throughput combined with a permanent liquid nitrogen supply 24 hours a day. To overcome these limitations we used a novel approach to measure a complete filter area in less than 1 hour. The Analysis of MP was performed using a quantum cascade laser (QCL) based microscope, Daylight Solution Spero QT. This system has three advantages compared to existing FTIR imaging systems: 1) liquid nitrogen is obsolete while, 2) 230.400 spectra are measured simultaneously on an area of 2 × 2 mm² using a focal plane array detector with 480 × 480 detector elements (pixel resolution = 4.2 µm) 3) within 1 minute including data storage. Compared to FTIR a smaller spectral range of 1800 – 950 cm^{-1} is available while a larger number of spectra is collected. To investigate the performance of the instrument, it was first validated against known polymer samples. In the next step, a database for the automated analysis of these datasets similar to FTIR imaging was developed and finally the results were compared to those achieved by FTIR imaging. A QCL-based microscope achieves similar results compared to FTIR imaging, but with higher spatial resolution and much shorter measurement times. Typically, the measurement of a 12 × 12 mm² area took only 36 minutes. The smaller wavelength had few disadvantages but mainly advantages on chemical identification. Combining automated analysis with the new system we realized a novel fast and versatile tool to identify MP similar to FTIR imaging, while allowing the measurement of large sample sets at high resolution in a significantly shorter time.

3.04P.10

Analysis and evaluation of elemental composition of plastic items

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The input of plastics into the oceans is high and is expected to increase. Plastic input from land into oceans was estimated between 4.8 – 12.7 million metric tons [1]. While large numbers of studies on the chemical analysis of plastics and on the toxicological effects were published to date, one aspect has rarely been considered: Is it possible to identify plastic items based on their specific elemental composition? In this study, a total of 173 polymer samples of different polymer types and applications were analyzed for their elemental composition. Aged plastic samples were collected from urban environment, from a lake reservoir and during an expedition through the pacific. Pristine plastic samples were collected from household items as well as from electrics supplies. Furthermore, 30 tire tread samples from used tires were obtained. Samples were cut to small pieces of approximately 1 mm length and dissolved using microwave-assisted acid digestion. Analysis of the digest was conducted using ICP-MS and ICP-OES. The concentration results were statistically assessed by the means of multivariate statistics (principal component analysis (PCA) and k-means clustering). PCA revealed that the first two principal components could only explain approximately 30% of the total variation and differentiation between the samples was hardly possible. Therefore, k-means clustering was performed on the dataset, leading to a total of 12 clusters. Here, two clusters were identified that consisted only of tire rubber samples and did not include any other plastic sample. Approximately 80% of the remaining samples clustered together in the same cluster, regardless of polymer type, application or sample origin, while several clusters consisted only of one or two samples, indicating outliers. A hierarchical clustering method led to a similar outcome. Eventually, support vector machines were used to identify groups of samples using the polymer type as response vector, i.e. as a grouping variable. This approach, however, did not lead to a successful classification of plastic samples based on their elemental composition either, possibly due to a limited sample number. To summarize, identification of plastic items based on their elemental composition was not possible in the available dataset with the exception of tire rubber, which was clearly separated from all other plastic samples. [1] Jambeck, J. R. et al. 2015. Plastic waste inputs from land into the ocean. Science 347(6223), pp. 768–771.

3.04P.12

Qualitative Analysis Of Microplastics: A Problem To Be solved

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Trace detection of finely divided plastics, usually called microplastics, is a growing problem especially due to the accumulation of this type of waste in marine environments. They are considered potential contaminats, but it is not known exactly how it affects our health. Many studies have been carried out on the presence of milliplastics (mm), especially in seawater, marine environment, wastewater and drinking water but there is no standardized methodology. Nowadays, most of the applied techniques for microplastics analysis have been techniques of counting using magnifying glasses, fluorescence microscopy with Congo red staining, FTIR microscopy, Raman, and TGA and DTS thermo gravimetric analysis, coupled or not to other instrumental techniques. IPROMA has developed a method using Automated Thermal Desorption (ATD) coupled to Gas Chromatography (GC) and Mass Detection (MS) that consists at the controlled thermal degradation of different kind of plastics to get a pattern of them. This technique allows us to know the type of plastics present in the sample, as well as, the concentration of them. This method is not based on the measurement of particles; it is based on the intrinsic property that plastics have to breakdown the polymer at a certain temperature, originating different compounds. This property has been described by several authors (D.J. Hurston, 2010), (Craig L. Beyler), (M. Sarker, 2013) on studies about degradation and thermal decomposition of polymers. The chromatographic pattern and the thermal degradation products for each of the compunds object of study is obtained. From these degradation products it is possible to generate a calibration curve. This new methodology can be applied to atmospheric samples, sea salt, sediments, beach sand and, of course, water.

3.04P.13

How can we answer the question "How many MP particles do we have in our sample?" accurately in an acceptable timeframe?

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Microplastic (MP) is a heterogeneous particulate impurity derived from plastic as it is subject to environmental influences such as UV irradiation and mechanical strain. The main characteristics of the analyte are the variety of polymer types (at

differing stages of degradation), sizes (1 µm - 1 mm) and shapes (fragments, fibers, films, and spheres). These already complex samples have notoriously low microplastic contents, compared to the native particles and are famously prone to contamination. Consequently, to deliver a satisfactory answer all chemical and morphological characteristics of MP should be analyzed and quantified for each sample.[1] The general scheme for single particle analysis of MP is to workup the sample and then deposit all remaining particles on a smooth filter surface. Thereafter, an image is taken using a microscope and the particles are recognized in the image either by a commercial software or by an open source alternative. The problem at this stage is, that the validation of the image recognition is often impossible or missing. Which is why we developed *TUM-ParticleTyper*, a calibratable image processing tool, and a validation protocol that can be applied to most particle detection software. With this software only a morphological assessment of the sample is possible, which delivers a size distribution and shape classification. But to answer our question the sample must still be chemically analyzed, for example via Raman Microspectroscopy. To achieve this, the coordinates for an automated measurement at the particle centers must be calculated, which is done by the software. Furthermore, a subsample needs to be selected as the measurement of all particles is impossible, as the timeframe for the measurement may become excessively large. Therefore, a random sampling tool was implemented in the software enabling us to answer "How many MP particles do we have in our sample?" in an acceptable timeframe (2 days). [1] P. M. Anger, E. von der Esch, T. Baumann, M. Elsner, R. Niessner, N. P. Ivleva, *Trends Anal. Chem.* **2018**, *109*, 214-226.

3.04P.14

Simple Generation of Suspensible Secondary Microplastic Reference Particles via Ultrasound Treatment

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How many microplastic particles are in my sample? This seemingly easy question is posed by many environmental chemists. But do we really have the appropriate methods to answer this question? To test and advance microplastic detection, reference particles, which mimic the properties of the microplastic found in the environment, are necessary. The challenge herein is that most microplastic detected in environmental samples originates from weathering and fragmentation of larger plastic debris. This leads to a heterogeneous mixture of polymers in broad size ranges, with multiple shapes, which have altered surfaces trough ageing. Furthermore, these particles are suspensible in water and sediment according to their respective density. To produce such particles a sonication procedure in alkaline medium was developed. It was tested with three different polymers (polylactic acid, polyethylene therephtalate and polystyrene) and the reproducibility was accessed for triplicates. The fragments were chemically and morphologically characterized via single particle Raman microspectroscopy, which reviled that the fragmentation delivers up to 105/15 mL high purity (68.4 – 81.6%) polymer fragments in various shapes. The morphology of the fragments was further characterized by scanning electron microscopy to reveal the eroded surfaces of the produced fragments. The in situ ageing of the fragments was characterized by comparing the spectra from attenuated total reflection FTIR and reflectance micro FTIR analysis, to environmentally aged microplastic. The result was that similar additional bands (from OH, C=O & COO) could be found. Since the fragments are formed in aqueous conditions they are already suspended. Our results show, that all important properties of environmental microplastic can be mimicked and that the procedure described yields reproducible results [1]. Reference: [1] von der Esch E, Lanzinger M, Kohles AJ, Schwaferts C, Weisser J, Hofmann T, Glas K, Elsner M, Ivleva NP. Front Chem.; under review

3.04P.15

Nanoplastic analysis by Raman microscopy on-line coupled to field-flow fractionation via optical tweezers

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Environmental nanoplastic is a recent issue that emerged from the plastic contamination problem and is of high interest due to the newly arising difficulties in the analysis of these very small particles. Physical characterization can be provided by established nanoparticle techniques, such as field-flow fractionation (FFF), but especially the determination of their chemical identity is essential to provide a reliable assessment of plastic particle contamination. For that vibrational spectroscopy, specifically Raman microspectroscopy (RM), can supply this information and enable the distinction from other (in)organic materials from naturally occurring colloids. Here we present the on-line coupling of Raman microspectroscopy to FFF for the spectroscopic analysis for separated particles. This on-line coupling has been enabled by an optical tweezer-based flow cell, which captures eluting particles and increases their interaction with the Raman laser for sufficient spectra acquisition, thereby circumventing the limitation of reduced sensitivity in diluted suspension. The flow-cell was validated for suspensions in the concentration range of around 1 mg/L (109 particles/L), a size range of 200 nm – 5 µm and different particle materials. It was then used to couple RM to two variants of FFF: Asymmetric flow field-flow fractionation (AF4) and Centrifugal field-flow fractionation (CF3). The resulting setup with RM, UV and multi angle light scattering detectors (FFF-UV-MALS-Raman) provided (i) particle separation by FFF, (ii) size information by MALS, and (iii) chemical identification by Raman spectroscopy. This on-line coupling could provide a high-throughput analysis of nanoplastic in facile samples such as drinking water. And, provided that an improvement of the methods enables samples that are rich in organic material, also environmental samples will be able to be analyzed.

3.04P.16

Nano-FTIR - plastics identification at the nanoscale

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Trends in size distributions of microplastics point strongly towards higher abundances at smaller sizes. This suggests a large reservoir of currently undetected nanoplastic (NP) particles with diameters < 1 µm in these sensitive environments. Such small particles may pass cell membranes, remain in organs and accumulate upwards in the food web. To date, little is known on where and in which state nanoplastics can be found in marine samples. An important factor in this is the identification of the particles. Nano-FTIR is a novel technique that has a high potential to help solving these questions. It combines the nanoscale local resolution of atomic force microscopy with near-field infrared spectroscopy resulting in the unprecedented combination of simultaneous size determination and material identification on a nanometre scale. Despite the small sampled volume, we here demonstrate the suitability of nano-FTIR recorded data for the identification of common polymers by library search procedures as applied for MPs. Measurement parameters as well as the role of data treatment will be addressed. Finally, the detection of plastics in samples obtained from the marine environment is shown.

3.04P.17

Nanoparticle Separation via Crossflow Filtration

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The omnipresence of microplastics (MP) in the worldÏ_s oceans is well established.

Advancing detection and analysis techniques make the size-dependent quantification of MP possible. A growing number of publications show a (strongly) increasing number of MP with decreasing particle size.

Even though detection and analysis of small MP with only a few µm in diameter has been achieved, sub-micrometer scale nanoplastics (NP) remained undetected/unquantified thus far.

We at the Institute of Marine Research (Bergen/Norway) attempt to approach the problem of NP analysis by the combination of fractionized crossflow filtration followed by pyrolysis gas chromatography/mass-spectrometry (py-GC/MS). Crossflow filtration is a process of high importance for a large spectrum of industrial users; from the food and beverages industry to water treatment and gas separation. However, crossflow filtration also showed its versatility in a fair number of laboratory applications. Membranes featuring various pore sizes (in the ranges of micro-, ultra- and nanofiltration) are available exhibiting narrow pore size distributions.

Our approach has its starting point at chemically or enzymatically digested seafood samples, which are coarsely (pre-)filtered over a 10 µm membrane. Thereafter, the filtrate is subjected to several stages of crossflow filtration utilizing ceramic membranes to create size fractions. The procedure includes a washing and up-concentration step, in which the amount of dissolved molecular matter and the sample's volume are being reduced. Finally, the resulting sample fractions are freeze-dried and the resulting residues are analysed for NP employing py-GC/MS.

3.04P.18

Towards a standardized method for the extraction and characterization of nanoplastics in complex matrices

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Micro and nanoscale plastic particulates are generally acknowledged as having a potential to be harmful to the environment and possibly also humans [1]. In this work a new approach will be described in which a novel micro-machined sensing surface has been used to aid in the extraction and subsequent μ-Raman analysis of sub-micrometer sized plastic particles from a liquid matrix of enzymatically digested biological tissue. The surface used is composed of an array of holes with sub-micrometer depth and diameter onto which droplets of the analyte are spotted and allowed to dry. The resulting organized array of particles can then be more effectively analysed using Confocal Raman Microscopy by facilitating the detection and identification of individual sub-micrometer plastic particles located within the holes. As a proof of concept, the technique has been applied to the detection of nanoplastics in the digested tissues of salt water mussels.

3.04P.19

Quantitative characterization of the hydrophobicity of nanoplastic models

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Hydrophobicity is an important parameter for the risk assessment of chemicals, but standardised quantitative methods for the determination of hydrophobicity cannot be applied to nanomaterials. Here we describe a method for the direct quantification of the surface energy and hydrophobicity of nanomaterials, applied to the characterisation of different types of nanoplastics. A first group of sub-micrometric polystyrene particles was synthesized using a surfactant free emulsion copolymerisation process of styrene and acrylic acid (AA) or methacrylic acid (MAA), with different surface properties. A second type of plastic was produced by mechanical degradation of primary polystyrene for more environmental relevance. The different models were characterized in size and zeta-potential. The quantitative characterization of the nanoplastics hydrophobicity is based on the measurement of the particles binding affinity to engineered surfaces, using a Dark-Field microscope. The surfaces, called collectors, are molecularly smooth and characterized by specific surface charges and surface energy components. The surface energy potential acting between each nanoplastic and collector was then calculated using the XDLVO theory. The energy barrier represents the potential energy at which the particles are repelled by the collector surface. It depends on several known parameters (properties of the collectors and the particles) and some unknown parameters including the polar component of the surface free energy of the particles (ΓP), which directly quantifies the hydrophobicity of the particles. The values for ΓP were calculated using the binding rate on the collectors for all the studied particles and could also be converted in equivalent contact angle with water. The proposed method allows the quantitative characterization of the nanoplastics hydrophobicity in solution. Hydrophobicity is considered a highly relevant physico-chemical parameter for the safety assessment of nanoplastics, as well as for nanomaterials in general, influencing their fate, bioaccumulation and potential toxicity. The results here shown confirm the validity of the method with a robust set of particles exhibiting tuned hydrophobic character. The potential of this method for nanoplastics characterization is thus demonstrated. [1] Valsesia A, Desmet C, Ojea-Jiménez I, Oddo A, Capomaccio R, Rossi F, Colpo P. 2018. Direct quantification of nanoparticle surface hydrophobicity. Commun Chem 1:53.

3.04P.21

"The Plastic Nile": First evidence of microplastic contamination in fish from the River Nile (Cairo, Egypt)

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The presence of microplastics (MPs) in the world's longest river, the River Nile, has yet to be reported. This small-scale study aimed to provide the first information about MPs in the River Nile by sampling the digestive tracts of two fish species, Nile perch (*Oreochromis niloticus*, n=29) and catfish (*Bagrus bayad*, n=14). Fish were purchased from local sellers in Cairo and then the gastrointestinal tracts were dissected and examined for MPs. Over 75% of the fish sampled contained MPs in their digestive tract (MP prevalence of 75.9% and 78.6% for Nile tilapia and catfish, respectively). Fibers were the most abundant MP type (65%), followed by films (26.5%) and the remaining MPs were fragments. Polyethylene (PE), polyethylene terephthalate (PET) and polypropylene (PP) were all identified non-destructively by Attenuated Total Reflectance Fourier Transform Infrared spectroscopy. Comparison with similar studies from marine and freshwater environments show that this high level of MP ingestion is rarely found and that fish sampled from the River Nile in Cairo are potentially amongst the most in danger of consuming MPs worldwide. Further research needs to be conducted but in order to mitigate microplastic pollution in the River Nile we must act now.

3.04P.22

Centrifugal field flow fractionation and coupled detectors in the analysis of nano- and microplastics

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Spectroscopy methods are among the most popular techniques for identification and quantification of microplastics. However, Raman spectroscopy can be limited by the presence of fluorescent components in the samples, while the sensitivity and resolution of FT-IR microscopy becomes problematic in the size range below 10 μm. In contrast, this is exactly the size range where Centrifugal Field Flow Fractionation (CF3) is expected to perform well. CF3 is a powerful method to use for the characterisation of micro(nano)plastics in the sub-10-micron size range. It permits the components of complex mixtures in aqueous media to be separated based on their size and density and subsequently analysed by a range of on-line coupled detectors. By combining elution times with hydrodynamic diameters it becomes possible to derive information about particle density. On-line coupled Single Particle Extinction and Scattering (SPES) measurements give information on the refractive index of the particles while providing also number based size distributions. Combining this information with that from other on-line analysis methods (e.g. DLS, MALS) allows a better characterization of the components in complex mixtures of polymeric particulates whose size normally makes their identification extremely challenging. Moreover, as the fractions eluted from the CF3 can be collected for later off-line analysis, it presents new opportunities for the information (polymer identity and quantity) from confirmatory techniques such Py-GCMS to be linked to known size ranges.

Assessment of the Exposure and Effects of Contaminants of Emerging Concern in Drinking and Wastewater Systems and Assays to Evaluate their Removal (P)

3.05P.1

Nitrosamines in drinking water: Case study in the Metropolitan Region Area of Campinas-SP, Brazil

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Nitrosamines are a class of semi-volatile organic chemical compounds characterized by a nitroso group attached to an amine group. The detection of nitrosamines as disinfection by-products (DBPs) in drinking water has attracted considerable attention in recent years due to their high carcinogenicity, frequency of detection and their potential regulation in Europe and United States.This work aimed to develop and validate a selective and sensitive analytical method for 7 nitrosamines (NDMA, NMEA, NPYR, NDEA, NPIP, NDPA and NDBA) in drinking water at nanogram per liter levels. Sample preparation employed solid phase extraction and nitrosamines were determined using gas chromatography mass spectrometry with electron ionization (GC-EI-MS). The method was validated according to INMETRO guidelines using the follow figures of merit: selectivity, linear range, linearity, instrumental limit of detection, instrumental limit of quantification, trueness and precision. Compounds NDMA, NPYR, NDEA, NPIP, NDPA and NDBA showed good linear range between 1 and 200 $\mu g\ L^{-1}$ and NMEA between 5 and 200 $\mu g\ L^{-1}$. Determination coefficients for all compounds were greater than 0.995 and instrumental limits of detection and quantification ranged from 0.3 to 2.0 $\mu g\ L^{-1}$ and 1.0 to 5.0 $\mu g\ L^{-1}$, respectively. Mean recoveries varied from 67 to 79% for ultrapure water and 46 to 72% for drinking water. The relative standard deviation for ultrapure water and drinking water ranged from 3 to 6% and from 4 to 7%, respectively. The first preliminary case study for the occurrence of nitrosamines in drinking water in the Metropolitan Region of Campinas was done. Samples were collected and analysed from households of thirteen different cities in the metropolitan region (Americana, Campinas, Cosmópolis, Hortolândia, Indaiatuba, Itatiba, Jaguariúna, Monte Mor, Paulínia, Santa Bárbara D'Oeste, Sumaré, Valinhos and Vinhedo). Results from the case study showed that NDMA was the most frequently detected nitrosamine (87%) and NMEA was the least frequently detected nitrosamine (20%). The minimum, medium and maximum concentrations of nitrosamines ranged from < LOD to 12 $ng\ L^{-1}$, 10 to 22 $ng\ L^{-1}$ and 14 to 67 $ng\ L^{-1}$, respectively. Although this sampling can be extended, the concentrations found so far present a potential risk for the population supplied according to international regulatory entities. INCTAA (FAPESP 2014/50951-4, CNPq 465768/2014-8).

3.05P.2

Mapping degradation pathways of pharmaceuticals in advanced oxidation with liquid chromatography - UV/MS

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One of the most relevant topics in today's environmental analytical chemistry is water quality. To assess the water quality, concentrations of organic pollutants, such as pharmaceuticals, should be determined. Since there is a high usage of these compounds, a lot ends up in waste water effluents. For this reason, water treatment facilities use multiple approaches to clean the water from these contaminants, such as filtration or reversed osmosis. Another tool is advanced oxidation (AOPs), which does not remove, but transforms the contaminants with UV light and hydrogen peroxide. Applying AOPs in water-treatment facilities results into an even broader range of transformation products. Since the goal of AOPs is to decrease the concentration of the targeted contaminants, degradation products were initially receiving less attention. However, studies have shown that after medium-pressure UV/H_2O_2-treatment mutagenic substances can be formed and this resulted in a shift towards transformation product analysis. Because every contaminant degrades in multiple transformation products, the complexity of the sample is increasing during degradation, which results in several analytical problems. For example, the increase in complexity requires the chromatographic separation to be able to separate chemically-similar degradation products. Another problem is that the degradation may be dependent on the properties in the water sample, such as the dissolved organic matter (DOM) and the peroxide concentration. In this presentation, the research performed in the TooCOLD project (Toolbox for studying the Chemistry Of Light-induced Degradation), conducted within several institutes at the University of Amsterdam (UvA) and the Free University Amsterdam (VUA), will be presented. This project aims to develop a system that can both separate the complex mixture of interest and degrade the separated components in a light-degradation cell, after which the transformation may subsequently be further analysed. In the project presented, the effect of UV-light, hydrogen peroxide and the NOM concentration on the degradation pathways of several pharmaceuticals will be shown. This information may yield an improved understanding of degradation in AOPs and will help the design of the light-degradation cell that is developed in the TooCOLD project. Next to that, the analytical strategies optimized for the pharmaceuticals and their degradation products will be shown.

3.05P.3

Structural Investigations of Transformation Products of the Anticoagulant Drug and Rodenticide Warfarin

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Warfarin (Coumadin) is one of the most popular anticoagulant drugs used as a therapeutic in humans to prevent thrombosis, atrial fibrosis, and fibrillation since the 1950s. Because of its ability to hinder blood coagulation by blocking vitamin K-depending carboxylation of blood clotting precursors, it is also used as a rodenticide worldwide. Until today it has been partially substituted by far more potent anticoagulant rodenticides (ARs), so-called superwarfarins.[1] Numerous studies are confirming secondary and tertiary poisoning with ARs in non-target-animals and wildlife. Up to now, relatively little is known about persistence and toxicity of ARs and naturally, nor technically formed transformation products (TPs) in the environment, food chain, and transformations occurring during wastewater treatment.[2] Herein, we present liquid and gas chromatographic methods coupled to (high-resolution) mass spectrometry for the analysis of warfarin's transformation products. Methodologies such as UV-irradiation, ozonation, and chlorination were utilised to simulate technical water treatment. Resulting compounds were elucidated and examined by numerous analytical methods. Moreover, the oxidative phase I metabolism was mimicked by an electrochemical flow cell to synthesise and confirm major metabolites.[3-5] The further aim is the toxicological assessment of all substances formed, as well as, the quantification of warfarin and its TPs under environmentally relevant conditions employing the introduced methods.

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3.05P.4

Formation of iodinated trihalomethanes during chlorination and chloramination: an update

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Trihalomethanes (THMs) are carcinogenic compounds that are considered environmental pollutants. THMs can be released in the environment from multiple sources, but are also formed during water disinfection processes. Iodinated trihalomethanes (I-THMs), including dibromoiodomethane ($CHBr_2I$), bromodiiodomethane ($CHBrI_2$), bromochloroiodomethane (CHBrClI), dichloroiodomethane ($CHCl_2I$), diiodochloromethane ($CHClI_2$), iodoform (CHI_3) are usually more cytotoxic and genotoxic than the chlorinated and brominated haloforms. The presence of CHI_3 (which is usually the most abundant I-THM) in drinking water can result in medicinal odors and tastes. Formation of I-THM is conditioned by the presence of naturally occurring iodide (I^-), iodate (IO_3^-) or iodinated organic compounds, which can undergo transformation to one another. Various factors can influence I-THM formation besides the concentration of I^- or iodine containing compounds, including pH, UV light, temperature, chlorine or chloramines concentrations. During chlorination or chloramination, I^- is oxidized to hypoiodous acid (HIO) or to IO_3^-. HIO reacts with natural organic matter (NOM) and forms I-THMs or other iodinated disinfection byproducts. I-THMs are usually obtained in higher concentrations during chloramination than during chlorination, while IO_3^- is formed in significant amounts during chlorination. NOM consists of compounds with various functional groups, among which carbonyl groups are proven to contribute to I-THM formation. During chloramination, carbonyl groups undergo enolization. HIO reacts with the enol and forms an α-iodomethyl carbonyl group first, then further iodination takes place in two sequential steps, until an α-triiodomethyl group is formed. The compound with the α-triiodomethyl group can be hydrolyzed to CHI_3 and a carboxylic acid or it can be oxidized to triiodoacetic acid. Other pathways for I-THMs formation are possible, but need to be investigated in detail. Further research is required, especially on methods to reduce I-THMs formation.

3.05P.5

Pilot Scale Evaluation of Removal of Contaminants of Emerging Concern (CEC) Using Ozonation and Activated Carbon Filtration

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Contaminants of emerging concern (CEC) is a diverse group compounds, natural or synthetic, with the common denominator that they are (semi-)persistent and potentially bioactive/toxic. The CECs can be detected in natural and drinking waters at levels ranging from pg/L to µg/L, and some of them have been shown to have adverse health effects or pose significant risk even at very low concentrations and doses. A range of CECs have been detected in finished drinking water because conventional drinking water treatment techniques, including coagulation and sand filtration, are not efficient in removing the bulk of these compounds. Therefore, more efficient treatment approaches are needed to prevent human exposure to CECs. Ozonation combined with activated carbon filtration is a promising approach for dealing with the unknown challenges of emerging pollutants. In this study, a combination of ozonation and granulated activated carbon (GAC) filtration was tested during a 1-year pilot scale study to evaluate the efficiency of CEC removal. The specific objectives were to study: 1) how combination of ozone and GAC compares to only GAC with respect to removal efficiency of CECs and dissolved organic carbon (DOC), 2) how CEC removal efficiency differs between two commercially available GAC types, and 3) how CEC removal efficiency is affected by the simultaneous loading of DOC. In total 99 CECs were targeted, together representing a range of different compound categories including 66 pharmaceuticals, 13 poly- and perfluoroalkyl substances (PFASs) and 20 others (e.g. personal care products, food additives, industrial chemicals). The ozone treatment was shown effective for degrading all CECs except poly- and perfluoroalkyl substances (PFASs). The total concentration was reduced by on average (± SD) 82 ± 6%, 18 ± 24 % and 92 ± 2 % (n=12) for pharmaceuticals, PFASs, and others, respectively. The breakthrough of most CECs was delayed when ozonation was implemented as a pre-treatment. DOC removal was more efficient using Filtrasorb 400 compared to Norit 1240W, while the removal of CECs was similar with the two types of GAC.

3.05P.6

A study of photo-Fenton disinfection at near neutral pH

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Kris O'Dowd. Professor Suresh C. Pillai The Fenton process utilises hydroxyl radicals that are created by the decomposition of hydrogen peroxide by a Fe2+ catalyst [1]. The hydroxyl radicals are powerful oxidants that are non-selective and as result can be used to remove antimicrobial organisms form drinking water [2]. During the process Fe 2+ is converted to Fe 3+ [3] and the iron source required for the process is depleted. An advancement on this process is the photo-Fenton process that utilises light to reduce Fe 3+ back to Fe 2+ to recycle the catalyst and decrease the concentration of regents needed. This process has been limited by its requirement for an acidic pH needed to keep the homogenous iron catalyst in solution [4]. The use of a heterogeneous catalyst would negate the need for the acid pH and allow the process to be carried out at a near neutral pH. Zinc ferrite is a cubic [5] spinel oxide that will be examined as a heterogeneous iron catalyst for the photo-Fenton process. The ability to use near neutral pH reduces water treatment and cost. Parameter optimisation of the process would examine several key factors that alter the process's inactivation of micro-organisms. The inactivation rate when using the neutral pH of the water will need to be examined. Any change in pH that occurs as the process proceeds will also need to be studied to ensure that the final pH has reverted back to its original. The effect of temperature and irradiance on the inactivation rate and the optimal value for each will be presented. The parameter of reagent concentration is a key optimisation point. The reagent concentrations required for sufficient inactivation will also be presented, with the ratio of hydrogen peroxide to chemical oxygen demand and the ratio of zinc ferrite to hydrogen being optimised. 1. Leifeld, V., et al., Ferrous ions reused as catalysts in Fenton-like reactions for remediation of agro-food industrial wastewater. Journal of Environmental Management, 2018. 222: p. 284-292. 2. Buxton, G.V., et al., CRITICAL-REVIEW OF RATE CONSTANTS FOR REACTIONS OF HYDRATED ELECTRONS, HYDROGEN-ATOMS AND HYDROXYL RADICALS (.OH/.O-) IN AQUEOUS-SOLUTION. Journal of Physical and Chemical Reference Data, 1988. 17(2): p. 513-886. 3. Baxendale, J.H., M.G. Evans, and G.S. Park, THE MECHANISM AND KINETICS OF THE INITIATION OF POLYMERISATION BY SYSTEMS CONTAINING HYDROGEN PEROXIDE. Transactions of the Faraday Society, 1946. 42(3-4): p. 155-169. 4. De la Obra, I., et al., Microcontaminant removal in secondary effluents by solar photo-Fenton at circumneutral pH in raceway pond reactors. Catalysis Today, 2017. 287: p. 10-14. 5. Anchieta, C.G., et al., Rapid and facile preparation of zinc ferrite (ZnFe2O4) oxide by microwave-solvothermal technique and its catalytic activity in heterogeneous photo-Fenton reaction. Materials Chemistry and Physics, 2015. 160: p. 141-147. 6. Biglarijoo, N., et al., Optimization of Fenton process using response surface methodology and analytic hierarchy process for landfill leachate treatment. Process Safety and Environmental Protection, 2016. 104: p. 150-160.

3.05P.7

Pilot-scale assessment of tertiary treatment technologies for pharmaceuticals and personal care products removal from municipal wastewater

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In recent years, the occurrence, fate and effects of emerging contaminants have been increasingly investigated, indicating the growing need for effective technologies to control the spread of these pollutants into the environment. Several technological opportunities emerged for removing compounds refractory to biological processes. Among them, degradation by ozone and adsorption on activated carbon were identified as sustainable alternatives. Since the application of these technologies is consolidated for conventional contaminants, commercial solutions are already available. Nevertheless, more data about the removal of emerging contaminants under realistic conditions are required**.** In the present work, ozonation and adsorption on powdered activated carbon (PAC) were studied separately as tertiary treatments in two municipal wastewater treatment plants (WWTPs) at the pilot-scale over a long-lasting experimental campaign for assessing the removal of 19 compounds, including pharmaceuticals, personal care products and some of their biodegradation products. Moreover, the influence of operating conditions and wastewater characteristics was assessed, in the view of defining robust guidelines for processes design and operation. The ozone contactor consisted of two identical sequential columns (0.84 m^3 in total) providing ozone mass flowrate up to 8.5 gO_3/h for treating a wastewater flowrate from 1 to 2 m^3/h. The PAC was dosed in an Actiflo® Carb system, a patented Veolia technology combining coagulation, flocculation and sedimentation on lamella plates. Following operating conditions were studied on 2 PAC types: PAC dosage 5-20 mg/L, system PAC concentration 0.2-1 g/L, wastewater flowrate 6-9 m^3/h. For both technologies experimental results evidenced excellent removal efficiencies (80-100%, >95% in most cases) for most of investigated compounds. However, lower removal efficiencies (between 20% and 80%) were observed for several pollutants (e.g. gabapentin-lactam, galaxolidone, irbesartan, lamotrigine and tonalide for ozone, diclofenac, gabapentin-lactam, irbesartan, propyphenazone and sulfamethoxazole for PAC) with significant worsening under sub-optimal operating conditions. The influence of operating conditions was clearly identified for both technologies, being ozone and PAC dosages the most influential ones, and predictive models were proposed. The limited variability in wastewater characteristics resulted in weak indications about detrimental effect on processes.

3.05P.8

Influence of Wastewater Treatment Technology on Removal Efficiency of Organic Contaminants

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Wastewater treatment plants (WWTPs) are the primary line of defense for protecting the natural environment from many chemicals used in the anthroposphere. The diverse structures of anthropogenic chemicals in municipal wastewater poses a great challenge to WWTP design. Other factors, such as climate and economic resources, lead to a wide range of technologies in operating WWTPs. Understanding the link between WWTP technology and removal efficiency is key to further developing environmental protection strategies and targeting upstream measures including chemicals regulation to restrict discharge of chemicals to wastewater. In this work we quantified the breakthrough of ~300 chemicals in 15 Australian WWTPs that were selected for diversity in treatment technology. Influent and effluent samples were collected during census week in August 2016 using flow proportional or time proportional sampling. The samples were analyzed via direct injection with ultrahigh performance liquid chromatography coupled to a Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometer. The HRMS data were processed using CompoundDiscoverer employing seamless online-searching of the database mzCloud. Breakthrough was calculated as the quotient of the signal in effluent and influent. Cluster analysis of the chemical breakthrough data revealed 4 poorly performing WWTPs and 4 highly performing WWTPs. Chemicals that were effectively removed by just the high performing plants were identified, as well as chemicals that were poorly removed by just the poorly performing plants. These chemical subsets were used to explore how chemical structure is related to contaminant removal in primary, secondary, and advanced treatment. Benchmark chemicals were identified that could serve as indicators of different levels of WWTP treatment efficiency.

3.05P.9

UV Irradiation Decreases Ecotoxicity of Glyphosate

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Glyphosate [N-(phosphonomethyl)glycine] is the most widely used active ingredient in broad-spectrum herbicide formulations. Glyphosate is approved for both professional and non-professional use in many countries and is frequently detected as a contaminant in surface and groundwater. The environmental toxicity of glyphosate to non-target organisms has been considered low but adverse effects have been observed in some organisms including bacteria, algae, crustaceans, amphibians, and fish. In this study, we examined the effect of UV-A, UV-B and UV-C exposure of aqueous glyphosate on toxicity to aquatic test organisms from different trophic levels. A test battery with the bacteria *Bacillus subtilis* and *Aliivibrio fischeri*, the green microalgae *Raphidocelis subcapitata,* and the crustacean *Daphnia magna* was used to integrate biological effect of all constituents before and after UV exposure including degradation products. Exposure of glyphosate to UV-C irradiation (254 nm) significantly decreased toxicity to aquatic test organisms as indicated by increasing EC50 values. The effect was observed in different water types and at different UV doses (Joule/cm^2). Exposure of glyphosate to UV-B irradiation (302 nm) showed no effect at low UV doses but significant lower toxicity after exposure to higher UV doses. In contrast, extended UV-A exposure (365 nm) did not affect the apparent aquatic toxicity of glyphosate. The main photolysis products of glyphosate were determined by LC-HRMS after UV irradiation. The study suggest that UV-C and UV-B mediated photolysis of glyphosate can decrease concentrations of the parent compound and decrease the overall ecotoxicity.

3.05P.10

Removal of Emerging Micropollutants Through Biochar Enhanced Soil Retention Filters - an Additional Tool for Wastewater Treatment?

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The ubiquitous occurrence of emerging micropollutants (EMPs) in the environment represents a growing challenge for our society. Not only because of their low concentration and unknown chronic effects on organisms and ecosystems but also because of their expensive and complicated removal whereby they present even a greater risk for low socio-economic regions. A relatively cost and work efficient way to treat wastewaters and thereby remove pollutants are soil retention filters (SRF) which are used in wastewater treatment plants. SRFs are also capable of mitigating the flooding effects of rain-overflow-basins. The general working principle of SRFs is the filtration and removal of pollutants during the movement of water through the soil by adsorption and microbial biodegradation. To enhance the removal capacity of SRF biochar (BC) is investigated as a potential additive in this work. BC is produced by the pyrolysis of different biogenic feedstocks resulting in a low cost product with characteristics similar to activated carbon. Namely a large specific surface area with a great degree of aromaticity which influences the adsorption and bioavailability of EMPs. Moreover, BC also possesses a positive influence on soil parameters through improvement of the nutrition and water balance, soil pH, the cation exchange capacity and has a potential for use as an additional CO_2-sequestration method. Thus, BC is a promising and diverse tool for the future in environmental practices. The aim of this work is to analyse the partitioning, metabolization and removal of organic pollutants in the matrix of a SRF with and without BC by radioanalytics. Therefore, a set of substances was screened in terms of the sorption to BC as well as the general degradation capability in a SRF. To simulate the processes in a SRF we use soil material from a SRF in use and conduct degradation experiments in aquatic sediments according to the OECD guideline 308. Based on our findings we hope to draw additional attention to the potential of BC in wastewater treatment for the future.

3.05P.12

Toxicity of disinfected wastewater effluents probed by genetically modified bioluminescent bacteria and cyanobacteria

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The regular disinfection practices like chlorination and ozonation lead to the formation of toxic disinfection by products (DBPs) in water upon interaction with natural organic matter (OM) precursors. This study is attempted to assess the toxicity of whole chlorinated (1 and 10 mg L^{-1} of active chlorine) and ozonized (3-4 mg L^{-1}) treated wastewater effluents of three types collected from wastewater treatment plants in Israel (TWW1, TWW2 and TWW3) on three genetically modified bioluminescent bacteria obtained as a gift from Prof. S. Belkin (Hebrew University of Jerusalem, Israel). These bacterial strains receive plasmid fusion with promotor response to specific reporter genes *lux CDABE*. Genotoxicity strain contains *recA* promotor sensitive to SOS-DNA damages, cytotoxicity strain contains heat-shock *grpE* promoter sensitive to the cytotoxic substances causing metabolic changes in bacteria, and ROS strain exhibits *micF* promotor responds to superoxide reactive oxygen species (ROS) stress. Being an effective oxidant, ozonation showed a sharp decomposition in the atomicity of OM revealed by UV Abs_{254}, SUVA (specific UV absorbance), and fluorescence Ex/Em intensities, whereas chlorination showed very minimal effect. Chlorination increased the genotoxicity than cytotoxicity of TWW effluents, while ozonation showed an opposite trend, indicating that ozonation can degrade the genotoxic compounds present as well as the formed DBPs during disinfection. Both the chlorination and ozonation does not contribute to the production of reactive oxygen-mediated toxicity responses. The toxicity induction after chlorination and ozonation between three effluents were very diverse and depended mainly on the effluent OM composition and characteristics. These findings highlight that genetically modified bioluminescent bacteria can be utilized as a sensitive biomonitoring tool and toxicity indicators of water chlorination and ozonation.

3.05P.13

From pharmacy to field: predicting the exposure and fate of antiepileptic drugs in agricultural systems

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In an effort to address the pressures on the agricultural system associated with growing food demand and climate change, reclaimed wastewater is increasingly used to irrigate crops across the globe. However, despite recent advancement in wastewater treatment technology, pollutants including pharmaceutical compounds, persist in wastewater and are thus introduced into agroecosystems as mixtures of many pharmaceutical compounds. Among these pharmaceuticals are antiepileptic compounds of which 32 different compounds are prescribed for use in the United Kingdom. Of these, only 3 compounds have previously been investigated in terms of their fate and toxicity in soil-plant systems. Derived from the last 15 years of annual prescription data from the United Kingdom's National Health Service, antiepileptic compounds have been prioritized according to calculated predicted environmental concentrations. Initial studies using Single Cell Mass-Spectrometry to analyse the vacuolar contents of plants exposed to high-priority antiepileptic drugs are also presented here. Developed with the aim of evaluating accumulation and transformation of antiepileptics in plants, an outline of the developing method and preliminary findings gives an insight into the early stages of this research. Results from this work will help inform as to the potential risks associated with the use of treated wastewater for crop irrigation.

3.05P.14

EU-Interreg project CWPharma - Biotests evaluate contaminats reduction via advanced waste water treatment

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Active pharmaceutical ingredients (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. In the European Union´s Water Framework Directive the contamination of water with API residues is considered as an emerging environmental concern. The Baltic Sea has a large catchment area with 85 million inhabitants. Residues of various pharmaceutical ingredients – e.g. hormones, antibiotics, anti-inflammatory drugs and other analgesics – have been detected in the Baltic Sea water and fish, but it is currently difficult to make reliable emission estimations and risk assessment of APIs in the Baltic Sea Region (BSR). The EU interreg project 'Clear Waters from Pharmaceuticals' (CWPharma) focuses on filling the gaps of knowledge, e.g., regarding environmental concentrations and ecotoxicological effects of APIs. The project tries to increase the awareness of policy-makers, regulators and permitting authorities on environmental risks and implementation of the emission reduction structures. Therefore, CWPharma will give tools and recommendations to policy makers, authorities and municipalities on the best ways to reduce emissions of APIs in the BSR. In work package 3 "advanced wastewater treatment" technical solutions such as ozonation and different post treatments are evaluated and compared in pilot studies at waste water treatment plants in Kalundborg (DK), Linköping (SE) and Berlin (DE). Several ecotoxicological tests with waste water samples of secondary effluent before and after ozonation and with additional filters or biofilm reactors have been conducted regarding endocrine, genotoxic, mutagenic, neurotoxic or growth and reproductive inhibitory effects. The present contribution will give insights in the ecotoxicological results of test with native and enriched samples (SPE enrichment) and will evaluate the effectiveness of different API emission reduction measures.

3.05P.15

Effect-based monitoring for water safety planning: Why and how?

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Source waters used for drinking water production may contain many different organic micropollutants, while transformation products and disinfection by-products may form during specific treatment processes. This can result in a very complex mixture of chemicals present at low concentrations in drinking water with targeted chemical analysis unable to detect them all. Effect-based methods (EBMs) (i.e., bioanalytical tools or *in vitro* bioassays) can be used to complement chemical analysis as they measure the integrated effect of the chemical mixture in a water sample. The Global Water Research Coalition project "Effect-based monitoring in Water Safety Planning" will put current assays into practice and develop guidelines to support the implementation of EBMs for water quality monitoring. This will be achieved through the development of decision-making tools to select appropriate bioassays and sampling methods, selection of applicable interpretation methods (including effect-based trigger values) and development of protocols to assist with EBM implementation in the context of Water Safety Plans. Further, case studies and demonstration sites will be used to show how EBMs can be used to assess source quality, treatment efficiency and specific treatment processes. The overall aim of this project is that EBMs become operational tools for the management of health risks related to chemical mixtures.

3.05P.16

EDC-WFD: A project to deliver reliable measurements for better monitoring survey and risks assessments

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Monitoring programs should generate high-quality data on the concentrations of substances and other pollutants in the aquatic environment to enable reliable risk assessment. Furthermore, the need for comparability over space and time is critical for analysis of trends and evaluation of restoration of natural environment. Additionally, research work and exercises at the European level have highlighted that reliable measurements of estrogenic substances at the PNEC level are still challenging to achieve.The project EDC-WFD Metrology for monitoring endocrine disrupting compounds under the EU Water Framework Directive aims to develop traceable analytical methods for determining endocrine disrupting compounds and their effects, with a specific focus on three estrogens of the first watch list (17-beta-estradiol (17βE2), 17-alpha-ethinylestradiol (EE2), and estrone (E1)). Estrogens 17-alpha-estradiol (17□E2) and estriol (E3) will be included to demonstrate the reliability of the developed methods and to support the requirements of Directive 2013/39/EC, Directive 2009/90/EC and Commission Implementation Decision (EU) 2018/840, hence improving the comparability and compatibility of measurement results within Europe. During the EDC-WFD project four selected effect-based methods (EBM) will be deeply investigated in order to improve their rationale use and their support in water quality assessment. In particular, the EBM sensitivity, specificity and accuracy on reference materials with single or mixture solutions of the five substances at a concentration of EQS values will be explored.This contribution will present the objectives and methods applied within the EDC-WFD project.

3.05P.17
Managed aquifer recharge with reactive barriers (rbMAR): achievements and future challenges
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Climate change and population increase is a major worldwide threat for sustainable and safe drinking water supplies. The fact that groundwater is the only water resource available in many regions has led to groundwater depletion at local and regional scales. In this context, artificial recharge of aquifers (MAR) is an excellent option to increase available water sources. MAR systems, based on water filtration through the soil, have proven to retain suspended solids, bacteria, viruses, and other constituents, such as contaminants of emerging concern (CECs) and inorganic nutrients, able to be adsorbed on the soil particles surface or biodegradable by the existing microbial community. However, pathogens have been eventually detected in groundwater. In order to improve chemical and ecological, water quality, during MAR reactive barriers aiming to foster CECs removal and pathogens retention are urgently needed.This study demonstrates that adding a reactive barrier in the infiltration step improves the removal of CECs. Still, pathogen removal is significant (2-5 log units in our case), but was not particularly improved with respect to usual infiltration. Therefore, further improvement in the design of the barrier and the operation of the system is required to achieve a higher extent of pathogens retention and/or inactivation. Considering general chemical parameters monitored and CECs and pathogens it appears obvious that a fair and realistic regulation for reclaimed wastewater use in MAR, ensuring the good quality and quantity of groundwater is key for the smooth and wider implementation of MAR.

3.05P.18
Effect of pH and Storage Times on the Leaching of Potentially Toxic Metals (PTMs) from Different Types of Used Cooking Pots
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Humans are exposed to Potentially Toxic Metals (PTMs) via many routes. Cooking food in wares prone to material leaching is an exposure route. The leaching of PTMs from cookware during cooking depends on the material it is made of. This study assessed the effect of pH and storage time on the leaching of selected PTMs (Al, Cr, Fe, Ni, Pb, and Zn) from clay, non-stick, stainless steel, cast pot (*koko- Irin*), aluminium and glass pots. The pH and storage times were varied from 3 to 7h and 24 to 48 h respectively. Deionized water was boiled for 30 minutes and stored in used cooking pots obtained from homes in Nigeria. PTMs leached from the pots were determined by Inductively Couple Plasma-Optical Emission Spectrophotometer (ICP-OES). The result showed that aluminium pot leached Zn (11.7 µg/l) at pH 3 and 1.75µg/l at pH 4. Cast pot leached only Al at pH 3 to 7 with concentration ranging from 11.47-2273.15 µg/l. Clay pot leached Al, Cr, Fe and Zn at all the pH levels studied. Nonstick pot only leached Zn (24.39 µg/l) at pH 3. Stainless steel pot leached Al (53.18µg/l), Fe (236.63µg/l), Pb (29.87µg/l) and Zn (1.0 µg/l) at pH 3. In Aluminum pot, the concentration of Al and Zn increased at 48 hours of storage time. For the cast pot the concentration of Al, Cr and Zn increased while that of Fe and Pb reduced and Ni was not detected furthermore for clay pot, the concentration of Cr and Pb increased while the concentration of Al, Fe, Ni and Zn reduced at 48 hours storage time. For Stainless steel and non-stick pots, the concentration of all the metals of interest increased at 48 hours of storage time except for Ni that was not detected in the non-stick pot.

3.05P.19
Comparison of three ISO standardized in vitro bioassay investigating estrogenic activity in waste water effluents
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Bioanalytical tools, with consistent operating protocols and standardized data analysis, are promising tools for a tiered approach in environmental water quality monitoring. Last year, three variants of *in vitro* estrogen receptor transactivation assays (ERTAs) were adopted as ISO standards. With these bioassays the estrogenic potential of water and waste water can be determined (ISO 19040 1-3: 2018). We evaluated responses of the three ERTAs: the human cell line-based ERα-CALUX, and two yeast estrogen screens, one with *Saccharomyces cerevisiae* the other with *Arxula adeninivorans*. Bioassay results were compared with eachother and with chemical analysis. As samples we used extracts of treated sewage effluent collected from 20 different waste water treatment plants. Each treatment plant was sampled three times: in spring, summer and winter. We choose effluent samples as sample matrix from various plants to 1) have diversity across samples and 2) have expected estrogenic activity above the quantification limits of the assays (i.e. 0.6-30 pg/L 17β-estradiol equivalents [EEQ] after 500times enrichment of the effluents). This approach allowed for a representative and robust comparison between the four methods. While, the ISO protocols recommend the testing of native water samples over sample enrichment prior to analysis, yet water samples are often enriched during routine analysis to increase sensitivity and lower quantification limits of the bioassays. In this study, we enriched the effluent samples, but re-dissolved these organic extracts in nanopure water and subsequently exposed the cells to aqueous extracts. Based on the first results, all three bioassays displayed similar trends and there was good agreement with analytical chemistry results. This suggests that no loss of estrogenic chemicals occurred during sample manipulation steps, such as enrichment and solvent exchange for the aqueous testing of the organic extracts.

3.05P.20
EVALUATION OF THE PHYSICO-CHEMICAL PROPERTIES OF DRUGS, BASED ON THE QSAR MODEL, TO IDENTIFY THE POTENTIAL OF BIOACCUMULATION OF THESE SUBSTANCES IN THE ENVIRONMENT
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Currently, there is a growing concern about "emerging pollutants", and it has been shown in several articles that these compounds are present in the environment and can be detected in sources of water supply, groundwater, sewage sludge) and even in drinking water. Among the emerging contaminants, the occurrence of residual drugs in domestic sewage and natural waters is an important international topic. Data demonstrate that the existence of pharmaceutical compounds in the environment is generally small when compared to other chemicals. However, the high persistence and, in some cases, the low concentration of the drugs present in the environment is compensated by the continuous replacement of these compounds, which maintains the risk of chronic exposure to aquatic organisms as well as to humans. The present work aims to evaluate the structure of previously selected compounds, based on the Ecological Structure Activity Relationships (ECOSAR), especially in relation to the probable compartments and the respective extension of their distribution, as well as data on ecotoxicity, the most costly and time-consuming analytical and toxicity tests. The fate and behavior of compounds in the environment and in TEEs are influenced by their physicochemical properties, which govern the partition in water, soil or biota. Compounds with low solubility and high octanol / water partition coefficient (Kow) are generally present in biota fatty tissues, which promotes bioaccumulation in the food chain. According to the data obtained by the QSAR's program the octanol / water partition coefficient and according to ZAGATTO (2006) the drug ibuprofen presents log Kow 3,97 and can be classified as potentially cumulative. Since the other compounds have log Kow lower than 3, therefore, do not have substantial potential for bioaccumulation, the data obtained for the respective drugs are: atenolol (log kow = -0.03); paracetamol (log kow = 0.49). Considering the presence of these compounds in the various environmental matrices, it is important to highlight that the increasing implementation of the use of sludge generated in sewage treatments, especially the disposal in the soil, recognizes that a standard procedure for the environmental risk assessment of drugs needs to be developed. or even the implantation of complementary treatment systems for the removal or reduction of these compounds from the environment.

3.05P.21
BEHAVIOR AND POTENTIAL OF DRUG MAKING IN COMPOSTED BIOSOID FROM ETE AND ITS IMPLICATIONS IN PUBLIC HEALTH
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Health; M.L. Caminada, UNIFAJ-Centro Universitário de Jaguariuna; M.M. Bocchiglieri, Basic Sanitation Company of the State of São Paulo (SABESP); W.d. Paganini, Faculty of Public , University of São Paulo (USP) / Department of Environmental Health;

Considering the need to find alternatives for the beneficial use of sludge generated in sewage treatment plants (TEEs), mainly agricultural use, the present work aims to evaluate the structure and behavior of the drugs in sludge from TEE, submitted to composting process. The physico-chemical properties of selected drugs and their behavior in TEEs, considering the tendency of persistence in the sludge, and the ecotoxicological potential when disposed in the soil, using the QSAR-ECOTOX-EPA model. Sludge composting line installation: • Characterization of crude and composted sludge; • Quantification of drugs by HPLC and their behavior in composting. The evaluated drugs are in the most consumed compounds list in Brazil 2016/17 (ANVISA, 2018) According to AQUINO (2013): log Kow < 2.5: high hydrofibicity and low absorption tendency in biomass and lipid fractions of suspended solids. 2.5 ≤ log Kow ≤ 4.0: moderate tendency of absorption in these matrices. log Kow> 4.0: highly hydrophobic and high sorption potential in the solids present in the sewage Table 1: Physical-chemical properties - QSAR's model ***Active principle Log Kow pka Solubility in water (mg/L) Sorption tendency Bioaccumulation potential Atenolol*** 0,16 59,6 13.300 low no ***Carbamazepina*** 2,30 13,9 112 ***Clonazepan*** 2,53 1,5 100 ***Paracetamol*** 0,46 9,38 14.000 ***Ibuprofeno*** 3,79 5,2 21 moderate yes ***Sinvastatina*** 4,68 n.m 0,03 high Bastos (2013) states that the agricultural use of biosolids is a controversial issue due to potentially adverse impacts on human health, and strict standards, high quality criteria and restrictions of use are imposed in the name of safety. These norms need to be based on risk assessment, since there is a lack of data on the subject, as well as the establishment of a risk pattern, because there is still no clarity as to what constitutes a tolerable risk in the Brazilian context.

3.05P.22

Can spatial distribution of organic contaminants in Gammarus pulex explain toxic effects?

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Environmental risk assessment is often based on exposure concentrations of contaminants. However, internal concentrations at the toxicological target sites are the actual parameter determining an adverse effect towards an organism. Currently applied sample workup for aquatic invertebrates, such as *G. pulex*, includes a sample homogenisation and solvent extraction. This way, all spatial information is lost. To investigate the spatial distribution of contaminants, specimens of *G. pulex* were exposed via the aquatic phase to a mix of pesticides (cyprodinil, tebuconazole, thiacloprid) and pharmaceuticals (citalopram). After 24 h, gammarids were snap frozen and cryosectioned (10-20 µm). The produced cross sections were analysed by matrix-assisted laser desorption ionisation mass spectrometry imaging (MALDI-MSI) with 2,5-dihydroxybenzoic acid as matrix. All compounds could be detected in the analysed samples, but their spatial distribution differed. Whereas citalopram, cyprodinil and tebuconazole were detected within different organ compartments of the organism, thiacloprid was detected only associated with the exoskeleton of *G. pulex*. The biotransformation product N-desmethyl citalopram was observed following the same distribution pattern as the parent compound. The present study demonstrates the suitability of MALDI-MSI for investigations on the spatial distribution of organic contaminants in *G. pulex*. However, for a more precise correlation of tissue types and MS-spectra, dissection methods need to be optimised for crustaceans. The observed association of thiacloprid with the exoskeleton may explain, the delayed toxic effects in aqueous exposure biotests (Betekov & Liess, 2008) and why differences in toxicity were observed for the same body burden of thiacloprid (Englert et al. 2017), after exposure via different uptake pathways (aqueous, dietary). Beketov, M. A., & Liess, M. (2008). Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods. *Environmental Toxicology and Chemistry: An International Journal*, *27*(2), 461-470. Englert, D., Zubrod, J. P., Pietz, S., Stefani, S., Krauss, M., Schulz, R., & Bundschuh, M. (2017). Relative importance of dietary uptake and waterborne exposure for a leaf-shredding amphipod exposed to thiacloprid-contaminated leaves. *Scientific reports*, *7*(1), 1-10.

Chemical Exposome of Human and Wildlife - Advancements in Non-targeted Analytical Methodology and Modelling Approaches (P)

3.06P.1

Transition-state rate theory sheds light on 'black-box' chemical fate algorithms

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Evaluation of chemical fate is common practice in environmental risk assessment. When data is lacking, fate can be estimated from molecular characteristics via BIOWIN/AOPWIN (EPI Suite™), CATABOL/CATALOGIC etc. With variable performance depending on the endpoint, the vast majority of such models are empirical or semi-empirical (‘fit the data-’ or ‘black-box-’) models. Using numerous incomprehensive parameters, they apply only to specific media/inoculi and have fundamentally limited applicability (domains). More precise values relate to ‘first principle’, mechanistic modelling of *in situ* (bio)degradation. We applied a ‘mechanism-based’ degradation formula stemming from transition-state rate theory with explicit terms for diffusion and reaction. Statistical evaluation with biotic and abiotic experimental degradation data for organic chemicals validated that molecular volume (~diffusion coefficient) and a characteristic distance function describe diffusion-limited biodegradation. Diffusion, along with quantum-chemical parameters characterize the frequency factor (collision theory). Reaction-limited (bio)degradation can be efficiently be related to linear-free-energy (LFER) parameters such as the reaction driving force and transition-state delocalization. The parameters used in our approach are intuitive and computationally inexpensive. The case studies and controls shown apply to various degradation mechanisms/pathways (H-abstraction, mono- and dioxygenation, C-N cleavage, decarboxylation, etc). Based on statistical evaluation, our approach does not necessarily perform worse than ‘established’ ‘black-box’/empirical methods. Thus, the work shows potential to improve our understanding of (bio)degradation of chemicals via ‘first principles’ to unravel the causal mechanisms of chemical fate in complex matrices. This aids a more precise and comprehensive environmental risk assessment.

3.06P.2

Honey Bee (Apis mellifera) Exposomes and Biological Pathways Associated with Nosema ceranae Infection

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Xenobiotic burden was determined to be associated with N. ceranae infection. Co-variate analysis determined both anthropogenic and naturally occurring chemicals in the bee exposomes. Biological pathways analyses putatively identified 10 dysregulated pathways as well as the presence of the P450 oxidative metabolism of naphthalene for detoxification. Based on these results, it is evident that the integration of genetic disease screening with discovery-based exposomics provides a promising multi-omic platform to identify adverse biological effects to bees occurring from exposures to chemicals and parasites. In addition, this approach will generate new hypotheses for targeted follow-up studies to examine bee health.

3.06P.4

Evaluation of in vitro assays for investigation of fish metabolism

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Fish metabolism is gaining relevance in human and environmental risk assessment of plant protection products. In addition to ecotoxicological testing in fish including studies to determine the bio-concentration factor, the metabolism needs to be investigated for dietary risk assessment as described in Commission Regulation (EU) 283/2013 as well as the working document SANCO/11187/2013. For environmental safety, comparison of metabolism in different fish species is needed and will contribute to the mechanistic understanding of active compounds. Experimetal data will be used for PBTK models. This study was performed to evaluate the ability of fish in vitro assays to deliver robust data on fish metabolism. Hepatocytes, S9 fractions and microsomes from several fish species were incubated with radio-labelled and non-labelled plant protection products. Analysis of samples was performed by radio-HPLC as well as LC-HRMS. The following investigations were performed: • Comparison of the metabolite pattern of rainbow trout and common carp hepatocyte incubations using radiolabelled test compunds and compare to in-vivo fish bio-concentration factor studies and other regulatory animal and in-vitro studies. • Comparison of the metabolic pathway of three test compounds using trout S9 fraction and hepatocytes • Investigation of metabolic stability of radio-labelled test compounds in several fish species (bluegill sunfish, rainbow trout, zebra fish, medaka, fathead minnow) and comparison of the metabolitic profile of microsomes and S9 fraction The results of the investigations show that fish liver in vitro assays are a valuable tool for comparison of metabolism in different fish species and to assess the transformation products in fish compared to e.g. mammals or other livestock animals. Different fish species show similar metabolic reactions and varying metabolic activity. Hepatocytes were shown to be the most powerful in-vitro system to evalute phase 1 as well as phase 2 metabolism. The establishment of a fish hepatocyte assay will deliver valuable data on fish liver metabolism, give more mechanistic insight into acitive compounds and reduce the number of vertebrate tests. The outcome of these tests may be further supported by PBTK modelling approaches for non-target species interpolation.

Chlorinated Paraffins - State of Science, Insights, Challenges

and the Way Forward (P)

3.07P.1
What's in a name? Understanding the complexities and impact of chloro alkane nomenclature around the globe.
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As part of the EU assessment of chloro alkanes under REACH, industry submitted a series of studies providing data on the persistency and bioaccumulative status of medium chain chlorinated paraffin (MCCP). These studies compliment a raft of other data points on the biodegradability and degradation pathways of these materials when they are in the environment as well as the inherent complexities when applying certain regulatory tests to sparsely soluble industrial chemicals. These activities are now informing the US assessment of these substances which will take place in the coming years. Recent regulatory testing also highlighted how nomenclature and understanding of how these chemicals are made, used and registered is creating confusion and leading to the wastage of resources which could be better utilised in addressing issues with imported products. This session will discuss the recent testing and present industries current requirements on chloro alkane analytics. It will also provide possible ways in which industry can work with academia to improve understanding of chloro alkanes to provide better definition for these substances.

3.07P.2
Testing and substance evaluation of Medium Chain Chlorinated Paraffin (MCCP) under REACH
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Medium-chain chlorinated paraffins (MCCPs, C14-17 alkanes, chloro) are currently registered under the EU REACH regulation and have undergone substance evaluation (SEV) that included additional testing on biodegradation and bioaccumulation potential. MCCP is a complex (UVCB) substance due to the random and variable nature of the chlorine substitution on the hydrocarbon backbone. MCCP's chemical complexity creates challenges for their determination and thus evaluation. For example, for separation between MCCPs as well as other compounds (e.g. chlorinated olefins) by MS a resolution of R>21,000 is needed. In addition, the lack of suitable isomer standards and the use of mixtures that are not well-characterised further complicates congener group (isomers with the same molecular formula) specific analysis. Recent advances in analytical chemistry and the provision of single carbon chain mixtures have allowed evaluation of congener groups in MCCP products as well as the testing on biodegradation and bioaccumulation potential. This work will present the results of the substance evaluation, including the results of the latest testing. For this, a matrix-approach was adopted comparing individual carbon-chain length mixtures by chlorination levels (e.g. C_{14}, 40-≤50% Cl by wt.). It will also present final conclusions of the SEV on MCCPs and further steps that need to be taken.

3.07P.3
CHLOFFIN - answering the urgent call for chlorinated paraffins standards
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One of the (many) challenges researchers face when determining the high volume production chemicals chlorinated paraffins (CPs) in environmental matrices is the lack of suitable standards and matrix reference materials. Current commercially available individual standards (native and labelled) have a chlorine pattern that is different than those found in technical mixtures and the environment. CP mixtures used for quantification are not well-characterised nor purity assessed and this results in semi-quantitative and not traceable data. Certified matrix reference materials (CRMs) are needed to validate a method. Considering the high annual production volumes (>2 million tonnes per yr) of CPs and their recognised impact on the environment and human health, the urgent need for standards and CRMs enabling the validation of methods for their accurate analysis became clear. The EU Eurostars project entitled CHLOFFIN started in October 2019. In this project, we aim to produce around 40 individual standards of CPs, 8 labelled individual CPs, 10 congener mixtures that are well-characterised and purity assessed and one matrix CRM. This work presents the first steps towards the synthesis of these standards as well as the certification of the RM and discusses the challenges and potential solutions for a successful realisation of this project.

3.07P.4
Analysis of Short chain chlorinated paraffins by high-resolution GC/Q-TOF
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Persistent organic pollutants (POPs) like Dioxins, Furans and Chlorinated paraffins (CPs) are regulated and restricted from production globally as declared by the UN Stockholm Convention. CPs are complex mixtures of polychlorinated alkanes with alkane lengths C10 to C30 and various degrees of chlorination, typically 30 to 75%. Short Chain Chlorinated Paraffins (SCCPs, C10-C13) are bioaccumulative and persistent in the environment. Analysis of these compounds represents substantial challenge due to their self-interference as well as an interference with other components of complex industrial matrices. Therefore, to ensure both high selectivity and high sensitivity for the analysis of the SCCP with wide range of chlorination, we used a high-resolution GC/Q-TOF operated in two ionization modes: negative chemical ionization (NCI) as well as low energy electron ionization (low energy EI). The pure congener standards were first used to evaluate the individual spectra. NCI spectra of SCCPs exhibited minor fragmentation with no significant breakdown of the carbon backbone. Low energy EI data indicated higher degree of fragmentation of the SCCPs as compared to negative CI. However, using this technique allowed the sensitive detection the SCCP species with low number of chlorine atoms. Both NCI and low energy EI techniques were later applied to analyze complex SCCP mixtures

3.07P.5
Congener-specific partition properties of chlorinated paraffins evaluated with COSMOtherm and GC-retention indices
J. Hammer, National Institute for Environmental Studies / IRAS; S. Endo, National Institute for Environmental Studies (NIES) / Center for Health and Environmental Risk Research
Short-Chain Chlorinated Paraffins (SCCPs) are high volume production chemicals that are produced for their high chemical stability and used various products such as plasticizers, coolants and flame retardants. SCCPs are considered persistent organic pollutants (POPs) and are found in various organisms including humans and in remote regions such as the Arctic. Because of their environmental issues, it is important to develop fate and effect models for these compounds. For these models, the physical-chemical properties of SCCPs need to be determined. Unfortunately, because SCCP mixtures are highly complex and consist of different congeners and (stereo)isomers, property values are currently only available for a range of SCCP congeners. In this study, gas chromatographic (GC) retention times were measured on various polar and nonpolar columns using commercially available analytical standards of individual SCCP congeners, in order to investigate the relationships between the SCCP structures and the molecular interaction properties (e.g., van der Waals, hydrogen-bonding). Simultaneously, partitioning coefficients (e.g. octanol-water, octanol-air partitioning) for individual SCCP congeners, including possible stereoisomers, were calculated using the quantum chemical prediction tool COSMOtherm and compared to experimental data from literature. GC retention measurements show that even analytical standards of individual SCCPs can contain several stereoisomers, and that stereoisomerism plays an important role in the physico-chemical properties of some SCCP molecules. Technical mixtures of SCCPs can therefore have a range of properties depending not only on the 2D structural diversity (i.e., substitution patterns) but also the presence of stereoisomers, which explains a part of the variation found in experimental partition data for SCCP products. Predicted parameters from COSMOtherm show that the largest discrepancy is found across stereoisomers of molecules with more than 60% chlorine in weight. While it is currently difficult to determine which substitution patterns and stereoisomers of SCCPs are exactly present in a mixture, COSMOtherm can help by predicting the range of properties we can expect.

3.07P.6
New standards for chlorinated paraffins - Eurostars CHLOFFIN project
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Chlorinated paraffins (CPs) is a class of industrial chemicals used as high-temperature lubricants in metal-working machinery and as flame retardant plasticizers in vinyl plastics. Less common applications include the use as flame retardants in rubber, paints, adhesives and as sealants. The total global production remains largely unknown but is believed to exceed at least two million metric tonnes per year. CPs show resistance to degradation, and some show bioaccumulation and toxic potential, and are suspected carcinogenic to humans. For example, short-chain CPs have been prohibited by the POP Regulation in the EU since 2017 and placed on several monitoring list such as the European Water Framework Directive. CPs need to be continuously monitored – and no suitable and generally accepted reference standards are commercially available yet.Industrially, the CPs are synthesized by direct chlorination of *n*-alkane feedstock with molecular chlorine at elevated temperatures and pressures, and sometimes in the presence of UV-light. CP individual reference standards were developed at Chiron in the early 2000s, some of them are recommended as

internal standards in the ISO 12010 method for water quality determination. But these standards have a chlorine pattern that is different than those found in technical mixtures and the environment. CP mixtures are also used today for quantification, but the mixtures are not well characterised nor purity assessed, bringing to semi-quantitative and not traceable data. In 2019 we launched the EU Eurostars project entitled; CHLOFFIN. One of the ultimate goals of CHLOFFIN is to develop standards with defined composition and response factors which are similar to the industrial mixtures. We aim to produce around 40 individual standards of CPs, 8 ^{13}C-labelled individual CPs, 10 congener mixtures that are well-characterised and purity assessed and one matrix CRM. This work presents the first step; synthesized standards which are single molecule compounds of SCCPs with hexa-chlorines. These standards are useful in the quantification of CPs as well as helping in distinguishing the various congener groups according to carbon chain length and chlorine content.

3.07P.7

Assessment of Human Exposure to Chlorinated Paraffins in a Norwegian Cohort

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Chlorinated paraffins (CPs) pose a considerable risk to humans and the environment, given that CPs are persistent, bioaccumulative, and cause adverse health effects. Despite the recent addition of short-chain CPs (SCCPs, C10-13) to the Stockholm Convention on persistent organic pollutants (POPs), medium- and long-chain CPs (MCCPs, C14-17; LCCPs, C>17) continue to be manufactured in increasing quantities, highlighting an urgent need for the exposure assessment and biomonitoring. Herein, we assessed human dermal exposure to CPs in a Norwegian cohort based on the amounts of CPs found in hand wipes. The hand wipe samples were collected between 2013 and 2014 from 60 adults (45 women and 15 men) from a cohort from Oslo, Norway. CPs were found to be the most abundant flame retardants in the hand wipes compared to PBDEs, HBCDDs, TBBPA, and OPEs. The total masses of CPs were in the range of 43-18000 ng/participant (median: 950 ng), which were generally mixtures of SCCPs, MCCPs, LCCPs, as well as very-short-chain CPs (vSCCPs, C< 10). The CP patterns varied between participants with most having predominantly MCCPs (mean: 58% across all the samples), but 10 of the 60 samples had patterns predominated by LCCPs (mean: 22% across all the samples). The 95th percentile daily dermal exposure values were 0.045, 3.6, 11, and 5.5 ng/kg body weight/day for vS/S/M/LCCPs, respectively, which were several orders of magnitude lower than their oral reference doses. Mean daily dietary intakes of S/M/LCCPs for adults in Sweden are available for a tentative evaluation of the relative contributions of the dermal exposure via hand contact, and these were 18, 39, and 2.0 ng/kg bw/d, respectively. Dermal exposure via hand contact appears to be an important human exposure pathway for LCCPs. Our results demonstrate the need for additional studies exploring the relative contributions of various exposure pathways to these ubiquitous environmental contaminants.

Emerging Environmental Contaminants - Status, Trends, Challenges and Knowledge Gaps (P)

3.08P.1

Toxicity Assessment Of Two Metallic Nanoparticles On Aquatic Biota

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The recent increase in the use of nanomaterials, notably nanoparticles in the day-to-day context, is due to the emergence and development of the nanotechnology area from the mid-1980s when their bases began to be established. From this point onwards, there was a great interest of the scientific community to explore this new type of material with interesting and promising properties, given its high resistance, reduced size, anti-microbial properties, ease of synthesis, among many others. With advances in this field, these materials have become of common usage and now have multiple applications not only in the most varied fields of science, but also in practical life, where they began to integrate personal hygiene products, containers, infrastructures, food and even part of biomedical techniques in the treatment of cancer or in simple MRI scans. This fact makes clear that with the increase of its use, the increase of its disposal also occurs, which in turn, unfortunately may threaten biota inhabiting the most varied environmental compartments, where they may reach. The present work aimed at assessing the influence of chemical composition and coating agents in the toxicity of metallic nanoparticles. It was studied two nanoparticles (Fe3Se4 - iron selenide and Fe3O4 - iron oxide) functionalized with dopamine and levodopamine (to promote its stability and internalization). For each nanoparticle, a battery of toxicity assays was carried out by using five freshwater species representative of different trophic levels, ranging from producers to secondary consumers. In terms of chemical composition, Fe3Se4 were the most toxic ones, while in terms of their coating agents, Levodopamine exerted the highest toxicity. The more sensible endpoints were H. viridissima feeding activity (EC50=57.03 mg/L) for Fe3Se4@L-Dopa, L. minor growth rate (EC50=28.30 mg/L) and yield (EC50=47.89 mg/L) for the same nanoparticle and B. calyciflorus mortality (EC50=16.17 mg/L) for Fe3Se4@Dopa. Referring to literature data, it may be possible to assume that these particles are much less toxic than others commonly used, therefore the use for which they were synthesized is probably safe, however a greater interest and attention should be paid to them in order to deepen knowledge on their possible impacts in the ecosystems.

3.08P.2

Monitoring of antibiotics, pollutants, and AMR in formal and informal settlements: Back-calculation of antibiotic usage in the absence of prescription data

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Antimicrobial resistance (AMR) is a major global topic, concerning the increasing pathogenic tolerance to antibiotics. Excessive and inappropriate antibiotic use heightens the emergence of antimicrobial resistance genes in pathogenic organisms, resulting in reduced drug susceptibility. The usage, excretion, and fate of antibiotics is an important aspect of AMR dissemination. In regions where prescription data / pharmacy records are not collated or publically available, an estimation of usage via back calculation from environmental concentrations can be vital. A novel analytical method was developed for broad- and narrow-spectrum antibiotics, via UPLC-ESI-TQD-MS/MS. Longitudinal monitoring of these 59 drugs and 25 drug metabolites, other pollutants, and metagenomic data, is used to aid our understanding of AMR dissemination. River and wastewater samples (both aqueous and solid) were collected from 10 sites within Stellenbosch town, SA, across 7 sampling campaigns between May 2018 and June 2019. Estimation of antibiotic usage requires several parameters, including flow data to establish daily loads; knowledge of prescribed drug compositions; percentage drug elimination; route and mechanism of elimination; and the drug's physio-chemical fate (e.g. solid/liquid partitioning). These estimations are achieved using antibiotic concentrations from >500 aqueous samples and >60 solid samples, together with the analysis of spatial and temporal trends. However, environmental drug persistence and the presence of other pollutants, such as heavy metals and biocides, are equally important to monitor – particularly when considering antimicrobial resistance. Exposure to sub-inhibitory concentrations of antibiotics is likely to impose selection favouring the presence of AMR genes; and acquisition through lateral gene transfer can lead to the emergence of AMR genes in the environment via co-selection. Subsequently, incidences of AMR dissemination are established by correlating the antibiotic and metagenomic data. Regulated and appropriate antibiotic usage is vital for controlling the spread of AMR. In the absence of records, wastewater based epidemiology is used to back calculate these data from the main town (via wastewater influent) as well as informal settlements without waste infrastructure (via pseudo-wastewater river samples). **Acknowledgements** We would like to acknowledge the Wolfaardt group, (Stellenbosch University) for sample collection, processing, and shipment to the University of Bath; the Stellenbosch University Central Analytical Facilities team (ICP-MS & XRF unit) for assistance with the ICP sample preparation and instrumental analysis; the members of the ReNEW project research team (www.gcrf-renew.co.uk); and our funders GCRF (Global Challenges Research Fund).

3.08P.3

High throughput method revealing the specific toxic effects of azithromycin to Raphidocelis subcapitata

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In the past decade concerns have been growing on the adverse effects of pharmaceuticals to aquatic organisms. Besides being designed to stimulate a physiological response in organisms, these substances are only partially metabolized after administration, being a large amount excreted unaltered or as active metabolites reaching the aquatic environment. Additionally, there is still insufficient ecotoxicological data on antibiotics such as for azithromycin. This macrolide has already been detected at high concentrations in the aquatic environment. Therefore, it can constitute a threat to aquatic organisms, namely for microalgae that are at the basis of the aquatic ecosystem. *Raphidocelis subcapitata* is one of the species recommended for ecotoxicological bioassays, commonly found in freshwater, with a short generation time and high growth rate. This study demonstrated the successful use of a high-throughput method for rapidly screening the toxicity of azithromycin to *R. subcapitata*. Flow cytometry and PAM fluorometry were used as diagnostic tools. Several endpoints were analysed, including growth rate, natural pigments content, photosystem II performance, cell size, cell complexity, cell viability, cell cycle and DNA content, formation of reactive oxygen species (ROS), mitochondrial and cytoplasmatic membrane potential and lipid peroxidation (LPO). The specific toxic effects of azithromycin to key components of microalgae cells were observed, before an impact on growth became effective, therefore revealing its specific mode of action (MoA). The used methodology showed a great potential for being incorporated into future microalgal toxicity bioassays for testing contaminants, aiming to improve the currently used strategies for hazard assessment.

3.08P.4

Using a sensitive multiple biomarker response to improve environmental hazard assessment in the green microalgae Raphidocelis subcapitata

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Microalgal toxicity tests such as the algal growth inhibition use integrative endpoints to analyse the toxicity of potentially hazardous substances in the aquatic environment. However, these do not provide any information on the toxic mode of action by which contaminants affect microalgae. Bottled waters may be used to substitute culturing media but should not inflict any stress to the cultured organisms. However, certain chemical components may interfere with specific cell components, not revealed by general toxicity assays. This study analysed the sensitivity of flow cytometry to analyse sub-lethal effects of different bottled waters to the freshwater microalgae *Raphidocelis subcapitata*. Several endpoints were analysed including growth rate, natural pigments content, cell size, complexity, viability and cycle, reactive oxygen species (ROS) formation, mitochondrial membrane potential and lipid peroxidation (LPO). In Addition, photosystem II performance was also analysed by PAM fluorometry. Results revealed that the most sensitive endpoints were the oxidative stress endpoints ROS and LPO, pigment content and the morphological endpoints cell size, complexity and cycle. Growth rate was the least sensitive of the analysed endpoints. The toxicological effects observed in microalgae where related with the chemical elements Ca, Na, Mg, and NH_4. Although these are essential macronutrients for algal growth, they may have detrimental effects when present at high concentrations. The applied high throughput methodology allowed to gather information on the morphological, biochemical, and physiological status of microalgal cells, revealing mechanistic information on the toxic mode of action of the bottled waters before an effect on algal growth was observed. The used methodology showed a good potential for being integrated into future microalgal toxicity bioassays for testing substances, helping to improve the hazard information obtained from the currently approved test guidelines.

3.08P.5

Determination of chemicals of emerging concern in livers of white-tailed sea eagle from Germany using novel and complementary High Resolution Mass Spectrometry techniques

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Investigating the distribution of chemicals of emerging concern in top predators is important not only for understanding exposures within food webs, but also for improving risk management in order to contribute to species conservation. The term “chemicals of emerging concern” (CECs) has been established for chemicals, which are not subjected to marketing restrictions and regulatory monitoring programmes, but are candidates for future regulation, due to their frequent detection in environmental matrices and potential hazardous properties. Their broad use in combination with their physicochemical properties has shown to result in environmental exposures in marine, freshwater and/or terrestrial compartments. However, there is limited information available on their occurrence in top predators, as well as, on their potential accumulation within food webs.Biomonitoring studies using wildlife sentinels represents an important means for the assessment of environmental exposures and has shown to serve as warning system for potential adverse effects as well for human exposures. There are many key characteristics that make raptors good sentinels for environmental contaminants, such as: their high position in food webs, their relatively long lifespan over which they accumulate CECs, the integration of exposures both over time and relatively large spatial areas and the relative ease with which populations can be quantified and monitored.This study focuses on the investigation on CECs’ occurrence in livers of white-tailed sea eagle (*Haliaeetus albicilla*). For this purpose, 30 livers samples, collected over the last 5 years from 3 different federal states in Northern Germany, were analysed following state-of-the-art wide-scope target and non-target screening methodologies. The analytes were extracted from freeze-dried matrices through validated, generic protocols of sample preparation. The final extracts were analysed by different chromatographic and ionization techniques coupled to High Resolution Mass Spectrometry (LC-ESI-QToF MS and GC-APCI-QToF MS), in order to broaden the chemical domain accessible to wide-scope target screening of more than 2,400 and non-target screening of more than 60,000 CECs. Furthermore, all chromatograms were digitally archived in the NORMAN Digital Sample Freezing Platform for further retrospective suspect screening.

3.08P.6

Rare earth element organotropism in European eel (Anguilla anguilla): gender and life stage influence

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Rare Earth Elements (REEs) are a group of 17 metallic elements with close chemical and physical properties: scandium, yttrium and 15 lanthanides from lanthanum to lutetium. REEs are prevalently used in many industrial sectors as in the production of supermagnet and luminophore, petroleum catalysis, pigmentation of plastics, metallurgical alloys, nuclear and MRI. The REE consumption was evaluated at 120 kt in 2014 and is increasing by 6% each year (Bru et al., 2015). Hence these activities are causing REE releases into the environment and particularly in natural aquatic ecosystems. REEs are able to bioaccumulate in aquatic organisms (Censi et al., 2013; Mayfield and Fairbrother, 2015; Strady et al., 2015) and enter in the food chain. With the REE emergence it is important to better know their bioaccumulation and their distribution in organisms such as fish. The aims of the present study were i) to investigate the REE organotropism in the European eel caught in the wild, by determining REE concentrations in muscles, skin, gills, brain, blood, spleen, kidneys, liver, gallbladder and gonads, ii) to study the influence of life stage and gender on the REE organotropism. For both yellow and silver eels, the liver and kidneys appeared to be the largest REE accumulator organs which is consistent with their role in the detoxification. The gills also accumulated a large amount of REEs and this can be related to the fact that they filter the water and suspended particles potentially charged in REEs. REE organotropism was different between yellow eels and female/ male silver eels, showing that sexual maturity and gender influenced the distribution of these elements in the Anguilla anguilla species. The study of the sexual maturity influence on the REE bioaccumulation showed that females accumulated significantly more than yellow eels in kidneys, muscles, gonads, spleen and liver. However, yellow eels accumulated more in gills. These differences can be attributed to the morphological and physiological changes that occur during silvering (Adam et al., 2008). The study of the gender influence on the REE bioaccumulation showed that males accumulated significantly more REEs in muscles and gills while females accumulated significantly more REEs in gonads. The gender could change REE bioaccumulation, by influencing morphology, physiology, comportment, food preference and detoxification mechanisms (Burger, 2007). Cerium is often the REE present in higher concentration but this predominance can vary between organs, sexual maturity and gender.

3.08P.7

Ecological and human health risk assessment of selected veterinary pharmaceuticals' residues in livestock production effluents and surface water samples

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Pharmaceutical residues in environmental samples have been classified as emerging contaminants. They often occur at low levels in the environment, they are biologically active at trace concentrations. They have been linked to many human and ecological issues such as antibiotic resistance and endocrine disruption in man and animals. Information about associated exposure risk(s), mechanism of toxicity induction, and the magnitude of potential impacts of these compounds, singly and in combination with others is scanty and not well understood. There is therefore need for an integrated approach, such as target and non-target screening as well as bioassays to complement analytical monitoring, in order to measure their ecological impact and characterize the risk they pose to man and the environment. Bioassays were used to assess potential ecological and human health risks of some pharmaceutical compounds detected in livestock farms’ effluents. Cocktails of the most frequently occurring pharmaceuticals in effluents were prepared using the lower (LOD) and upper (ULD) limits of detection in surface water samples. Whole effluent samples were also collected for exposure studies using *Pseudokirchneriella subcapitata and Daphnia magna* toxicity bioassays. Whole effluents were screened for toxicities using Ames mutagenicity testing and recombinant yeast bioassay (YES) for oestrogenic activity assessment. The lowest observable effect concentration (LOEC) and no observed effect concentration (NOEC) values for *Pseudokirchneriella subcapitata* growth inhibition were ≤

6.250 (

3.08P.8

Whole Genome Sequence Analysis of Multidrug Resistant K. pneumoniae and E. coli Isolated from Human Stool Samples in West Africa

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Background: K. pneumoniae and E. coli strains resistant to extended-spectrum beta-lactams are a major risk to public health through infections with limited or no available treatment options. In addition to high level of resistance, ESBL-strains of Enterobacteriaceae with elevated level of virulence genes are being observed in increasing numbers. In a global scale, the most significant information gaps concerning the occurrence and sources of resistant bacteria are in West African countries where no surveillance system of resistance exists. Materials/methods: Five children in Burkina Faso and Mali were studied for their fecal carriage of resistant Enterobacteriaceae. K. pneumoniae and E. coli isolates from stool samples were whole genome sequenced using Illumina short-read sequencing. Their genomes were studied for antimicrobial resistance genes, virulence genes and their association to mobile genetic elements. Expression of the resistance genes was studied by comparison to their phenotypic susceptibility patterns. Results: According to the phenotypic resistance profiles, a 16-month old Burkinabe child was a carrier of one ESBL-producing K. pneumoniae and one ESBL-positive E. coli with resistance to multiple other antibiotics. Genome wide analysis of the K. pneumoniae isolate of sequence type 45 confirmed the presence of globally the most significantly spread ESBL gene CTX-M-15. In addition to the multiple resistance genes, its virulence gene profile showed high similarity to the ones previously described as pathogenic K. pneumoniae causing neonatal sepsis. CTX-M-15 encoding E. coli of ST38 carried by a Malian child showed resistance to five different classes of antimicrobials, in addition to the 3rd generation cephalosporins. At the same time, numerous virulence genes associated to several different E. coli pathotypes could be detected. Most worryingly the isolate encoded capsular type K1 genes among other genes associated with E. coli that cause neonatal meningitis. Conclusions: We described the presence of previously elsewhere reported nosocomial strains ST45 K. pneumoniae and ST38 E. coli in Burkina Faso and in Mali, carrying a number of antimicrobial resistance and virulence genes. Our results show alarming examples of pathogens that potentially cause severe infections, have extremely narrow treatment options and are carried by infants.

3.08P.9

Effects of sertraline on fish embryos

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The use of antidepressants has been increasing resulting in its presence in the aquatic environment. This environmental release raises concerns on potential effects to non traget organisms that have physiological systems regulated by these pharmaceuticals. Sertraline (SER) is an antidepressant belonging to the serotonin reuptake inhibitor class (SSRIs) that has a high consumption rate. However, there is little knowledge about the toxicological effects of SER in aquatic ecosystems. Thus, this study aimed to evaluate the effects of SER (0.1 up to 3000 μg.L-1) on zebrafish (Danio rerio) focusing on different biological endpoints (e.g. heartbeat rate, swimming behaviour and biochemical enpoints associated with neurotransmission, antioxidant defenses and energy metabolism). Overall, embryos demonstrated a high sensitivity to SER (e.g. promoting heartbeat rate increase and decreased swimming behaviour. These findings support further research on the long-term effects of antidepressants such as SER to aquatic biota and new methodologies to efficiently remove them from the environment.

3.08P.10

Antagonistic modulators of the Aryl Hydrocarbon Receptor (AhR) from a chemical-structural outlook

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The aryl hydrocarbon receptor (AhR) mediates cellular signals in vertebrates and invertebrates with its main transcriptional regulatory function being the up-regulation of cytochrome P450 family 1 (CYP1) of metabolizing enzymes. Although several chemicals have been reported as antagonists of AhR expression, knowledge gaps exist on the chemical-structural and functional features of AhR antagonistic modulators. In the present study, a chemical-activated luciferase gene expression (AhR-CALUX) bioassay was performed for a set of known contaminants in addition to an extensive literature search, to construct a diverse and broad database for modeling the AhR antagonistic activity. Subsequently, QSAR models and toxicophoric hypotheses were built using simple chemical structural fingerprints which allowed the elucidation of the structural and physicochemical features that influence the AhR blockage potential of chemical compounds. Despite the different conceptual basis of the employed methods, complementarity between their outcomes was observed. The models proposed herein were validated showing a high prediction capacity and robustness. Therefore, they could be useful in the prediction of the AhR antagonistic potential of chemical compounds with toxicological and pharmacological applications.

3.08P.11

Direct analysis of river water for monitoring emerging contaminant influx from agricultural runoff and wastewater treatment plant effluent

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High spatial frequency monitoring of selected river water catchments for >100 emerging contaminants (ECs) such as pharmaceuticals, personal care products, illicit drugs, herbicides and insecticides using direct injection liquid chromatography tandem mass spectrometry (LC-MS/MS) is presented herein to gain insight into the impacts of rural and urban communities on receiving waters across Germany, the UK and Ireland. This particular analytical method was suitable for ng L^{-1} EC determinations in river water using a rapid 4 min gradient on a 5 x 3 mm, 2.7 µm particle size biphenyl cartridge making high sample throughput possible (~50-100/day quantitatively). Method performance including linearity (R^2 ≥0.99), precision/accuracy (< 15 % for each), limits of detection/quantitation (from 1 ng L^{-1}) were all excellent in general and the method only required 10 mL of sample to routinely perform both matrix-matched calibration (10-points) and several replicate measurements. Both large and small river catchments with different population sizes were selected including from River Emsbach (Selters/Taunus, Hesse, Germany, population ~8200; small river); River Schwarzach (Schwarzenbruck, Bavaria Germany, population ~8,500; small river), River Barrow (New Ross, Co. Wexford, Ireland, population ~8,000; large river); London (UK, a 100-km portion of the River Thames, population ~8.6 million; large river) were studied. For example, in the Schwarzach, up to 30 ECs were determined between limits of quantification (~ 4ng/L) and 1000 ng/L at discharge points from a local sewage treatment works. Therefore the use of such high-throughput, direct analysis methods provides near real-time information on receiving water system contamination at high spatial coverage as needed. In perspective, frequently performed river water analysis could be used as a way to determine changes in the health and wellbeing of river ecology, as well as a potential indicator for any emerging impact of new and changing anthropogenic activities.

3.08P.12

Examination of embryotoxic potential in sediments by means of the sediment contact test with Danio rerio

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The European Water Framework Directive (EU WFD) obligates the member states to achieve good ecological and chemical status of all European water bodies. The long-time experience under the EU WFD shows that we also need to consider the sediments. Sediments can be a sink for many pollutants. Nevertheless, not all of them are bioavailable. Hence, by flood events bounded pollutants can become bioavailable. According to this contaminated sediments may impact biota, for example sensitive larval stages, living in the sediment phase. The current study took place within the $DemO_3^{AC}$ project aimed at investigation of the ecological and chemical status of the River Wurm in Aachen (North Rhine-Westphalia, Germany). It is focused on examination of embryotoxic potential to zebrafish larvae in sediment by means of the sediment contact test. Sediment samples were collected 2017 and 2018, both in summer, at 7 different locations. The sampling sites were chosen in according to their localization regarding local waste water treatment plants, rain overflow basins (ROB) as well as depending on the environment. Especially the release of ROBs may have significant impact on sediment toxicity acting like a flood- like event. While the tests with native samples are represent real environmental exposure, freeze- dried sediments mimic the worst-case scenario, which can take place during flood events. The native samples did not cause any effects in the test system. Whereas the freeze- dried samples of a few locations caused effects on the embryos. The experiments are currently in progress. However, the results will be available before the conference.

3.08P.13

Determination of 40 contaminants of emerging concern in sediment samples from the Trondheim fjord, Norway

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Parabens, bisphenols, benzophenones, benzothiazoles, benzotriazoles and triclocarban are compounds showing endocrine disrupting potential, classifying them as environmental contaminants of emerging concern. A solid-phase extraction (SPE) protocol was developed and applied for the simultaneous extraction of 40 of these contaminants from sea-sediment. The analysis was performed by multi-residue UPLC-MS/MS methods. Overall, 64 sediment samples were collected from 10 different locations in the Trondheim Fjord, Norway; 9 of those locations were on a grid in proximity to a wastewater treatment plant (WWTP), while 1 was a remote location that was obtained as representative for background concentrations of the fjord. The sum concentrations of parabens and their transformation products (âř(9)Parab) ranged 57.0-380.8 (median 82.8) ng/g dry weight (d.w.). The sum concentrations of benzothiazoles (∑(9)BTHs) and benzotriazoles (∑(7)BTRs) ranged 9.32-152 (median 62.3) and 3.48-67.9 (median 7.73) ng/g d.w., respectively. The sum concentrations of bisphenols (∑(9)BPs) and benzophenones (∑(5)BzPs) ranged 0.67-12.1 (median 3.15) and 0.25-34.7 (median 1.27) ng/g d.w., respectively. Triclocarban concentrations ranged 0.53-3.65 (median 1.40) ng/g d.w. 36 compounds demonstrated reproducibility ranging 0.90-10.0 % relative standard deviation (RSD %), while the remaining 4 compounds ranged 14-39 % (RSD %). The lower limit of quantification (LLOQ) ranged 0.08-1.08 ng/g d.w. for 37 compounds, with 3 compounds ranging 10-14 ng/g d.w (benzothiazole, 4-hydroxybenzoic acid and methyl protocatechuic acid). 24 chemicals demonstrated absolute recoveries ranging 60.0-102 %, 9 compounds ranged 40.0-60.0 % and 7 compounds ranged < 30 %. For the sediment samples from the Trondheim Fjord, some chemicals were linked to the WWTP, especially the paraben transformation products, benzotriazoles and benzophenones. However, many chemicals showed higher concentrations in the remote location, attributed to the high boat traffic in the fjord. To our knowledge, this is the first time these emerging contaminants are reported in the Trondheim Fjord.

3.08P.14

The global database "Pharmaceuticals in the Environment" - Update and new analysis

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Pharmaceutical residues occur globally in the environment. This is demonstrated in the updated database “Pharmaceuticals in the Environment”. Residues of pharmaceuticals in the environment have been measured in 75 countries in all UN regions. Worldwide 771 active substances or their transformation products were reported and 596 for the European Union. The residues of pharmaceuticals are a potential risk to the environment and their occurrence raised an increasing concern. In the last years, studies on this potential risk increased. Hence, the exposure of the natural environment is characterized much better. To organize the huge amount of information caused by the global environmental exposure situation, the German Environment Agency (UBA) initiated this project to collect all these data within one publicly available database. Besides the database evaluation on the global distribution of the active pharmaceuticals substances from the European Watch List (WL) for emerging water pollutants and the main antibiotic groups is presented in a corresponding report and exemplary on the poster. The database now contains 178,708 entries. Most of these substances were found in the effluents of wastewater treatment plants (liquid emission – worldwide: 613, EU: 474). 528 substances were detected worldwide in surface water, groundwater and drinking water. 19 substances were detected in surface water, groundwater or drinking water in all five UN regions. For the database 1,519 publications and 240 review articles were comprehensively reviewed and analysed. Environmental concentrations of human and veterinary pharmaceutical residues could be collected for 53 matrices. The data were transferred from the publications, reports and other data sources into the database (MEC database). You are invited to browse the 178,708 data entries from 1,519 publications in the publicly available database for download as Microsoft Excel© or as Microsoft Access© file.

3.08P.15

Understanding chemical contaminants in urban freshwater environment

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Pharmaceuticals and personal care products (PPCPs) are detected in surface water, groundwater and drinking water all around the world. The presence of PPCPs may result in harmful effects on human health (e.g. antibiotic resistance) and on wildlife populations (e.g. endocrine compounds causing feminisation of fishes and amphibians). Fresh water pollution is particularly important in urban environments where chemical use is high because of high population density. Recently, a large focus was put on pharmaceuticals in fresh water systems, potentially missing other chemical molecules used in cities with potential harmful effects. The aim of this research was to identify the types of chemical contaminants detected in cities from previous studies. Sixty seven articles were identified exploring the problem that had detected a total, 748 chemicals in the fresh water system in cities all around the world. The chemicals included 266 pharmaceuticals, 212 industrial chemicals (flame retardants, metals, petroleum, plasticizers, solvent, technical additives and terpenoids), 112 pesticides (fungicides, insecticides and herbicides), 71 personals care products (preservatives, fragrances materials, UV filter), 21 illicit drugs, 20 household chemicals (detergents, surfactants), 11 biomarkers (bacteria and viruses) and 9 food chemicals molecules (food additives and food sweeteners). This study of suspected chemicals in cities allows for a broader vision of urban water pollution, pollution not only restricted to pharmaceuticals. The next step of this research is to find associations between socio-economics data of a city (e.g. population, wastewater treatment system, GDP, manufactures around the city) with chemical emissions. This research is particularly relevant to anticipate chemical emissions in fresh water systems under climatic change, and global change scenarios that are forecast to occur over the next 30 years. Climate change will result in water scarcity, intense climatic events and increase spread and risks of diseases. Demographic change (demographic growth, aging population and expanding urbanisation), technology change and individual’s life style change will also influence consumption of chemical molecules and consequently chemical emissions. These changes must be considered for risks and mitigations strategies for chemical emissions in the future.

3.08P.16

Occurrence of Plastic Waste Dumps in the Lagos Lagoon, Nigeria and Stakeholders' Ecological Risk Perception

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Plastics are synthetic or semi-synthetic organic compounds which are lightweight, durable and can be moulded into solid materials. They have become a menace in aquatic ecosystems due to their inability to degrade thereby posing a threat to aquatic biota. However, data on the occurrence of plastic in Nigerian aquatic ecosystems is scanty. Hence, in this study, we assessed and characterised plastic waste dumps in the Lagos lagoon, Nigeria and evaluated stakeholders’ environmental risk perception of the plastics in the Lagoon through questionnaire administration. Six (6) major sites with high plastic wastes load due to deliberate dumping and tidal activities were recorded at Oworonshoki, Obeshe, Oreta, Apapa, Ebute Ilaje and Iddo sites of the Lagos lagoon. 83.3% of the stakeholders (residents) dispose of their plastic wastes into the lagoon while 60% of the residents have no knowledge of the risks posed by the plastic waste dumps in the Lagos lagoon. Polyethylene terephthalate was the highest in plastic waste dumps with 16.7% found on the coastal body. The results indicate the unregulated practice of plastic waste dumping into the lagoon with potential risks posed to biota. Further studies are recommended to evaluate the occurrence of microplastics in various matrices of the lagoon including biota as well as evaluation of potential biological effects posed to aquatic biota. This will provide evidence-based data for targeted interventions to sustain life below water as contained in the United Nations Sustainable Development Goal 14.

3.08P.17

Investigating the occurrence and trend of emerging contaminants in Asopos river basin using LC-QToF-MS and advanced chemometric tools

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Monitoring activities over the last two decades have revealed the ubiquitous presence of organic contaminants in the environmental compartments. In this study, the river basin of Asopos, north region of Athens, where most of the industrial and agricultural activities are taking place, was monitored. Although previous literature focused on the occurrence of heavy metals in the aforementioned territory, there is lack of information about the presence of emerging contaminants. The aim of this study is to develop an advanced HRMS based methodology for the determination of emerging contaminants with wide-scope target and non-target screening with the use of prioritization tools. The sampling campaign was coordinated on two sites, where one was close to the estuaries of the river and one was near to the industrial and agricultural activities, to detect the occurrence of any spills. 24h composite samples were collected on a monthly (30 consecutive days) and seasonal (7 consecutive days, beginning of every season) basis. Solid phase extraction with mixed-mode sorbents was used during the sample preparation of river water samples to ensure the extraction of various classes of compounds. The extracts were analyzed by complimentary chromatographic techniques, including RP & HILIC coupled to QToF-MS. Specific focus was given to create a new chemometric tool to reveal the fluctuations in both sampling points based on the trend of occurrence given by each detected m/z values. This prioritization tool was able to detect single point changes (either drop or increase in the trend), continuous increase/decrease and inverse relationship between two trends (due to different levels of contamination in two sampling sites). Furthermore, those m/z values with interesting trends were followed by non-target screening approach for their structure elucidation. From wide-scope target screening method, including 2785 emerging contaminants, several classes of compounds belonging to surfactants, plant protection products (PPPs) as well as biocides and PPCPs have been detected. A weekly trend analysis revealed the presence of industrial chemicals such as benzothiazoles, benzotriazoles and PFASs in both sampling points, during the working days. PPPs were present, potentially originating from the nearby farming land, washed out after rainfall. The results provided an overview of the organic contaminants detected in the Asopos river basin highlighting the potential sources of concern.

3.08P.18

The use of Pollution Induced Community Tolerance (PICT) for soil Cu decontamination techniques efficiency assessment

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The evaluation of soil Cu decontamination efficiency after the application of remediation techniques such as Cu immobilization isn´t an easy task. One option is the measurement of soluble Cu, bioavailable Cu, etc. However, these measurements are not specific and the knowledge about what levels are or not tolerable for soil organisms is still unknown. Pollution Induced Community Tolerance is a concept based on the existence of an increase of any organisms (such as bacterial communities) tolerance to a soil contamination if i caused a toxic pressure. Therefore, if Cu pollution caused toxicity on soil bacteria, they will develop tolerance to Cu. We hypothesize that if a remediation technique became effective (such as Cu immobilization) the toxic pressure will decrease, and therefore the bacterial community tolerance to Cu will also decrease. In the present work, we spiked a natural soil with different concentrations of Cu (0, 500, 1000 and 2000 mg kg^{-1}) under laboratory conditions, resulting in 4 microcosms. After an incubation period (2 months), we applied two remediation treatments to those soil microcosms polluted with Cu; amendment with two different by-products to the soil samples (48 g kg^{-1}): pine bark and crushed mussel shell. Also, microcosms with no remediation treatments were used, resulting in a total of 12 microcosms. Then the soils microcosms were incubated for another 2 months and the bacterial community tolerance to Cu measured. In the soil samples non-amended with any by-product, the bacterial community tolerance to Cu increased as high the Cu concentration was. In the soils amended with pine bark, the bacterial community tolerance to Cu also increased in response to Cu additions, but the magnitude of the increases was much lower than for the non-amended soil. However, for the soil samples amended with crushed mussel shell, the bacterial community tolerance to Cu was not significantly different than for the control soil. Therefore, the bacterial community tolerance to Cu may be a good tool to assess the efficiency of the techniques used for Cu remediation.

Mass Spectrometry Screening Strategies to Evaluate Environmental Exposures: What Have We Learned and Where Are We Going? (P)

3.09P.1

Community engagement and perceptions of targeted and non-targeted chemicals of concern for municipal wastewater reuse onto human food crops.

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This project used participatory science, HRMS suspect-screening approaches, and community participants to understand community perceptions of municipal wastewater irrigation onto human food crops. The technical team, in collaboration with the City of Jacksonville, North Carolina (USA), monitored organic chemicals of concern in wastewater and on-site/off-site surface waters and groundwater for a municipal land application system. The 2,900 hectare land application system irrigates 810 hectares of forest with secondary-treated municipal wastewater year around at total wastewater volumes equivalent to normal annual rainfall for the site. The social science team engaged city residents and farmers adjacent to the land treatment systems on the science of HRMS suspect-screening analyses, risk analysis for decision-making, and monitoring results of known and unknown chemical features for monitored waters. This presentation will summarize the technical HRSM findings of off-site and on-site waters since 2017 but also present social science findings of community engagement dynamics between HRMS experts and community participants over the last three years. Technical experts gained valuable input from community participants on the efficacy of HRMS public science communication, participant perceptions of HRMS information for decision-making, and participants' decisions of how HRMS information could be used for future decision-making to gain community acceptance of irrigating human food crops with municipal wastewater from the site.

3.09P.2

Non-target screening using two-dimensional GC coupled to high-resolution MS reveals complex pattern of contamination in Arctic marine zooplankton

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The image of the pristine Arctic has in the last decades shattered. While sparsely populated and industrialized, the Arctic environment is impacted by global sources of pollution through long-range transport. The accumulation of persistent pollutants in Arctic food chains is by now well known. More recently, focus is being given to so-called "emerging contaminants". These chemicals are not necessarily "new", but their persistency (P), bioaccumulation (B) and toxicity (T) properties have not been given focus previously and thus they have typically gone under the radar of regulatory monitoring. Although increasing, there is still limited knowledge of the presence of such chemicals in marine biota, particularly in lower trophic species. In addition to long-range transport, increased population, transport and tourism in the Arctic region causes concern for the potential effects of a wider range of chemicals on Arctic biota than previously addressed. Only limited data is available regarding the presence of emerging contaminants in the Arctic food chain, and in particular Arctic invertebrates. In the present study, we have applied a novel pipeline to investigate the presence of contaminants in a variety of benthic and pelagic low-trophic organisms benthic and pelagic amphipods, copepods, arrow worms and krill. Samples were collected in Kongsfjorden in Svalbard (79.0°N, 12.0°E) in 2018, subject to solvent extraction and two-dimensional gas chromatography coupled to high-resolution mass spectrometry (GCxGC-HRMS). To allow the investigation of a large sample set, data processing, including spectra deconvolution, library matching, logical filtering and searches against online PBT databases, has been to a large extent automated. The amphipods and lipid-rich copepods contained the highest abundance of identified and tentatively identified compounds in comparison to the other test species. Tentatively identified compounds included plasticizers and other plastic additive compounds, flame retardants precursors and production solvents and chemicals, food flavoring ingredients, fragrance compounds, UV-stabilizers, antioxidants, antifungal and antimicrobial chemicals, insecticides, and some pharmaceuticals, including a synthetic estrogen. The significance of these discoveries is discussed in light of the potential for detrimental effects on species and ecosystems caused by these chemicals, as well as suggested local and distant sources of the components to the Arctic environment.

3.09P.4

Mass spectrometry-based approaches to characterize chemodiversity of chlor(am)inated water

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The formation of disinfection byproducts (DBPs) is the main unintended consequence of the use of chemicals to disinfected water, and more knowledge is needed to characterize the DBP mixtures formed in chemically disinfected water. DBP mixtures have been to date partially charecerized, and many of the known DBPs have shown to be highly cytotoxic and genotoxic to mammalian cells in in vitro assays [1]. A recent study indicates that the toxicity potential of the non-volatile fraction of the AOX, still largely unknown, may be more relevant than that of the volatile fraction [2]. The toxicity of the DBP mixture is directly related to the DBPs that get formed, that depends on the organic and inorganic constituents of the water and the disinfection conditions [3]. In this context, the present study aimed at characterizing the chemodiversity of halogenated DBP mixtures, and assessing the limitations of the methods used for this. To achieve the study objectives, chemically disinfected waters (with chlorine or chloramine) from four diferent drinking water treatment plants (DWTPs) in Sweden were investigated using different analytical approaches: targeted analysis of selected halogenated DBPs by means of gas chromatography-low resolution mass spectrometry (GC-MS), adsorbable organic halogens (AOX) measurements, and non-targeted screening of halogenated mixtures by means of ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Targeted analysis only was capable of capturing 27% of the halogenated material formed during water disinfection. AOX confirmed that although less halogenated material formed during chloramination than during chlorination, the composition of chloraminated DBP mixtures is largely unknown. Targeted and non-targeted analyses confirmed the low formation of Br-DBPs during chloramination of source waters with the highest bromide levels, where higher levels were expected. Non-targeted analysis with FT-ICR-MS evidenced the wide chemodiversity of halogenated DBP mixtures, but it is only capable of capturing one fraction of it, i.e., non-volatile, medium to low polarity, and oxygen containing compounds. However, there is no one single analytical solution to capture the chemodiversity of chemically disinfected water, due to the varied nature of its components. Acknowledgements - CP acknowledges support from the Swedish University of Agricultural Sciences (August T Larsson Guest Researcher Programme) and the Fundación General del CSIC (ComFuturo Programme, 2nd edition). This work was supported by the Government of Catalonia (Consolidated Research Groups 2017 SGR 1404) and by the Swedish Research Council FORMAS (grant 2013-1077).

3.09P.6

Seasonal trends of xenobiotic compounds in wastewater treatment plants effluents from Madrid (Spain): target and suspect screening

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Municipal wastewater contains a complex mixture of xenobiotic organic compounds (XOCs) originating from personal care products, pharmaceuticals, excreted hormones, household and industrial chemicals, among other emerging organic compounds. Within this context, the objective of the present work was the application of a multi-residue analysis of up to 200 polar xenobiotics and the suspect screening of thousands of compounds in wastewater treatment plants (WWTPs) effluents from Madrid (Spain). Grab samples were taken from five WWTPs located in Madrid, Spain, during two sampling campaigns (July and November 2017) and preconcentrated (500 mL of each one, n=3) by means of solid phase extraction (SPE) protocol using in-house prepared PTFE cartridges by filled with 300 mg Strata HR-X (top) and 100 mg of both Strata ZT-WAX and ZT-WCX (bottom), conditioned with 10 mL of ethyl acetate: methanol (1:1, v:v) and 10 mL of Milli-Q water). After sample loading, cartridges were dried and eluted with 12 mL of methanol:ethyl acetate (1:1, v:v) containing 2% ammonia (v/v) and 12 mL of methanol:ethyl acetate (1:1, v:v) containing 1.7% (v/v) formic acid. To detect site-specific, suspected and formerly unknown contaminants in the samples, we applied a screening procedure based on liquid chromatography high-resolution mass spectrometry (LC-qOrbitrap) with stepwise identification schemes. Based on automated structure searches from the NORMAN list (Network of reference laboratories, research centres and related organizations for monitoring of emerging environmental substances) suspected site-specific and documented water contaminants was reduced to those amenable to LC-qOrbitrap (Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific™ Q Exactive™ Focus quadrupole-Orbitrap mass spectrometer, Thermo, CA, USA). Measurements were performed in the Full scan-data dependant MS2 (ddMS2) discovery method in the positive (pH=3) and negative (pH=7) modes. After peak picking for exact masses of suspects, presumably false positive detections were stepwise excluded by retention time prediction, the evaluation of isotope patterns, ionization behaviour, and HRMS/MS spectra. The different profiles of xenobiotics observed in the five different WWTPs were studied, as well as the differences observed for a dry (July) and rainy (November) periods. Apart from xenobiotics, transformation products (TPs) were also screened by including a list of suspect TPs of the xenobiotics included in the NORMAN list. Finally, when pure standards were available, quantification of the detected compounds was also performed and differences among samples were not only assessed in terms of the presence or absence of a compound but also in terms of concentration.

3.09P.7

Advancing throughput for comprehensive HRMS screening of drinking water: Combined approach of online SPE and direct injection

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Water safety and quality are fundamental to human development and well-being. Therefore, safe and readily available water is important for public health, whether it is used for drinking, domestic use, food production or recreational purposes. Environmental regulations throughout the world currently focus on monitoring a limited number of well-known compounds that are assumed to be responsible for significant ecological and human health related risks. However, there is a continuously growing interest to extend the well-defined fraction of anthropogenic chemicals, the priority pollutants declared by the regulations, to allow the surveillance screening for contaminants of emerging concern and thus provide comprehensive data on the chemical quality of drinking and surface waters. Therefore, targeted analytical methods are increasingly complemented by untargeted acquisition methods using high resolution accurate mass LC/MS. While regulated compounds often require enrichment to comply with sensitivity requirements, this approach usually complicates the determination of polar and semi-polar compounds in a single analytical run as polar compounds pass rapidly through the SPE cartridge resulting in a partial or complete loss during LC/MS detection. The aim of the current work was the development of a target and suspect screening LC/MS method for the determination of contaminants of emerging concern with a broad range of physicochemical properties in drinking water. The combination of a customized online SPE setup with direct injection capabilities in conjunction with high resolution accurate mass Q-TOF LC/MS allowed the identification and quantification of polar and non-polar analytes with high quality in a single injection. The method has been implemented in a routine screening environment and increased throughput has been facilitated. Sensitivity in the low ng/L range was observed for compounds amenable for enrichment without discriminating polar compounds. Moreover, improved retention and peak shapes have been observed for polar compounds compared to direct injection.

3.09P.8

Photodegradation products of poly- and perfluorinated alkyl substances (PFAS) on agricultural soils identified by LC-HRMS

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The class of poly- and perfluorinated alkyl substances (PFAS) comprises more than 3000 compounds which have a broad application area, from industrial processes to consumer products. For example, PFAS are used to produce paper and card board with water and grease repelling properties. Paper sludge from impregnated paper products was applied on agricultural soils during a period of ten years and caused contamination of several millions of square meters on a site in Southwest Germany. Original PFAS chemicals and transformation products have been identified in soil samples by liquid chromatography-high-resolution mass spectrometry (LC-HRMS) screening approaches. Polyfluoroalkyl phosphate diesters (diPAPs) are a major source of contamination. Typical degradation products of diPAPs are fluorotelomer alcohols (FTOHS) and perfluorinated carboxylic acids (PFCAs) which are also found in groundwaters of the contaminated sites. There is still missing information on the importance and kinetics of prevailing environmental processes and the resulting transformation products (TPs) of PFAS on the contaminated site. Therefore, we investigated in this study the photodegradation of diPAPs on mineral particles. Simulated sunlight was used to photodegrade 6:2/6:2 diPAP and 8:2/8:2 diPAP on Goethite particles in laboratory experiments within a few hours. LC-HRMS screening and electrochemical degradation experiments allowed the identification of several degradation products from the compound classes of PFCAs and unsaturated fluorotelomer carboxylic acids (FTUCA) as well as two so far not identified TPs at m/z 293 and 393. The results clearly show that diPAPs are photodegradable under environmental conditions and reveal the occurrence of degradation products which should be further considered in the field. Acknowledgement: The authors thank the state of Baden-Württemberg for financial support of the projects L7517012 and BWPFC19010 (program BWPLUS).

3.09P.9

Biotic and abiotic transformation products of the antidepressant fluoxetine identified by LC-HRMS

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The consumption of antidepressants has been increasing worldwide. Fluoxetine (FLX; trade name Prozac) is a frequently used compound of the class of selective serotonin re-uptake inhibitors (SSRI) and ranges among the 20 most prescribed pharmaceuticals in USA. Norfluoxetine is a well-known human metabolite. In a recent study further transformation products have been identified by LC-quadrupole-time-of-flight-mass spectrometry (LC-QTOF-MS) after laboratory experiments of direct and indirect photodegradation with simulated sunlight and biodegradation in Zebra fish embryos with activated sludge. 27 TPs could be tentatively identified from photodegradation experiments. TP formation occurred mainly by the following processes: demethylation, N-acylation, hydroxylation and/or loss of the trifluoromethylphenol group. In zebrafish embryos, 9 of the identified photodegradation TPs and further 2 TPs have been identified with norfluoxetine and hydroxyl-FLX as the main metabolites. Generally, in photochemical processes low-molecular weight TPs have been produced due to loss of moieties. In biotic processes, adduct formation was dominant (e.g. N-acetylation). The relevance of the occurrence of biotic and abiotic TPs and suitable precursors for adduct formation in surface water is still unknown and will be addressed in the ongoing study by field and laboratory investigations. Results demonstrate the importance of TPs to fully understand the environmental fate and ecotoxicological effects of FLX. Acknowledgement: The authors thank the Ministry of Science, Research and Art of the Land Baden-Württemberg, Germany for funding the project Effect Network in Water Research (Grant No. 33-5733-2511t32/2).

3.09P.10

Evaluation of reprotoxic effects in the model cell line JEG-3 by means of an LC-MS/MS based aproach

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The human placental cells JEG-3 are suitable to evaluate reprotoxicity of contaminants because compounds may enter into the cells and interfere with the routes involved in hormone synthesis. The ability of contaminants to interfere with aromatase activity, a key enzyme involved in the biosynthesis of estrogens, is commonly determined by measuring the amount of tritiated water (^3H_2O) formed during the aromatization of 1β-^{3}H-androst-4-ene-3,17-dione (^{3}H-AD). However, the analysis of tritium has associated health, safety and environmental risks. In this context, the present works aimed at developing a greener and safer methodology for this purpose. For this, liquid-chromatography coupled to tandem mass spectrometry (LC-MS/MS) was explored to determine trace levels of steroids in culture medium, as this technique is capable of delivering the high selectivity and sensitivity that the aforementioned application requires. After optimizing LC-MS conditions for the simultaneous determination in a single analytical run of the steroids of interest, namely estrone, β-estradiol, estriol, testosterone, and androstenedione, the performance of different extraction approaches (i.e., protein precipitation, liquid-liquid extraction, and Turboflow™ clean-up) was also evaluated Turboflow™ technology provided the best results in terms of accuracy and precision without compromising the method sensitivity, which could be attributed to the associated minimum sample handling. Thus, this technology was fully validated for the analysis of targeted steroids in culture medium and applied to evaluate the endocrine disruption potential of selected disinfection by-products in the framework of the ENFOCAR project. This work summarizes the results obtained regarding method performance and application and hence, the suitability of the automated Turboflow™-LC-MS approach to determine trace levels of steroids in culture medium. *Acknowledgments:* CP acknowledges support from the Fundación General del CSIC through the ComFuturo Programme (2nd edition).This work was supported by the Government of Catalonia (Consolidated Research Groups 2017 SGR 01404- Water and Soil Quality Unit) and the project PGC2018-097513-B-I00 (MCIU/AEI/FEDER, UE)

3.09P.11

Carbamazepine exposure in the sea anemones Anemonia sulcata and Actinia equina: metabolite identification and physiological responses

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Pharmaceuticals and other emerging compounds present in the coastal waters increasingly affects species, not yet all studied. In this study, we have focused attention on species (*Anemonia sulcata* and *Actinia equina*) that have been little studied but with an interest in their conservation and biotechnological application. These organisms belong to the class of the Anthozoa (Cnidaria Phylum), commonly known as sea anemones. Both are widely distributed in the Mediterranean Sea. The objectives of this work have been to determine the physiological and biochemical effects of carbamazepine (CBZ) exposure and characterize the degradation routes followed by this compound in water and tissues (biotransformation) of the two anemones species. Anemones provided by Oceanografic of Valencia were acclimatized to laboratory conditions for two weeks and exposed to 1 µg/L and 100 µg/L of CBZ in artificial sea water in a semi-static system. The experiment lasted 8 days. Small portion of the tentacles were taken the 2˚ and 8˚ day of experiment. Water samples were taken of the all aquaria with CBZ after 3˚, 6˚, 8˚ day. Ion transport (measured as N, K, ATPase activity), energetic metabolism (measured as glucose and lactate levels) and nitrogen excretion (measured as ammonia concentration in tissues) were determined. Treated samples of *A. sulcata* and *A. equina* were analysed by ultra-high-performance liquid chromatography high- resolution mass spectrometry on a quadrupole-time-of-flight (QqTOF). The lactate and glucose concentrations in tentacles of both anemones were modified by CBZ exposure. The structures of nine metabolites have been tentatively identified using HRMS and HRMS/MS data and three of this, were confirmed by Medline database program. The current work constitutes the first study on the identification of CBZ metabolites in Cnidaria, of species belonging to the Anthozoa Class. *Keywords* - sea anemones; biochemical determinations; physiological responses; mass spectrometry.

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3.09P.12

Intraweek occurrence and fate of approximately 200 contaminants of emerging concern, including antibiotics, in wastewater samples from 5 wastewater treatment plants in Cyprus by LC-QTOF-MS/MS

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Urban wastewater treatment plants (UWTPs) are a unique interface between human society and the environment. Analysis of various classes of contaminants of emerging concern (CECs) in wastewater samples along with improvements in analytical high-resolution mass spectrometry (HR-MS) methodologies should allow for future application of promising wastewater tracers, providing UWTP operators and regulatory authorities a more definitive toolbox with which to assess contamination associated with wastewater reuse and discharge practices.The aim of this study was a comprehensive quantitative target screening of approximately 200 CECs in 24-hour composite flow-proportional samples of influent wastewater (IWW) and treated effluent wastewater (EWW), collected in April 2018, during 7 consecutive days from 5 UWTPs located in Cyprus, employing conventional activated sludge (CAS) process or membrane bioreactor (MBR) technology. It involved a generic sample preparation, an UltraHigh-Performance Liquid Chromatography coupled with Quadrupole-Time of Flight Mass Spectrometry (UPLC-QTOF-MS/MS) method and post-acquisition data evaluation. An in-house database was built with information of retention time, MS and MS/MS ions for the target compounds which comprises pharmaceuticals, estrogens, illicit drugs, industrial chemicals and artificial sweeteners as well as some human metabolites and transformation products (TPs).Numerous compounds have been detected in high abundance in both IWW and EWW samples, including antibiotics (e.g. trimethoprim, sulfamethoxazole and sulfadoxine), beta-blockers (e.g. atenolol, metoprolol and sotalol), other pharmaceuticals (e.g. diclofenac, lamotrigine and valsartan) and industrial chemicals (e.g. benzotriazole and tolytriazole). Several CECs presented high removal rate during secondary treatment, i.e. paracetamol, ephedrine, caffeine, theophylline, naproxen and ranitidine, and thus, were below the limit of detection in EWW samples. Moreover, a variety of pharmaceuticals detected in IWW, have been transformed upon CAS and MBR treatment processes generating several TPs. The following parent compounds and their associated TPs have been detected in IWW and EWW, respectively: metformin/guanylurea, clarithromycin/N-desmethyl clarithromycin, lidocaine/norlidocaine, citalopram/N-desmethyl citalopram and citalopram amide, venlafaxine/venlafaxine-N-oxide and N-desmethyl venlafaxine, tramadol/tramadol-N-oxide and carbamazepine/carbamazepine-10,11-epoxide.

3.09P.13

Quantification of Pharmaceuticals and Personal Care Products in Sediment, Seawater, and Biota Collected Around a Wastewater Discharge Point in Norway

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The consumption and use of pharmaceuticals and personal care products (PPCPs) in urban populations may pose a threat to ecosystem health as conventional wastewater treatment plants (WWTPs) do not completely remove these compounds from the discharged effluents. IVAR SNJ Biological WWTP, a regional WWTP in Norway, is an advanced biological treatment plant. The WWTP receives wastewater from both household and industrial sources, including a hospital. It is required by the Norwegian pollution regulation to remove at least 70% of all degradable biological matter before discharge. SNJ is able to reach 80% removal, however, no assessments of PPCPs in the effluents have been made. The residual contaminants released from WWTPs into marine waters undergo rapid dilution and are challenging to detect. These chemicals are often persistent and their effects on the environment are not yet fully understood. Advances in analytical chemistry, such as the development of highly sensitive, high throughput mass-spectrometer instrumentation, enable the quantification of very low concentrations in environmental samples. A recent study in our group evaluated 15 emerging contaminants, including 6 PPCPs (acetaminophen, diclofenac, lamivudine, phenytoin, carbamazepine and sulfamethoxazole), in seawater, sediments, seaweed, and biota. All 15 target compounds were quantified in all samples. For the biota samples, all PPCPs, except diclofenac, were found in the ngL-1 range. We decided to optimise the methods used for extraction and quantification in an environmental monitoring study in order to evaluate and quantify the presence of more than 50 PPCPs in the marine area around the sewage outfall of SNJ. Solid samples were ultrasonicated prior to SPE using Oasis HLB cartridges. Our samples represented three different fates; seawater, top layer of sediments and biota (invertebrates and crustaceans). The analysis was performed using five replicates per sample for both GC-MS and LC-MS/MS analyses. The advantage of GC-MS was the high reproducibility, due to the electron impact ionization process, while the LC-MS/MS had less extensive sample preparation procedures. Therefore, the LC-MS/MS was used to run a broader selection of samples in shorter time and at lower cost. The amounts of PPCPs recorded in the different station was representative of the contamination status of the area.

3.09P.14

Seasonal changes in emerging contaminant occurrence in the River Thames using passive sampling and liquid chromatography-mass spectrometry

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Occurrence of emerging containments was determined using Chemcatcher passive samplers deployed in the River Thames, UK for at least 14 days during winter (December/January) 2018/2019 and summer (August/September) 2019. Samplers were configured with HLB (divinylbenzene-co-N-vinylpyrolidone) sorbent disks as the receiving phase and a PES (Poly-ethersulfone) disks were used as the diffusion limiting membrane. Sampler extracts were analysed using two reversed-phase liquid chromatography (C18 and (C6H5)2) methods coupled to quadrupole-time of flight (Q-TOF) and a rapid triple-quadrupole (MS/MS) mass analysers. Contaminants were identified using both targeted analysis, based on reference material and suspect screening using accurate m/z and retention time libraries. A total of 135 compounds were found in common across both seasons using both screening analysis and targeted analysis when compared to a standard mix of 180 compounds. Of these 11.1% were drug metabolites, 8.9% were cardiovascular agents, 5.9% were fungicides, opioids, antidepressants, antibiotics and herbicides, and 5.2% were controlled drugs. When comparing compound abundance between the seasons, the top five classes (in order) during winter were; herbicides, drug metabolites, cardiovascular agents, controlled drugs and antidepressants. During summer the top classes were; drug metabolites, controlled drugs, cardiovascular agents, anti-depressants and fungicides. The bioconcentration factor for both invertebrates and fish were subsequently predicted using machine learning for all compounds identified. Identification of emerging containments in this catchment will enable the rapid prioritisation of ecotoxicological risk assessments. A previously developed 4-layer multi-layer perceptron-based machine learning model predicting the bioconcentration factor (BCF) from 14 molecular descriptors was applied. The model was trained, optimised and tested using 70:15:15 randomised spilt of the dataset into training, verification and blind test sets. The model achieved an adequate R2 performance for the verification and blind test sets. The model was externally validated for selected compounds using animal experimental data. This work represents the foundations of future work aiming to model BCF from in situ data collected using passive samplers.

3.09P.15

Profiling of human exposures to broad range of anticancer drugs by mass spectrometry techniques - monitoring of hospitals, pharmacies and patient households

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Anticancer therapies are classified as hazardous drugs, and have mutagenic, carcinogenic and teratogenic effects in hospital personnel during life-long occupational exposures. Their consumption is steadily increasing leading thus to potential spread out to the environment. The present study developed a multi-target UPLC-MS/MS method for a broad set of anticancer drugs (ADs) including commonly used markers of contamination like cyclophosphamide – CP and 5-fluorouracil - FU as well as less explored drugs (Paclitaxel - PX, Ifosfamid - IF, Irinotecan - IRI, Metotrexat - MET, Capecitabin, Imatinib, Sunitinib, Tamoxifen, Doxorubicin, and Everolimus). Total platinum (Pt) was also measured by ICP-MS as a marker of platinum-based drugs. The methods were employed in a large-scale monitoring of indoor areas in the Czech republic and Slovakia (more than 600 samples collected during 2018-2019) that included hospitals and pharmacies (25 large authorized institutions), small patient care units (12 clinics) as well as households of oncology patients (preliminary screening). Over 80% of samples were positive for at least one AD, the most frequently detected were the traditional markers of contamination CP, Pt and FU that often exceeded the recommended Threshold Guidance Values. Maximum values detected in hospitals reached up to 880,000 and 115,000 (pg/cm2) for CP and FU, respectively, which represent a major risk for employees. Newly analyzed ADs (namely PX, IRI, MET and IF) as well as active ingredients of orally administered therapies (sunitinib, imatinib, capecitabin) were also detected, especially on the floors of the toilets and patient administration wards. The present study is one of the first larger-scale monitorings documenting serious occurrence and contamination of indoor environments by a broad spectrum of different ADs, and showed that the levels of contamination were generally independent from the size of hospitals. Results from the monitoring are an important input to hospital managers helping to improve working procedures, protecting thus spread of these hazardous contaminants to the environment. [The research was supported by Ministry of Health of the Czech Republic, grant No. NV18-09-00188].

Measuring, Modelling and Monitoring of Pesticides Fate in a Regulatory Context (P)

3.10P.1

Improved parameterization of sediment trapping in VFSMOD

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The most widely implemented mitigation measure to reduce transfer of pesticides and other pollutants to surface water bodies via surface runoff are vegetative filter strips (VFS). To reliably model the reduction of surface runoff, eroded sediment and pesticide inputs into surface water by VFS in a risk assessment context, an event-based model is needed. The most commonly used dynamic, event-based model for this purpose is VFSMOD. VFSMOD simulates reduction of total inflow (ΔQ) and reduction of incoming eroded sediment load (ΔE) mechanistically. These variables are subsequently used to calculate the reduction of pesticide load by the VFS (ΔP). There are several options in VFSMOD to calculate ΔP, notably the empirical Sabbagh equation (either with original or revised regression coefficients) and a regression-free, mechanistic mass-balance approach (Reichenberger et al., 2017). Four studies with 16 hydrological events were selected from the experimental data compiled by Reichenberger et al. (2019), representing different levels of data availability and uncertainty. A first set of VFSMOD simulations, with parameterization according to the settings in the tool SWAN-VFSMOD, was run with the aim to compare the performance of the different pesticide trapping equations. The simulations yielded a general overestimation of ΔE, suggesting that the SWAN-VFSMOD parameterization of sediment filtration is too optimistic. However, a reliable prediction of ΔE is important for the reliability of predicted ΔP, in particular for strongly sorbing compounds. In a second step, a maximum-likelihood-based calibration and uncertainty analysis with the DREAM-ZS algorithm was performed for each hydrological event and the target variables ΔQ and ΔE. Overall a good match of measured ΔQ and ΔE was achieved, but only a few parameters could be well constrained. In a third step, in order to reduce the observed equifinality, the hydraulic parameters were fixed to the best parameter sets obtained during the second phase, and only sediment filtration parameters were calibrated with DREAM-ZS. The most important parameter characterizing the incoming sediment in VFSMOD is the median particle diameter DP. A set of empirical equations to predict DP from soil texture (Foster et al., 1985) was used as supporting information in the calibration of DP. The poster will present an improved, generic parameterization methodology for sediment trapping in VFSMOD that can be used for regulatory VFS scenarios.

3.10P.2

Degradation and adsorption of metsulfuron methyl and its metabolites in different types of soils commonly occuring in Europe

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In 2017 more than 25 thousand tons of plant protection products (in terms of active substance) were sold in Poland. Most of them, i.e. 54%, are herbicides, haulm destructors and moss killers, 28% are fungicides and bactericides, 9% are plant growth regulators, 7% are insecticides and acaricides, 2% are other plant protection products and molluscicides. Plant protection products have been known for decades and the unregulated and indiscriminate applications have generated an increased presence of residues in soils, surface waters, ground water or crops. A representative from the group of the most frequently sold plant protection products was selected for this study. Metsulfuron methyl is one of the most widely used of sulfonylurea herbicides. Its wide use is due to broad-spectrum weed control at very low rates and good crop selectivity with systemic and residual action. Metsulfuron methyl is widely used for the purposes of weed control in arable field spraying in agricultural production. Therefore, there is a risk that active substance

residues and its metabolites may leak into the soil and cause damage to soil fauna. Furthermore residues of pesticide can also get into groundwater due to its high water solubility, high mobility and slow degradation of metsulfuron methyl. There is no doubt that pesticides play an essential role in maintaining high levels of food production, but on the other hand, they affect soil fauna and many biological processes involving it. Hence, it is extremely important to determine the dynamics of metsulfuron methyl and its metabolites degradation in the soil and the impact on soil fauna. The aims of this study were to (i) determine the rate of degradation in three types of soils commonly found in Europe; (ii) assess the adsorption and desorption behaviour on five different soil types; (iii) determine the Kd, Koc, KFads, and Kdes. This study was conducted in accordance with OECD methods Test No. 307: Aerobic and Anaerobic Transformation in Soil and Test No. 106: Adsorption - Desorption Using a Batch Equilibrium Method. The obtained results, showed the dynamics of degradation of metsulfuron methyl and its metabolites in three different types of soils. The adsorption and desorption behaviour on five different soil types was also showed.

3.10P.3

Challenges with Ready Biodegradability Test of Poorly Water-Soluble Compounds

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Biodegradation is the process in which organic substances are decomposed by microorganisms (mainly aerobic bacteria) into simpler substances such as carbon dioxide, water and ammonia. Biodegradation is one of the most important factors in assessing the environmental fate of chemicals. Substances that are coming and subsequently getting in contact with the aquatic microorganisms, which is therefore the basis for the assessment of aquatic biodegradation. It describes the decomposition and mineralization by microorganisms in the environment. If this process is complete, the initial organic substances are entirely converted into simple inorganic molecules. The Closed Bottle Test is the classical test method for Ready Biodegradability. The advantage of the test is its simple performance, the possibility to test poorly soluble and volatile compounds, as well as the requirement of low test concentrations of test compound. When we are dealing with a poorly water-soluble compound, there comes the requirement of the use of an emulsifier or a solvent, as a solubilizing agent. Before using it, it is required to be check that, it should not be toxic to bacteria and should not be biodegradable and fulfils the validity criteria defined by the guideline. In this experiment we had tested total six different solubilizing agents, i.e., Tween 20, Tween 80, Triton, IGEPAL, SDS, and Silicon oil AR 20, along with Inoculum blank and run the test for 28 days. Sodium benzoate was also run simultaneously to check the validity of the test. The analysis results on 28 day showed that among six agents, only three solubilizing agents met the validity criteria. These three agents (Triton, IGEPAL and Silicon oil AR 20) may therefore be used as a solubilizing agent for the test.

3.10P.4

The Effect of Laboratory Lighting on the Isomerisation of Pesticides

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Concerns have been raised regarding possible differences in the effects, degradation and transformation of different isomeric forms of plant protection products. New guidance was issued by EFSA in July 2019[1] to ensure that the required information is available to complete the risk assessment of active substances of plant protection products which contain stereoisomers as components or impurities or may form transformation products which contain stereoisomers.In the course of performing environmental fate studies, it is important to know if any change in the isomeric form of an active ingredient is due to natural processes in the test system or if it has been brought about by the extraction and/or work-up of the samples in the laboratory.During an adsorption/desorption study (OECD 106) that was conducted on a pesticide active ingredient, an unknown degradate was observed which eluted very close to the parent peak on the radio-HPLC chromatogram.This poster shows how the unknown was identified as an isomer of the parent compound and that it was formed by the action of the laboratory lighting on the sample extracts during the work-up stages. [1] EFSA Journal 2019; 17(8):5804

3.10P.5

Comparison of different parameters and methods for the assessment of microbial activity of surface waters in OECD 309

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For persistency assessment in different regulations of chemicals, the degradation of chemicals is tested following OECD test guidelines, e.g. OECD 309 for determination of degradation in surface water. In a test according to OECD 309 the microbial activity of the test water is determined by the mineralisation of an easily degradable reference substance, usually 14C-labelled sodium benzoate or aniline. As validity criterion the guideline states that aniline or sodium benzoate shall "degrade within two weeks" or correlates with colony forming units (CFU) in the range of 103-104. However, no details are given about parameters such as the test temperature, the test setup (e.g. closed or flow-through) and no specific definition of what is meant by degradation (mineralization or parent disappearance) is provided. Thus, it is questionable whether the validity criterion is applicable for evaluation of the test validity. This is further complicated as in practical testing often different degradation rates of sodium benzoate, determined in form of mineralization, are observed in dependence of the applied test design (closed vs. flow through) or the test water (e.g. fresh vs. marine water). This is not addressed by the guideline. The aim of our project was (i) to systematically investigate the influence of the chosen test design (flow-through vs. closed setup), the temperature (12°C / 20°C), different label positions, as well as the seasonal variation of the test water on the degradation of sodium benzoate and (ii) to compare this data with microbial activity describing parameters of the test water. For the second objective not only the CFU were determined in the water samples but also the total active cell number determined using the principle of an optic/voltammetry platform. The results show that, while the CFU-values are varying during the seasons (while staying in the range of 103-104) the mineralization rate in closed systems is generally, but not always, slower than in flow-through systems, even though oxygen concentrations were still sufficiently high. Also nearly identical mineralization rates were observed for experiments conducted at different temperatures. Thus, our experiments show that the provided validity criterion of OECD 309 is not sufficient as long as no specifications for certain parameters are given.

3.10P.6

Sorption of epoxiconazole and tebuconazole in arable soils and its relationship to basic and advanced soil properties

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Conazole fungicides are currently and previously intentionally and carelessly used pesticides in various industrial and urban locations. Most of them enters the soil forming short- or long-term residues. In this study two representatives of triazole fungicides, epoxiconazole (EPC) and tebuconazole (TBC), have been tested with 20 soils from Czech Republic for their sorption. Sorption, by means of Kd coefficients, was compared to basic and advanced soil properties. EPC and TBC showed significant Pearson (P) and Spearman (S) correlation with $pH_{(H2O)}$ and TOC(%).Clay, silt, sand and cation exchange capacity (CEC) did not show reasonable linkage with adsorption. Recalculating Kd to Gibb's free energy (ΔG) provided more detailed explanation of adsorption mechanism. Small and negative ΔG values means that adsorption was feasible, spontaneous and exothermic. ΔG values gave much better correlation for same soil properties $pH_{(H2O)}$ and TOC(%) in most cases. Additionally ΔG revealed more binding characteristics of hudrogen (H)-bonds for EPC (H-donor, acceptor-0, 4) and TBC (H-donor, acceptor-1, 3) in sorption and degradability in water-soil systems. EPC sorption was not highly influenced with pH changes compared to TBC. Number and types of H-bonds with molecular geometry govern the leachability in soil, complying that TBC is more leachable than EPC for the same soil. *Acknowledgement - This research was supported by the RECETOX Research Infrastructure (LM2015051) and project of Czech Science Foundation (GF17-33820L)*

3.10P.7

Field Study to Assess the Influence of Tied Ridging Technology Used in Potato Cultivation on the Rate of Surface Runoff of a Herbicide

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On some agricultural fields planted with potatoes, water and sediment transportation as run-off and erosion can be problematic. Potatoes can be very vulnerable to soil erosion, especially just after planting. On sloping sites water run off along with soil particles can lead to enhanced loss of nutrients and plant protection products to adjacent surface water bodies. The recent use of tied ridges (micro-dams), established in the ridge bottom/furrow of potato beds, is one of several ways in which growers can help reduce surface runoff significantly while increasing time for infiltration to occur. A field study, in compliance with GLP standards, was set up to assess the influence of tied ridging on the rate of surface run-off (water and soil loss) of a herbicide, applied to bare soil, and monitored under realistic worst case conditions representative of the proposed use. A single application was made to a series of replicated plots (11 m by 5.5 m) planted with potatoes using field scale machinery considered typical of that used in high usage areas across Europe. Tied ridges were established in the ridge bottoms of three of the seven plots to assess the level of mitigation afforded by way of a reduction in surface runoff. Rainfall for the experimental site was reviewed continually and artificial irrigation applied to the site to simulate rainfall and run-off where necessary. The data generated from the study was intended for use as a higher tier refinement in modelling frameworks. The study design and set up as well as the post-treatment sample collection towards the assessment of pesticide and nutrient loss in surface runoff during the monitoring period will be described.

3.10P.8

Simulating rainfall to measure the effect of three cultivation methods on pesticide run-off

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Research has shown that reducing tillage intensity can be an effective means of protecting soils from erosion and thereby reducing surface runoff and associated pollutants at the edge of field. For seedbed preparation in advance of drilling row crops, three typical cultivation methods considered are conventional ploughing (inversion tillage), min-till and strip-till. Conventional ploughing is considered to be the most intensive cultivation method. Min-till typically involves a single cultivation pass over the entire soil surface to no more than 20 cm depth and incorporating crop residues. Strip-till is potentially the least intensive method, with one third of the total field area being cultivated or disturbed. Pesticide run-off concentrations are compared in a plot scale field study to assess the impacts of these different tillage intensities during simulated storm events. Run-off is measured/collected and both the aqueous and sediment bound pesticide concentrations are then determined. To facilitate the simulation of representative storm events, a bespoke rainfall rig has been designed and constructed with the aim to replicate raindrops of appropriate size and velocity expected during natural storm events to dislodge and transport soil particles, causing erosion. The bespoke rainfall rig consists of multiple wheel mounted gantries which are connected together to form a fixed structure covering the plot under study. Carefully chosen pivot sprinkler heads, fitted with the appropriate size nozzles are mounted on each gantry span, at the desired adjustable height above ground level. Pre-testing of individual sprinkler heads and gantries has been carried out to establish the number and spacing required to give the optimum plot coverage when simulating the representative storm events. To characterise output from the sprinkler nozzles, the flour box methodology was applied to determine droplet size and velocity. Rainfall collectors were deployed in a grid at 1 m spacing within the gantry area to determine simulated rainfall volume and intensity distribution. The data and conclusion(s) with respect to simulation of rainfall will be presented, including droplet size and velocity.

3.10P.9

Analysing exposure profiles based on 20-year FOCUS SW simulations

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The EFSA Working Group on FOCUS Surface Water Repair will provide a software package to run multi-year simulations (20 years with a uniform 6-year warm up period) to replace the current short-term simulations (12 to 16-month simulation period). The latter has been in place since 2003 due to its proven track-record of meeting the protection goals for aquatic ecosystems while the new multi-year simulations shall be equally protective and should provide an increased robustness and reliability to the PECsw simulations by using a 20 year simulation period. In the present investigation multi-year simulations for some active substances after application to several crops will be conducted with the aim to generate exposure profiles for the whole simulation period. In a first analysis step, the multi-year exposure concentration patterns will be analysed with the EPAT tool to check if a representative exposure pattern can be derived and if subsequently typical climate periods can be identified. Finally, the impact on the most sensitive aquatic species of the representative exposure profiles will be analysed by toxicokinetic-toxicodynamic (TK/TD) modelling using the general unified threshold model of survival (GUTS). This modelling approach was deemed ready to be used in RA by the EFSA scientific opinion on TK/TD effect models for aquatic organisms. Different possibilities for analysing the representative exposure profiles will be investigated.

3.10P.10

Sensitivity analysis of pesticide application timing on exposure estimates of the new 20-year FOCUS surface water drainage scenarios

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The EFSA draft scientific report on FOCUS multiyear surface water simulations questioned the use of a pesticide application timer (PAT) in the European risk assessment on surface water exposure. Currently, a PAT is used to determine actual application dates from a window of intended dates in order to prevent conditions which disagree with agronomical practice. Ignoring PAT rules could result in unrealistic predicted environmental concentrations (PECsw), e.g. due to the possibility of pesticide application on rainy days. Also, the avoidance of a PAT might increase the overall variability of simulation results. In the present study, we carried out a sensitivity analysis to assess the effect of different PAT rules on day-to-day PECsw of FOCUS surface water drainage scenarios. A set of hypothetical compounds recommended by EFSA Repair working group with varying K_{oc} and DT_{50} values were simulated. Three different options were evaluated: (i) no-PAT, applications were assumed to take place on every day of the year in each of the 26 assessment years and a modification of the current PAT with an application windows of (ii) 7 and (iii) 15 days centered on the intended day of application (± 3 and ± 7 days, respectively). The resulting day-to-day PECsw patterns were then compared to the no-PAT option. In addition, multi-year results using the PAT candidates were also compared to the current FOCUS single-year assessment. For scenarios D1, D2, D5 and D6, the day-to-day PECsw patterns showed that the PECsw obtained from no-PAT calculations were extremely sensitive to the application date and were generally larger than results obtained from using other PAT options. This observation emphasises the need for a PAT in FOCUS surface water simulations. The PAT candidate with an application window of 7 days still allowed rainfalls to occur close to application date. However, extreme rainfall events during the assessment period were avoided which resulted in smoother PECsw patterns. PECsw percentiles also showed poor agreement between the new multi-year assessment approach and the current FOCUS single-year approach. For scenario D2, the single-year FOCUS approach resulted in PECsw partly below the 47.5th percentile in comparison to the new multi-year approach.

3.10P.11

Landscape level simulation of off-field exposure by runoff - Hydrology, run-off generation and filtering

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In EFSAs (2017) scientific opinion on risk assessment for in-soil organisms specific protection goals are discussed for off-field areas in landscapes. This has two important aspects. Firstly, the relevant domain is the landscape, and secondly, effects have to be assessed in time and space. As a consequence, also exposure has to be assessed in a way to address its spatial and temporal variation in agricultural landscapes. Benchmark scenarios as used for example in FOCUS surface water employ edge-of-field assessments which are not sufficient to describe processes at the landscape level. Hence, the EFSA opinion states (section 8.1.2) "... appropriate off-field exposure scenarios that apply to a given percentile of the concentration distribution still need to be developed". In previous work a modelling framework was presented which is able to consider relevant processes at landscape level, i.e. interaction between landscape elements, and relevant exposure routes for off-field areas such as drift and run-off. In this work we focus on run-off and present details on the methods to derive overland flow directions from a digital elevation model to route water and mass flows through the landscape. Run-off generation is described using FOCUS-PRZM. Run-off filtering, i.e. the reduction of pesticide surface mass flux that causes local exposure, is considered for un-treated agricultural fields and off-field areas, using different reduction efficiencies. Off-field run-off filtering can be described by empirical functions derived from data given in the FOCUS Landscape and Mitigation report or using the mechanistic model VFSMOD. The modelling framework is applied to a 2x2km realistic landscape to illustrate the spatial distribution of run-off overland mass flow and deposition due to filtering. Besides the description of exposure in the landscape such assessments can be used to evaluate mitigations as for example in-field margins which improve filtering in-field and thereby reduce deposition off-field.

3.10P.12

On the use of EU-wide geo-databases for aquatic risk assessment - a case study on pH characteristics in surface water

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Fate, behavior and toxicity of plant protection products (PPP) in natural surface water bodies are controlled by various parameters and properties. For example, the pH level in surface water may be of great importance, as this may affect the stability of an active ingredient and its fate and effect in natural aquatic environments. On this account, we explored the information content of different EU-wide geo-databases on surface water characteristics and their applicability to support aquatic risk assessment. Particularly, the WISE database, a collection of monitoring data associated with the Water Framework Directive (WFD), was investigated in detail and combined with other EU- wide information (e.g. CORINE land cover, catchment characteristics). The WISE WFD database, contains data from the 1st and 2nd River Basin Management Plans reported by EU Member States and Norway. This includes information about ground water and surface water bodies (e.g. water body category, ecological or chemical status) structured by country, river basin district and sub-units. The data were checked for quality and consistency and subsequently evaluated with focus on the range and variability of pH observation in rivers and lakes from 2005 to 2018. An overlay with the CORINE land cover database (CLC) was used in order to delineate agricultural area as well as specific crops relevant for PPP application. The results indicate that for agricultural areas and specifically for areas with permanent crops a large part of the EU surface water locations is in neutral or alkaline range while acidic conditions mostly do not occur. For agricultural area, the median of annual pH levels in rivers and lakes is 7.9 (n =20211). For water bodies in the area of permanent crops (i.e. fruit trees, vineyards), the pH median is 8.0 (fruit trees; n=409) and 8.1 (vineyards; n=344) ranging between 7.6 (5th percentile) and 8.5 for fruit trees, resp. 8.4 for vineyards (95th percentile). This example shows the great potential of EU-wide data collections for aquatic risk assessment particularly when information is used in combination with other data sources, e.g. spatial land use data.

3.10P.13

Stepwise development of catchment hydrology for effect modelling in regulatory landscape-scale aquatic risk assessment

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The EFSA Guidance Document on Aquatic Risk Assessment (EFSA, 2013) assigns key a role for landscape-scale effect modelling in future aquatic risk characterization in a tiered risk assessment framework. The hydrologic catchment constitutes a logical assessment unit for landscape scale analyses. After selecting a catchment for analysis, the catchment hydrology needs to be set up with a high temporal (e.g., 1h timesteps) resolution and spatially explicit locations of fields and streams, according to effect modelling requirements. The hydrological model provides sensitive parameters for risk characterization, such as the water volume (substance dilution), the water surface area (receiving spray drift inputs) as well as the flow velocity (substance transport within the river network) for each stream segment dynamically in the catchment. Hence, the hydrologic parameters predictions presumably have a high influence on the concentration profiles used for effect modelling. Because adequate hydrological monitoring data for modelling are not always available at catchment scale, we introduce a stepwise modelling approach with different levels of (a) mechanistic process complexity and (b) data requirements to define specific regulatory hydrologic scenarios at landscape scale using the modular Catchment Modelling Framework (CMF https://philippkraft.github.io/cmf/). These distinctive levels start from small data requirements with limited predictive performance continuing to large data requirements with an expected high predictive performance. This enables running the hydrological model with publicly available data (local gauging stations) or even on the EU-level (e.g. WISE, EFSA spatial data 2.1, CORINE), as well as with detailed monitoring data. The companion presentation of Beltman et al. shows the build-up of the model for a drift assessment with publicly accessible data (gauging, land use). In the next step the catchment will be analysed for suitable hydrological monitoring methods and locations to validate the current hydrological simulation and improve the model setup with additional data to build the next level of hydrological robustness.

3.10P.14

Pan-European catchment modelling: an open data analysis

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In the process of authorization of plant protection products (PPPs) in the European Union, tiered approaches are followed in aquatic exposure and effect assessment. The EFSA Guidance of tiered risk assessment for edge-of-field surface waters (EFSA, 2013) includes landscape-level modelling, along with field studies, as an option for the highest tier of aquatic effect assessment. On the exposure side also a stepped approach is followed, but currently it stops at edge-of-field mitigation measures to reduce pesticide inputs into surface water via surface runoff and spray drift. Landscape-level aquatic exposure modelling (i.e. exposure modelling at the level of hydrological catchments) is not routinely performed for regulatory purposes yet, but is a promising option to (a) support stewardship projects and (b) to set monitoring data into context. Moreover, (c) the modelling of larger areas is helpful to identify likely hotspots in order to implement a detailed monitoring study. Nevertheless, no information is at hand which open data on the pan-European scale are suitable for use in catchment modelling. Hence, in this project we investigated the suitability of public hydrological datasets available at pan-European scale (as provided by EU authorities) to facilitate routine regulatory exposure modelling at the catchment scale. First, we analysed measured stream / river discharge records from gauging stations of the Water Information System in Europe (WISE) database, which was compiled and processed by the European Environment Agency (EEA). Second, we derived the upstream catchment areas for each gauging station using the watersheds of the Catchment Characterisation and Modelling (CCM2) database and estimated area-specific discharges using the MARS25 meteorological dataset. The poster will present a plausibility check of measured stream discharges and discuss the feasibility of regulatory catchment modelling using only open data (publicly available from EU authorities, e.g. EFSA and EEA) on the pan-European scale.

3.10P.16

Enhanced FOCUS MACRO groundwater calculations

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FOCUS MACRO is one of four models used to evaluate potential movement to groundwater in the EU registration process, including several national approaches (e.g. UK, Sweden, Denmark etc.). Beside the specific national scenarios only one single FOCUS scenario (Châteaudun) is available in FOCUS MACRO. If an intended crop is defined for this scenario, FOCUS MACRO simulations need to be run (EFSA PPR, 2013). This often leads to an extensive amount of modelling work, especially if soil metabolites have to be considered. FOCUS MACRO is only able to simulate one coupled metabolite at a time by running a parent, an intermediate and a metabolite simulation. A single run lasts around 30 to 45 minutes and depending on the hardware used this may result in a significantly long calculation time. Furthermore, FOCUS MACRO runs separate parent and intermediate simulations for each metabolite and generates identical data if crop, application rate and scenario remain the same. This significantly increases the data size and simulation time without any additional results. Our MACRO GW Automation Tool was developed to address both problems without making any changes in the input or output files. First, it launches multiple runs at the same time, starting with parent and intermediate runs simultaneously, followed by the metabolite runs as soon as the intermediate run is finished. Additionally, redundant parent and intermediate runs can be de-selected by the user, and the metabolite could be connected to another intermediate simulation, which significantly reduces the calculation time.

3.10P.18

A model-data integration study of plant uptake process using a virtual 3D soil-root system

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Plant uptake is an important factor in environmental exposure assessment for the authorization of plant protection products (PPPs). However, in all regulatory models, this process is oversimplified and lacks a mechanistic description. For a better mechanistic understanding, we present a novel process-based model for a 3D soil-root system. The model simulates root growth, water flow and solute transport in both soil and root system as well as plant uptake, not only at macroscopic scale but also at root segment scale. The virtual system simulates water flow and solute transport both in soil and in the 3D root system, as well as water and solute exchange between them. The dynamic growth of the 3D root system in the soil is simulated with the CRootBox model. In the model, the plant uptake process can be considered as an active process with Michaelis-Menten-kinetics (for fertilizers) or a passive one (for PPPs) which is caused by the diffusion and advection through the root membrane. In a case study, the virtual system was integrated with experimental data of rice plant uptake with different levels of Phosphate (P) fertilization in the topsoil. Using the virtual soil-root system, in silico experiments were subsequently set up and carried out under the same water regimes and P levels as in the real experiment. The 3D architectural root systems were reconstructed from the phenological root data (nodal root number, lateral types, interbranch distance, root diameters, root biomass allocation among soil layers). The environmental data such as irrigation and evaporation were used as the driving data for the 3D simulations. The results from in silico experiments displayed similar trends in total P uptake as the measured ones. The effects of drying cycles and P availability were well represented by the simulated P uptake. More interestingly, the simulations reveal the underlying processes such as the dynamic of the solute depletion zone in the rhizosphere during root growth as well as the contributions of specific roots in the uptake under contrasting scenarios. By model-data integration, the virtual soil-root system enhances the mechanistic understanding of the plant uptake across scales, which cannot be discovered by experimental observation only.

3.10P.20

Contributing to the evaluation of hydraulic pedotransfer functions: A case study from Central Europe

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The simulation of soil water movement and solute transport in the unsaturated zone for purposes of exposure modelling requires knowledge about hydraulic soil properties. Soil information collected during field studies or from soil profile databases often only provide physicochemical soil properties. However, hydraulic properties can be estimated from basic soil data by using pedotransfer functions (PTF). Deriving PTF requires comprehensive datasets which often exhibit a spatial bias for particular regions or countries. As an example, the HYPRES PTF were derived for the European scale but the data basis originated largely from a single country. In addition, commonly used PTF are only intended for the use in pan-national or continental scale assessments and may not be able to represent spatial heterogeneity at a field scale due to limited explained variance. This results in uncertainty about the accuracy of estimated hydraulic parameter values when applying PTF at a field level. We evaluated the performance of hydraulic PTF commonly used in European exposure risk assessments on soil samples from Central Europe. Soil samples were collected from agricultural fields and were analysed to determine undisturbed bulk density, soil texture, organic carbon content, saturated hydraulic conductivity, and water holding capacity at four distinct depths. Measured water content at five different suction pressures as well as hydraulic conductivity were compared to estimations derived from basic soil properties. Evaluated pedotransfer functions comprised HYPRES and ROSETTA as well as the PTF used for creation of the more recent 3D Soil Hydraulic Database of Europe (EU-SoilHydroGrids).

3.10P.22

Spatial and temporal distribution of the current-use and recently-banned pesticides in arable soils of the Czech Republic

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The study presents monitoring data on the spatial and temporal occurrence of pesticide residues in arable soils of the Czech Republic. In total, 34 soils were sampled consecutively in years 2014-2017 amounting 136 samples and analyzed for 60 pesticides and four transformation products. Of the pesticides currently in use, conazole fungicides were frequently present in soils above the limit of quantification and/or above the 0.01 mg/kg threshold. They appeared in the following order of positive findings (i.e., % of positive soils): epoxiconazole (56-62% depending on the sampling year) > tebuconazole (35-47%) > flusilazole (29-32%)~propiconazole (18-35%)~cyproconazole (18-29%) > difenoconazole (3-9%) > metconazole (0-3%). Of the other pesticide types, herbicides azoxystrobin, diflufenican, chlorotoluron, metolachlor, pendimethalin and terbuthylazine, fungicides carbendazim (banned in 2014), fenpropidin, fenpropimorph and prochloraz and the insecticide methoxyfenozide were found in ≥ 20% of soils at least in one sampling campaign. Soils typically (>50%) contained 2-7 residues with the maximum of 14. For the 136 samples, 116 different mixtures were identified. The multi-occurrence of chemicals resulted from the simultaneous presence of both current-use and formerly-used pesticides as well as transformation products such hydroxy-atrazine and hydroxy-terbuthylazine that were found in up to 71% and 74%, respectively, of soils sampled in the respective year. Occurrence of pesticides was driven by their annual consumption rates, half-lives in soils, and hydrophobicity. The collection of monitoring and application data revealed that 69% of pesticide residues was inherited from the previous growing season(s) while 63% of the applied pesticides did not persist till the following growing season. The monitoring results evidenced that pesticides´ former and current uses result in complex chemical burden in agri-soils that needs further attention in terms of defining adequate EU-wide monitoring tools, soil protection measures and pesticide management policies.

3.10P.23

Modelling Strategies For Arable Spray Drift Of Plant Protection Products In Europe

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Current spray drift curves supporting environmental risk assessments are derived from assemblages of field studies and do not allow for consideration of the influence of individual parameters that may be used in either statistically-derived models or mechanistic models for defining input parameters (e.g., wind speed, boom height, tractor speed, spray pressure, etc.). This presentation will summarise the current range of options for representation of drift, including presentation of statistical strategies for the development of a Bayesian drift representation model drawing upon an extensive dataset of experimental drift trials compiled as a component of the SETAC DRAW workshops. Strategies for the development of reference scenarios to support further development and testing of models (statistical and mechanistic) will also be presented drawing upon surveys and expert consultations. Recommendations will then be presented outlining future experimental efforts using a standard testing protocol developed as a component of the SETAC DRAW workshops, further efforts to reinforce understanding of application practice to support development of regulatory scenarios, and future options for flexible but more realistic implementation of drift mitigation with models.

3.10P.24

Using a higher-tier coupled modelling approach to support the evaluation of groundwater monitoring studies on a sub-catchment scale

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In the context of European regulation on plant protection products (PPP), a tiered approach is applied to assess the potential for leaching of active substances and their metabolites to shallow aquifers which allows for inclusion of groundwater monitoring studies as the highest tier (European Commission, 2014). The aim of our study was to support the evaluation of groundwater monitoring data on a sub-catchment scale and to address regulatory concerns with regard to representativeness of hydraulic connectivity and well/screen position using a higher-tier modelling approach. In the study the one-dimensional leaching model PEARL was coupled with the multi-dimensional scientific software OpenGeoSys in order to simulate the leaching of PPP and the subsequent transport in the saturated zone to a groundwater monitoring well. The modelled concentrations were compared with measured concentrations resulting at the well screen in catchments where upgradient agricultural fields were treated with the test substance. In addition, a sensitivity analysis was performed to evaluate the representativeness of the monitoring approach and to identify relevant factors for study design. The sensitivity analysis showed that hydrogeological properties, the arrangement of the treated fields and the screen position have different effects on the simulated concentrations.

3.10P.25

Detection and occurrence of 62 pesticides in a Typical Mediterranean Coastal Wetland by HPLC-MS/MS

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The use of pesticides is between two and three million tons per year, 70% of all pesticides being used in Europe and in The United States following by China (1). Their role is associated with the control of many insects, weeds and other organisms that can damage agricultural crops. The physicochemical properties of some of these compounds, such as their solubility in water, justifies the importance of monitoring their presence in surface water by runoff and in ground water by leaching (2). The purpose of this study was to establish the patterns and concentrations of pesticides in surface water from a typical Mediterranean wetland, the Albufera Natural Park (Valencia, Spain). To this end, this study was focused on the evaluation of the occurrence and environmental fate of 62 pesticides, some of the most widely used. This area consists of a highly eutrophic coastal lagoon surrounded mainly by rice fields. We took 52 samples in as many points of the Park. The sampling was performed two-time in the same year (2019), to value the different environmental distribution in the time. The extraction was based on solid-phase extraction (SPE) using cartridges of Reversed Phase (Strata-X). The determination was carried out by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a triple-quad using two precursor → product ion transitions for almost all compounds in the Dynamic Multiple selected Reaction Monitoring mode (MRM). Chromatographic separation was performed using Luna® 3μm C18 column. The mobile phase consisted of deionized water and methanol, both with ammonium formate10 mM (positive mode). The results evidenced the presence of many selected Emerging Contaminant (EC) in surface water. The most abundant ones are herbicides and fungicides, as the *prochloraz*. This work produced an accurate outlook of a basal state for the Albufera Natural Park and could be developed in the context of a chronic monitoring of this site. Furthermore, the results pinpointed the need of further studies on the short and long term ecotoxicological impact in animal and vegetal species.

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3.10P.26

Preparation and characterization of a nanoformulation of poly(3-hydroxybutyrate) loaded with tebuconazole

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The application of nanotechnology in the development of agricultural products has gained attention in recent years. One of these applications consists of the association of pesticides with polymeric nanocarriers resulting in nanoformulations whose enhanced properties could lead to increasing the effectiveness of the pesticides against the pest, as well as reducing its harmful effect on the environment compared to the conventional formulation. Some of these enhancements are the increase in water solubility, longer residence times and improved bioavailability of pesticides in soils as a consequence of the controlled release of active ingredient from the nanocarrier. Poly(3-hydroxybutyrate) is a very promising polymer to be used as a pesticide nanocarrier. This is mainly because this polymer is slowly degraded by microorganisms, which leads to a slow release of the pesticide from the nanocarrier. In this work, a nanoformulation of tebuconazole associated with poly(3-hydroxybutyrate) was prepared following the emulsion solvent evaporation method. After the preparation, the nanoformulation was characterized by the determination of particle size, polydispersity index and zeta potential using dynamic light scattering, particle morphology by scanning electron microscopy, and encapsulation efficiency by ultrafiltration. Finally, the release kinetics of the nanoformulation was assessed by dialysis using bag made of semipermeable cellulose membrane. The release experiment was performed using both undiluted and diluted nanoformulation to evaluate the effect of dilution on the release rate of tebuconazole from nanocarrier. The results showed that the nanoformulation preparation was successful, resulting in a suspension of spherical particles in the nanometric size range with high encapsulation efficiency. The release kinetics of tebuconaloze was significantly slower when the experiment was performed with the nanoformulation than with pure tebuconazole solution. This indicates that the association of the fungicide with the polymer meant a controlled release of tebuconazole from the nanocarrier. The dilution of the nanoformulation led to an increase in the release rate in comparison with the undiluted nanoformulation. This last result is crucial to evaluate in more realistic condition of dilution that would happen when the nanopesticide concentrate is diluted in the tank before its application on the field.

3.10P.27

Non-target toxicity of conazole fungicides to Caenorhabditis elegans and Chironomus riparius

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The aim of providing sophisticated chemical tools for controlling pests triggered the production of more complex chemicals that often contain an asymmetric center. Currently, chiral compounds consist 30% of the pesticides in use. Among them, conazole fungicides (CFs), acting as inhibitors of ergosterol synthesis, are almost exclusively produced and applied as mixtures of either enantiomers (optical isomers that are mirror images of each other) or diastereoisomers (in case more asymmetric centers are present in the structure, each diastereoisomer is made up of two enantiomers). For chiral pesticides, limited data are available with respect to their stereospecific fate and effects, which hampers to foreseen all the possible negative consequences of their use as well as to perform a reliable risk assessment. Therefore, in the present study, non-target effects of five chiral conazole fungicides were assessed including the testing of their racemic mixtures (enantiomers equally represented) and enantiomer-enriched mixtures. Two biological endpoints were tested: the nematode *Caenorhabditis elegans* that occur in both sediment and soil and the non-biting midge *Chironomus riparius,* extensively as a model for genome structure analysis in insects and also is used in toxicology tests and functional developmental genetic studies. The dose-respose curves were used to evaluate the effect values and for comparing the toxicity of the five conazole fungicides. In addition, the possible benefits arising from the use of enantiomer enriched mixtures over racemic mixtures were discussed based on the toxicity data gained for *rac*-uniconazole, *rac*-diniconazoe and their enantiomer-enriched mixtures of uniconazole-P and diniconazole-M. In this respect, the study enhances our understating of the non-target effects of widely used conazole fungicides, enlightens the role of enantioselectivity in toxicity and its importance for risk assessment of pesticides as well as provides the science with solid arguments for guiding the manufactures toward the production of more single- or enriched-enantiomer pesticides.

3.10P.28

THE APPLICATION OF 2D HPLC TO ADDRESS THE NEED TO INVESTIGATE THE CHIRAL PROFILE OF ACTIVE INGREDIENTS AND THEIR METABOLITES IN ENVIRONMENTAL FATE AND METABOLISM STUDIES

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The recently published guidance from EFSA[i] on risk assessments for active substances that contain stereoisomers provides recommended approaches to address and assess data requirements for active substances with the same molecular formula but different three-dimensional orientations. One approach is to study different isomers of an active ingredient and individually assess each isomer, but this approach is both costly and time consuming, requiring the synthesis of the individual isomers. Chromatography is a constantly evolving technique and while the concept and application of 2D-HPLC has been around for a number of decades, recent improvements in equipment and column technology allows the routine use of 2D-HPLC in a reliable and robust manner. The use of 2D-HPLC to couple reverse phase profiling methods with reverse phase chiral methods along with tandem mass spectrometry – with or without radiolabelled 0analysis – enables the investigation of chiral profiles of agrochemicals and their metabolites without the need for separate isomer studies or extensive sample isolation, workup, and subsequent chiral chromatography. This presentation discusses the practical application of 2D reverse phase-chiral separations and their potential to become a routine approach to analysis in environmental fate and metabolism studies when considering the data requirements to fulfil the new EFSA guidance. [i] EFSA Journal 2019; 17(8):5804

3.10P.29

Monitoring of pesticides in groundwater of Catalonia (NE Spain): evaluation of spatial and temporal trends of groundwater quality status, and environmental and human risk assessment

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Groundwater is one of the most vulnerable freshwater sources and its quality deterioration may affect ecosystems and human health. To protect groundwater quality, the European legislation (Directive 2006/118/EC) has established quality standards for pesticide pollution. Catalonia (NE Spain), is one of the Spanish regions where the consumption of pesticides is the highest, due to intensive agriculture and industrial activities, and a high population density, which has also associated an important demand of water. This study is part of the surveillance and operational monitoring network established within the Water Framework Directive (WFD), with the purpose of understanding the spatial and temporal variations of pesticides in Catalan aquifers, and to evaluate their compliance with European legislation in terms of groundwater quality. For this, 118 groundwater samples were collected in Catalonia between 2017 and 2018, and analyzed for 51 medium to highly polar pesticides and pesticide transformation products. Reliable of the results obtained is ensured by the use of a fully automated method based on on-line solid phase extraction-liquid chromatography-tandem mass spectrometry and isotopic dilution quantification. The results obtained showed that 43 pesticides were detected in at least one sample, with concentrations achieving the μg/L range, as in the case of bentazone (11 μg/L). A total of 18 compounds exceeded the quality standards of 100 ng/L for individual pesticides in 18% of the samples analysed, while the quality standard of 500 ng/L for the sum of pesticides was surpassed in 12 samples. The spatial and temporal distribution of pesticide pollution is highly related to the land use. After comparison of the results with previous campaigns carried out in Catalan aquifers, the total pesticides level seems to have increased, being neonicotinoids one of the classes that contributed the most to current total pesticide levels. Note that this chemical class was not previously investigated in this area. Some pesticides, like diazinon, metolachlor and terbuthylazine were found in all sampling campaigns, despite the fact that the use of diazinon and metolachlor is currently banned in Europe. The risk that measured pesticide concentrations may pose to ecosystems and human health is currently under assessment. Acknowledgements - This work was supported by the Government of Catalonia (Consolidated Research Groups 2017 SGR 1404- Water and Soil Quality Unit).

3.10P.30

More than one environmental risk assessment methodology for botanical substances in Plant Protection Products

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In the framework of the Guidance Document SANCO/11470/2012– rev. 8 of 20 March 2014, "*a botanical active substance consists of one or more components found in plants*". It is also recognised in this guidance that non-testing methods, read-across and bibliographic data may be applied to replace experimental data. Due to often complex composition and physico-chemical properties of botanical active substances, data requirements for approval under Regulation (EC) No 1107/2009 may be not all fulfilled, due to technical infeasibility of experimental studies which are usually mandatory to describe their fate and behaviour in the environment, such as OECD 307 and OECD 308 tests. In existing cases where no such experimental data are available, the methodology followed to assess environmental risk varies from one substance to another, and may also be different for a same substance when Competent Authorities evaluate the authorisation dossiers of Plant Protection Products. We will present a comparison of data for some approved botanical active substances and study case where the conclusions on the proposed alternative approach were different among Member States, highlighting the necessity of harmonisation at both EU and national levels for such natural complex substances.

Metal Speciation and Bioavailability: Mechanistic Links between Exposure and Effects in Multi Stressor Environments (P)

3.11P.1

Integrating Zn speciation and availability information about a Mediterranean stream

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The hegemonic paradigms in ecotoxicology postulate that some available fraction of a specific pollutant is better correlated with its impact than its total amount. So, there is a need to take into account the pollutant's speciation, i.e. how the total amount of pollutant is distributed amongst chemical forms which have different physicochemical and biochemical properties. In the case of the Free Ion Activity Model (FIAM) or the Biotic Ligand Model (BLM), the key role falls into the free concentration of the metal ion. However, exceptions to this claim support the relevance of identifying some (bio)available fraction, e.g. some labile fraction. Thus, special analytical techniques are needed to routinely measure reliable predictors of bioavailability and bio-uptake. No technique can claim to report all relevant information in all matrices and for all analytes. Rather, each technique provides some complementary information that has to be judiciously integrated in a sound interpretative framework to obtain a more representative picture of the analyzed medium. This will be presented for the particular case of the Osor river (Girona, Spain) which is highly impacted by an abandoned mine. Four techniques will be applied to the same water: AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) [1] and PIM (Polymer Inclusion Membranes) [2], for the free Zn concentration determination, and DGT (Diffusive Gradients in Thin films) [3] and LASV (Anodic Stripping Voltammetry with Linear stripping) [4], for the labile fractions. PIM and DGT are passive sampling techniques. A methodology [4], based on the splitting of the flux into a contribution from the free metal ion and another contribution from the complexes leads to the determination of the lability degree of the complexes [5]. References: 1. Companys, E., Galceran, J., Pinheiro, J.P., Puy, J., Salaün, P., *Current Opinion in Electrochemistry*, 3 (2017) 144-162. 2. Vera, R., Fontàs, C., Galceran, J., Serra, O., Anticó, E., *Sci. Total Envir.*, 622-623 (2018) 316-324. 3. Davison, W., Zhang, H., *Nature*, 367 (1994) 546-548. 4. Companys, E., Galceran, J., Puy, J., Sedó M., Vera, R., Anticó, E., Fontàs, C., *Anal. Chim. Acta*, 1035 (2018) 32-43. 5. Galceran, J., Puy, J., *Environ. Chem.*, 12 (2015) 112-122. The authors gratefully acknowledge financial support from the Spanish Ministry of Education and Science (project CTM2016-78798).

3.11P.2

Analysing the proton binding characteristics of a natural organic matter fraction extracted from brackish waters

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The binding of (micronutrient) trace metals to marine dissolved organic matter (DOM) is controlled by the acid-base properties of DOM and the element speciation in seawater, both depending on pH, ionic strength, temperature, and solution composition. E.g., dissolved Fe, an essential micronutrient in seawater, is bound in more than 99% to marine organic ligands [1]. Thus, interactions with DOM are key to understand the availability of trace metals in seawater and should be considered in global biogeochemical models to evaluate future climate change scenarios like ocean acidification. The effect of pH and dissolved organic carbon (DOC) on the binding of Fe to DOM was recently incorporated into a new global model [2], which combines Fe speciation data, generic NICA (non-ideal competitive adsorption) parameters for humic substances, and empirical Fe-DOM binding constants [3]. Despite the achieved improvement compared to previous models, an accurate assessment of the thermodynamics of proton/metal ion binding to marine DOM is still missing. The direct analysis of marine DOM has proved difficult due to its low concentration, especially in open-ocean environments. Yet concentration and isolation techniques such as solid-phase extraction (SPE), with DOC extraction recoveries above 30%, can provide fractions of marine DOM containing hydrophobic and some polar compounds which are highly representative of the original seawater sample [4]. We present a complete physicochemical study of a marine DOM fraction isolated from surface Baltic Seawater using SPE. We applied a potentiometric titration technique for identifying major classes of active chemical groups and measuring their proton binding properties. We use a combination of the NICA isotherm for the specific -chemical- ion binding, and the Donnan model for electrostatic effects. We compared the number of protonated chemical groups, Donnan volumes and proton affinity spectra in SPE-extracted marine DOM with generic parameters for fulvic acids. We expect our results to be representative for the acid-base behaviour of surface marine DOM in brackish waters and be used for modelling proposes. References: 1. Gledhill M, van den Berg CMG. Marine Chemistry, 47 (1994) 41-54. 2. Ye Y, Völker C, Gledhill M. Global Biogeochemical Cycles (2020), *submitted*. 3. Avendaño L, Gledhill M, et al. Frontiers in Marine Science 3 (2016) 58. 4. Li Y, Harir M, et al. Analytical Chemistry 88 (2016) 6680-6688.

3.11P.3

Lipids that contain arsenic in the Mediterranean mussel, Mytilus galloprovincialis

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Arsenic-containing lipids, arsenolipids, are widely found among marine organisms, but their origin and possible biochemical roles remain unknown. Arsenolipids in organisms might be 'purpose-built' playing some specific biochemical role, or their presence might simply reflect an error in the biochemical workings 3of the organism whereby a dimethylated arsenic species is accidentally incorporated into the synthetic pathway for essential lipids. This work describes the diversity and abundance of arsenolipids in the digestive gland and mantle of nine specimens of the Mediterranean mussel, *Mytilus galloprovincialis*. By using high performance liquid chromatography (HPLC) coupled to both elemental and high-resolution molecular mass spectrometry, we identified 36 arsenolipids including arsenic derivatives of fatty acids, hydrocarbons, arsenosugar-phospholipids and arsenosugar-phytol; 21 of these arsenolipids were identified for the first time and included a new group comprising arseno-ether-phospholipids.The arsenic compounds in the mussels show distinct profiles for mantle and digestive gland, which provide insights into the arsenolipid origin. The results suggest that the presence of some arsenolipids in the mussels is from direct uptake of the compounds, presumably from food, rather than biogenesis within the mussels.The wide diversity of arsenolipids in mussels reported in this study, and their structural similarity to non-arsenic lipids common to marine organisms, suggests accidental incorporation of arsenic into lipids. Nevertheless, and given the importance of lipids as structural and functional compounds, the presence of abnormal (wrong) compounds homologous to essential lipids could provoke impairment to the cell function and damage the organism.

3.11P.4

Bioaccessibility testing of metals, inorganic metal compounds, and complex metal-containing materials in selected artifical media simulating relevant human-chemical interactions

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The toxicity of most metals and metalloids is associated to a large degree with the release of metal ions, their uptake into an organism and their interactions at specific target organ sites (i.e. bioavailability). In the context of the European REACH regulation there is a demand of testing the bioavailability routinely for numerous materials. In a tiered approach in a first step simulation experiments are performed for determining the bioaccessible fraction of metal/metalloid species. Bioaccessibility is defined as the fraction of a substance (e.g., soluble metal ion) that dissolves under surrogate physiological conditions and therefore is "potentially available" for absorption into systemic circulation or for interactions in organisms. The resulting data (e.g., identified metal species, released mass fractions, kinetics) can be used to assess the potential bioavailability and toxicity. For example, the measured concentration of a relevant metal species can be compared to data from toxicity tests (e.g., tissue concentrations). The applied bioelution tests are in vitro methods, which apply simulated biological fluids mimicking physiological conditions (e.g., for uptake into the human body by inhalation or by ingestion into the gastro-intestinal tract). To this end, we used different physiological simulation media for testing the bioaccessibility of metal species from metals and inorganic metal compounds. Tested media are for example GST (Artificial gastric fluid; pH = 1.5) which mimics the very harsh digestion milieu of high acidity in the stomach or GMB (Gamble´s solution; pH = 7.4) which mimics interstitial fluid within deep lung under normal health conditions. The amount of tested metal or inorganic metal compound can be 0.2 and / or 2 g of test item per litre medium and samplings are performed generally after 2 and 24 hours. For lung media a third sampling can be performed after 7 days of incubation. All media are incubated at 37°C with continuous shaking during the whole period of testing. Additional for lung media a CO_2 atmosphere is applied for stabilizing the pH during the incubation. Undissolved test item is separated by filtration and centrifugal filtration and both fractions are measured for the specific metals by ICP-OES, ICP-MS or ICP-MS/MS. The feasibility of the approach will be discussed on basis of the experiences from tests with several compounds.

3.11P.5

Polymorphic variants of As3MT and GSTs on distribution of profiles urinary arsenic species in population exposures by drinking groundwater in

Colombia
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Objective: assess the effects of polymorphic variants of As3 metil- transferase and gutathione-S-transferase on profiles of urinary arsenic species. **Methods:** twenty-one wells of groundwater for human consumption from the municipalities of Margarita and San Fernando in Colombia were analyzed for concentration of arsenic. For the exposure assessment and risk characterization was used the lifetime average daily dose of arsenic (LADD µg/kg-day). Specific surveys on 151 individuals aged between 18 and 81 years old were applied to collect demographic information and other exposure factors. In addition, GSTO2-rs156697, GSTP1-rs1695, GSTT1-null, GSTM1-null, and As3MT-rs3740400, genetic polymorphisms were evaluated by real time PCR or by Restriction Fragment Length Polymorphism (PCR-RFLP). We examined arsenic speciation through concentrations of urinary metabolites related with exposure using Atomic Fluorescence Spectrophotometer (HPLC-HG-AFS): inorganic arsenic (InAs) (AsV and AsIII), monomethylarsonic acid (MMAV) and dimethylarsinic acid (DMAV) and Total urinary arsenic (TuAs). Also were calculated the ratios of arsenic metabolites as indicators of the metabolic capacity; primary methylation index (PMI) and secondary methylation index (SMI). The effects of polymorphisms were tested using a multivariate analysis, adjusted by potential confounders. **Results:** the arsenic concentrations in groundwater were on average 35.3±8.6 µg/L greater than US Environmental protection Agency guideline for arsenic (10 µg/L). Also the on average of AsIII was 21.5±7.5 µg/L and the concentration of AsV was 14.0±11.7 µg/L. Was observed a significant correlation among LADD of arsenic and TuAs (r=0.69; p =0.000). %DMA was associated with GSTO2, age, BMI, alcohol consumption, LADD and the interactions GSTO2*Age, GSTP1*BMI (R2 =0.43; likelihood-ratio test, p =0.000). %MMA was associated with As3MT, GSTP1, sex, BMI, Alcohol consumption and the interactions As3MT*GSTP1, As3MT*Sex, As3MT*BMI and As3MT*alcohol consumption (R2 =0.40; likelihoodratiotest, p=0.007). **Conclusions:** GSTO2 (TC + CC), As3MT (TG + GG) homozygotes/heterozygotes and LADD of arsenic could increase %MMA and decrease %DMA in people exposed to low and moderate levels of arsenic from drinking groundwater. However the explanatory models stronger showed the participation of some covariates that could to influence the effects of the polymorphisms on these exposure biomarkers to arsenic

3.11P.6
An assessment of heavy metals intake in four Danish dietary profiles - A need of policy instruments securing soil health and food safety
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Based on diverse and common dietary habits, people may result exposed to intake of different amounts of heavy metals contained in foods. This paper assesses the risks of being exposed to four heavy metals (Cd, Hg, Pb, and As) considering four frequent Danish dietary profiles (standard, vegetarian, vegan and carnivore). A modeling approach was performed to estimate the average intake of heavy metals for Danes by considering more than 456 representative Danish food products. Food products were grouped into six food categories (fruits and vegetables; grains; protein-rich; food rich in fats, sugar and alcohol; milk and dairy; oils and condiments) where a lognormal distribution of heavy metals was verified. A probability distribution for the total daily intake of heavy metals for all dietary profiles was obtained by performing a Monte Carlo analysis. Results were compared with the tolerance values defined by the European regulatory agencies. Preliminary results from the model show that except for the carnivore diet, the main source of Cd intake is in the food category grains for standard, vegetarian and vegan diets. Concerning Hg, the main source is protein-rich food mainly composed of meat and fish products, resulting in a substantial exposition in the carnivore. For Pb and As, the main source is the food category food rich in fats, sugar and alcohol that is more pronounced in the vegetarian and vegan diets. Except for the total As (organic + inorganic), the average concentration of heavy metals did not exceed legal limits. Indeed, our analysis found that the probability of surpassing the limits across the four dietary profiles was 60% (Cd), 17% (Hg), 16% (Pb) and 33% (As). Regulatory measures that limit the amount of heavy metals in food products need to be improved especially with regard to Cd. The main reasons behind Cd presence in grains crops are clearly dependent on agricultural soils. The increased use of mineral fertilizers in the EU has been directly associated with an increased amount of Cd in the soils. This research further explores the recently adopted new fertilizer regulation to investigate how the settings of Cd limits in fertilizers alone are not sufficient to fully-tackle Cd accumulation over time. We believe that a renegotiation of the withdrew EU Soil Framework Directive may be an effective complementary tool to limit Cd accumulation.

3.11P.7
Nickel and chromium marine ecotoxicity: relevant for risk assessment of enhanced olivine weathering in coastal zones
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Global warming should be kept well below 2°C in order to minimize the potential impacts of climate change. Reducing greenhouse gas emissions will not be sufficient to meet this goal; CO_2 will also have to be actively captured from the atmosphere by so called negative emission technologies (NETs). Enhanced silicate weathering (ESW) is a NET which could be applied in coastal environments. The mineral olivine ($Mg_{2x}Fe_{2(1-x)}SiO_4$) is a prime candidate for this application due to its fast dissolution rate and relatively widespread abundance. ESW aims to increase the CO_2 storage capacity of the oceans by artificially speeding up the chemical silicate weathering process ($Mg_{2x}Fe_{2(1-x)}SiO_4 + 4H^+$ à $2xMg^{2+} + 2(1-x)Fe^{2+} + H_4SiO_4$). However, associated trace metals are released upon weathering. Nickel (Ni) and chromium (Cr) are of highest concern and might have adverse effects on marine organisms if concentrations rise significantly above background levels. The first objective of this research was to assess the toxicity of Ni and Cr to marine organisms based on literature ecotoxicity data. Species sensitivity distributions (SSDs) were constructed using both external media concentrations and body concentrations. Additionally, bioconcentration and bioaccumulation factors were collected to determine the metal bioaccumulation potential. Finally, environmental exposure distributions for open-ocean and coastal zones were constructed using reported metal concentrations in seawater and sediments worldwide. The hazardous concentration for 5% of the species (HC5) derived from SSDs based on external media concentrations were 0.23 and 0.42 µM for Ni^{2+} and Cr^{6+}, respectively. These concentrations are approximately 60 and 145 times higher than ambient seawater concentrations for dissolved Ni^{2+} and Cr^{6+}, respectively. Nevertheless, trace metal concentrations resulting from enhanced olivine weathering could reach levels similar to or higher than the calculated HC5s for the individual metal ions in shallow water systems with a relatively long residence time. In addition, the potential for mixture toxicity effects must be taken into account. These preliminary results indicate that enhanced olivine weathering could have potential adverse effects on marine biota and care should be taken in setting up field experiments testing enhanced olivine weathering. This research will provide useful information necessary to evaluate one of the possible solutions to achieve the long-term climate goal.

3.11P.8
Effects of marine tailing exposure on the growth and development of Calanus finmarchicus
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Increasing mining activity worldwide due to high mineral demand produce large amounts of tailings that needs to be deposited. In Norway, several locations dispose their tailings directly into the fjord systems. There are many environmental challenges connected to marine tailing disposal (MTD), among others the spreading of fine particulate matter in the water column and the potential release of metals and process chemicals. In this study we comparatively investigated the potential impact of three different tailing compositions on the development of early life stages of the copepod *Calanus finmarchicus*. *Calanus finmarchicus* nauplii at the nauplii stage 6 (NVI) where exposed to pure calcium carbonate particles (Par), to tailings from a marble processing plant (Marble) and to tailings from a copper mine (Copper). The animals were exposed to high (H; nominally 750 000 particles/mL) and low (L; nominally 37500 particles/mL) tailing concentrations on rotating wheels for 20 days, with exposure dispersions being exchanged 3 times. At each sampling point (seven in total) one bottle from each treatment were sampled. Analysed endpoints included swimming activity, stage determination, biometry and respiration. By the end of the experiment, 34 days post egg laying, all treatments apart from the low particle exposure and both the marble tailing exposures had reached the copepodite 5 (CV) life stage. However, the percent CV were lower in exposed groups compared to the control group. In the control group, approximately 20 % CV were recorded, while only 8 % in ParH, 4 % for ParL and 9 % were determined in the CopperH exposure. In addition, for the control group and ParH treatment, copepodite stage 4 (CIV) were present at sampling point 5 (11 days post exposure), while in the other treatments no CIV where recorded until sampling point 6, three days later. At sampling point 6, respiration measurements (n=4) showed a significant difference between treatments (one-way Anova, F=11,96 df=6 p< 0,0001), with ParL, MarbleH and

CopperH having significantly lower respiration rates than control (p< 0,05). Our results show that *C. finmarchicus* develop slower in the presence of tailings. Interestingly, lower particle concentration seems to lead to slower development than higher particle concentration. We observed a tendency of the particles to flocculate faster in the higher particle load. This means that in the lower particle loads small particles remained available for the filter feeding copepods for a longer period and could possibly lead to a higher ingestion rate of particles and subsequent less energy for growth. In addition, marble tailings with the presence of process chemicals seems to impair growth more than copper tailings.

3.11P.9

Mining environmental liabilities: a potential source of metal contamination for freshwater ecosystems in Costa Rica

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Metal mining in Costa Rica, and the use of toxic substances in this activity, such as cyanide and mercury, has occasionally resulted in the negligent abandonment of waste structures containing significant amounts of toxic metals. These structures have been exposed to oxidation and weathering, resulting in the environmental release of metals, thus affecting the natural quality of the surrounding freshwater ecosystems. This research showed that abandoned mining liabilities in Líbano of Tilarán, Guanacaste, are a potential source of metal contamination the aquatic ecosystem (water, sediment and benthic macroinvertebrates of the San José and Cañas rivers). The study allowed quantitative and qualitative assessment of mining waste through an assessment of the area and volume of land contaminated and physical and chemical characterization of the contaminants. In addition, the quantity and quality of contaminant leachate generated from mining liabilities in the rainy season were examined with infiltration tests. A wet cell kinetic test allowed describing the processes and chemical reactions that are likely to occur in mining wastes during rainfall and high temperatures in dry season (27°C) that characterize the study area. The presence of pyrite (FeS_2), which facilitates the acidification of the waste sediments and the release of metals, was shown by X-ray diffraction tests carried out on samples of the wastes materials. Through the measurement of the flow rate and physicochemical parameters of the water, the study also contributed to determining the spatial distribution of metal concentrations with greater environmental impact, such as lead, arsenic, and cadmium. The results show a high content of metals (37.8 tons of lead, 20.2 tons of arsenic and 0.4 tons of cadmium) from the total ~106 tons in mining liabilities, and their relationship with the spatial distribution of these metals in sediments, as well as the presence of these metals in the benthic macroinvertebrate fauna. In the rainy season, the lead and arsenic sediment concentrations were above the international guidelines' quality criteria. These metals were identified in organisms of the Leptohyphidae family. Smallest individuals (< 0.4 mg, dry weight) presented the highest average concentrations of lead (1.30 µg/g), arsenic (10.62 µg/g) and cadmium (1.25 µg/g). The inadequate disposition and exposure of the liabilities to weathering have created the conditions for the dissolution of metals, which is a source of heavy metals pollution in the San José and Cañas rivers.

3.11P.10

The role of fish helminth parasites in monitoring metal pollution in aquatic ecosystems: a case study in the world's most productive platinum mining region

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Fish parasites are able to accumulate metals orders of magnitude higher than their hosts, thereby reflecting very low environmental exposure levels. In addition, several parasite taxa such as acanthocephalans and cestodes can indicate metal bioavailability due to free ion activity, rather than dietary uptake, due to their lack of a digestive system. Studies on Pt accumulation in parasite-host systems are limited, with only three studies published on acanthocephalans and a single one considering cestodes and nematodes, however, these studies were all done on Pt accumulation from automobile catalytic converters. Therefore, the aims of this study were (1) to determine Pt mining related metal accumulation in helminth fish parasites infecting different hosts between a reference and impacted impoundment and (2) to assess whether there is a difference between bioaccumulation of metals in parasitized and non-parasitized hosts. The cestode, *Atractolytocestus huronensis*, accumulated Pt two-fold higher than the acanthocephalan, *P aratenuisentis ambiguus*, while the nematode, *Contracaecum*sp., accumulated Pt 21-fold higher than the nematode, *Anguilicola crassus*. The higher Pt accumulation in parasites from the present study indicates that Pt derived from mining activities is more bioavailable for organisms than Pt derived from automobile catalytic converters. Infected fish accumulated lower metal concentrations compared to uninfected fish, while the parasites had significantly higher metal concentrations than their hosts. The parasites demonstrate the bioavailability of metals deriving from Pt mining effluent and have the ability to resists the toxic effects thereof.

Micro(nano)plastics Occurrence, Fate and Effects: Mechanistic Approaches to Study Risks to Environmental and Human Health (P)

3.12P.2

Distribution of microplastics in Surface waters of ponds and channels in South Ryadh and Al-Jubail (Saudi Arabia)

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Presence of microplastics in the environment has become a global concern [1,2]. The composition and distribution of microplastics (>80 µm in size) in surface water of the South of Ryadh and Al-Jubail (Saudi Arabia) were investigated in this study. The samples taken in the area of influence of the WWTP of South Ryadh including the artificial pond that receives the effluent and Al-Jubail –the largest industrial city in the Eastern province on the Persian Gulf coast of Saudi Arabia in 2019. The major shape types of microplastics can be classified to fibers and fragments, and 89.7 % of microplastics in the surface water were fibers. The microplastics < 100 µm were most abundant at all sample sites, which accounted for 81.2 % of microplastics. The composition of microplastics showed different polymers being identified as major polyethylene and polypropylene, according to infrared spectrometer analysis. The average abundances of microplastics were 150 and 292 item/L, in the South of Ryadh and Al-Jubail, respectively. The microplastic concentrations in the surface water decreased linearly with the distances from the urban agglomerations with rather high positive correlation coefficients ($r = 0.8021$, $p < .01$). The source and the density of microplastics may be the primary factors to influence the microplastic concentrations in the surface water. The research about distribution and nature of microplastics in artificial ponds and channels might provide useful information that could be used for establishing sources, transport and fate of microplastics. Acknowledgements The financial support from the project number (RSP-2019/11) King Saud University, Riyadh, Saudi Arabia. References [1] Y. Pico, A. Alfarhan, D. Barceló. Nano- and microplastic analysis: Focus on their occurrence in freshwater ecosystems and remediation technologies, TRaC-Trends Anal. Chem., 113 (2019), pp. 409-425 [2] Y. Pico, D. Barcelo. Analysis and prevention of microplastics pollution in water: current perspectives and future directions. ACS Omega, 4 (2019), pp. 6709-6719

3.12P.3

Assessment of the Effects of Chronic Exposure of Polystyrene Micro- and Nanoplastics to Daphnia magna

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The presence of plastic debris in the aquatic environment is a potential threat to aquatic organisms. The continual degradation of larger debris under natural environmental conditions and wastes from commercial production are sources of micro-sized and likely nano-sized plastic particles in natural waters. Studies have investigated the acute toxicity of micro- and nanoplastic particles to *Daphnia magna*. However, to date, information regarding their potential sublethal effects following chronic exposure is limited. This study assessed the chronic toxicity of 20 nm and 200 nm polystyrene micro- and nanoparticles (PS-MNPs) to *Daphnia magna*. The effect on survival, growth, swimming behavior, reproduction and feeding rate was investigated over 21 days using a static renewal method. During the chronic exposure, tested animals were reared in moderately hard water, fed with green algae (*Chlamydomonas reinhardtii*) and yeast, Cerophyll™ and trout chow (YCT) following the recommended feeding standard by Environment and Climate Change Canada for chronic toxicity studies with *Daphnia magna*. PS-MNP suspensions were added to the rearing media. The rearing media was changed three times per week, and the PS-MNPs were renewed. Survival and reproduction were monitored by a daily count for mortality, immobility, number of neonates, number of broods and first day to brood. Other effects such as differences in hopping frequency, heartbeat rate, post-abdominal curling, thoracic movement and swimming behaviour were also measured throughout the duration of the experiment. There was no significant difference in survival for animals exposed to both sizes of the PS-MNPs relative to the controls. However, exposed daphnids exhibited reduced feeding and growth rates. Also, the first day to brood was delayed and the number of broods was reduced for both particle sizes relative to the controls. The reduced growth rate may be attributed to the reduced feeding rates of the animals in the presence of the PS-MNPs. These results suggest that nanoplastics may have important impacts on the ecological system through delayed reproduction and reduced growth rate which may eventually lead to disruption in population size.

3.12P.4

Impact of PMMA nanoplastics in the microalgae Rhodomonas baltica: effects of surface functionalization

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Plastics as a form of marine litter are ubiquitous contaminants and have been globally recognized as an environmental problem. The interactions between small plastic particles and microalgae has been shown to trigger direct and indirect toxicity that is highly dependent on particle type, size, surface chemistry and charge. Nonetheless, it is still not well understood how nanoplastics (NPLs) properties, microalgae species and adaptative responses play a role in toxicity. So, this study aimed to evaluate the cellular and physiological effects caused by plain and negative surface charged polymethylmethacrylate nanoplastics (PMMA and PMMA-COOH, 50 nm) on the red microalgae Rhodomonas baltica. Exponentially growing R. baltica were exposed to PMMA and PMMA-COOH NPLs in batch cultures for 72 hours. After exposure, algal cells were analysed for growth, pigments content, cell size and complexity, cell viability, reactive oxygen species (ROS) formation, metabolic activity, mitochondrial membrane potential, lipid peroxidation (LPO), DNA content and photosystem II performance. PMMA behaviour in exposure media was evaluated by dynamic light scattering and nanoparticle tracking analysis. NPLs characterization showed a broader size distribution of PMMA in comparison with PMMA-COOH and a higher instability of the former in microalgae exposure media in comparison with Milli-Q water. PMMA caused a higher impact in cellular and physiological parameters of R. baltica than PMMA-COOH, even though a decrease in algal growth was only seen for the later. Overall, PMMA caused a significant decrease in cell viability, an increase in cell size and complexity, overproduction of pigments, loss of membrane integrity, hyperpolaryzation of the mitochondrial membrane, increased production of ROS and LPO, decrease in DNA content and reduced photosynthesis. On the other hand, the decrease in algal growth in PMMA-COOH is connected to an impairment in cell cycle and consequent decrease in cell viability, metabolic activity and photosynthetic performance. This study provided a first mechanistic understanding of the impacts of PMMA NPLs to microalgae, underlining the role of surface chemistry and size to the observed effects. Further research focusing on the specific interactions of PMMA and PMMA-COOH with cellular membranes, as well as mechanisms of internalization are underway, after which a conceptual adverse outcome pathway on the effects of PMMA NPLs in microalgae will be developed.

3.12P.5

Intracellular fate and toxicity of nano and microplastics and associated benzo(a)pyrene in mussel hemocytes in vitro

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Nano and microplastic pollution has become a global threat for the marine environment. Due to their hydrophobicity and large surface area, nano and microplastics can adsorb persistent organic pollutants (POPs) and behave as carriers of POPs to marine organisms In this study, *Mytilus galloprovincialis* hemocytes were used to investigate the intracellular fate and toxicity of polystyrene (PS) nanoplastics (NPs, 0.05 µm, 10^2-10^{12} part/mL) and microplastics (MPs, 0.5 and 4.5 µm, 10^2-10^9 and 10^2-10^8 part/mL, respectively) with or without associated benzo(a)pyrene (BaP, 1 µM). DLS indicated that 0.05 and 0.5 µm particles formed large aggregates in the exposure medium and that presence of BaP reduced the extent of aggregation for all particles. Uptake of MPs and fluorescently labelled NPs into hemocytes was confirmed by confocal microscopy. BaP fluorescence was detected in cells exposed to all tested PS particles with associated BaP and, for 0.05 µm NPs, part of BaP appeared in lysosomes. Cytotoxicity occurred only at the highest concentrations (starting at 10^{12} part/mL for 0.05 µm NPs, 10^8 part/mL for 0.5 µm MPs and 10^6 part/mL for 4.5 µm MPs, but at equivalent concentrations in terms of PS mass of approximately 50 mg/L). PS particles with associated BaP produced a more marked decrease of cell viability. Similarly, only the highest concentrations of virgin 0.05 and 0.5 µm particles produced a slight decrease in plasma membrane integrity whereas 4.5 µm particles both with and without BaP at the highest concentration caused a notable damage to plasma membrane integrity. PS particles with associated BaP triggered ROS production at the highest concentrations. Results indicate that tested PS particles were not highly toxic for mussel hemocytes and toxicity appeared to depend on mass concentration rather than on particle size or particle numbers. Toxicity occurred only at concentrations much higher than those considered environmentally relevant. The higher toxicity of PS particles with associated BaP compared to particles alone confirms that studied PS particles behave as carrier of BaP to mussel hemocytes. Overall, primary cultures of mussel hemocytes provide a valuable tool for high-throughput toxicity testing, useful for ERA of NPs and MPs in marine ecosystems. *Funded by Spanish MINECO (CTM2016-81130-R), Basque Government (grant to consolidated research group IT1302-19) and UPV/EHU (VRI grant). Work carried out in the frame of JPI Oceans project PLASTOX (005/2015).

3.12P.6

Rapid fragmentation of microplastics by the freshwater amphipod Gammarus duebeni (Lilj.)

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Microplastics have become ubiquitous in all environments. Yet, their environmental fate is still largely unknown. Plastic fragmentation is a key component of plastic degradation, which is mostly caused by abiotic processes over prolonged time scales. Here, it is shown that the freshwater amphipod Gammarus duebeni can rapidly fragment polyethylene microplastics, resulting in the formation of differently shaped and sized plastic fragments, including nanoplastics. Fragments comprised 65.7% of all observed microplastic particles accumulated in digestive tracts. Higher numbers of fragments were found in response to longer exposure times and/or higher microplastic concentrations. Furthermore, the proportion of plastic fragments was highest when food was present during the depuration process. It is concluded that G. duebeni can rapidly fragment polyethylene microplastics and that this is closely associated with the feeding process. These results highlight the crucial role, currently understudied, that biota may play in determining the fate of microplastics in aquatic ecosystems.

3.12P.7

Microplastics across Drake Passage

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Microplastics are ubiquitous in the marine environment, with evidence of their presence at the sea surface, within marine sediments, trapped within sea ice and ingested by marine organisms from the micro to macro scale. However, the challenge of mapping and assessing the full extent of the pervasiveness of marine microplastics is still ongoing. Many areas remain undersampled, particularly within the Southern Hemisphere, and the water column is underrepresented within the literature. Here we present the distribution of microplastics across Drake Passage, between Burdwood Bank and Elephant Island. Samples were collected during ORCHESTRA cruises JR17001 (November 2017) and JR18002 (November 2018) along the GO-SHIP line repeat hydrography line SR1b. Bulk water samples (between 5 and 10 L) were collected throughout the water column (at depths between 5 m and 4200 m) via a CTD rosette. Water samples were filtered using a vacuum pump in situ prior to analysis in the United Kingdom. Visual identification was initially conducted using a compound microscope. A subsample of microplastics identified were analysed using Fourier-transform infrared spectroscopy (FTIR) to confirm their compositional properties. Microplastics were identified at all depths sampled in both sampling years, the majority of which were microfibres. Microfibres can have a variety of sources, with vast quantities shown to be released through the washing of synthetic clothing, as well as from fishing gear such as ropes and nets. The presence of microplastics throughout the water column in Drake Passage suggests that small plastics may be capable of reaching the Antarctic continent, bypassing the Polar Fronts and Antarctic Circumpolar Current through deep water transport. These findings may reveal possible transport mechanisms through the water column and towards Antarctica.

3.12P.8

Development of Cost-Effective Methodologies to Identify and Quantify Microplastics in Seawater Samples

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The accumulation of plastic debris in marine environments and their potential negative effects on marine organisms have become issues of high priority for environmental policy. Microplastics (MPs; 5 mm - 1 µm) are reported to be within the most abundant pieces of litter found in the marine environment accumulating in the water column, on beaches, in sediments, in biota and in the air. Despite recent improvements on the procedures to detect and identify MPs, there is still a need to standardise methods and to produce guidelines for a cost-effective detection, identification and quantification of MPs. The standardisation and establishment of cost-effective methodologies is essential to facilitate comparison among studies, to enable a robust risk-exposure assessment of organisms and humans to plastic particles, and to support the establishment of long-term monitoring programmes. The goal of this work is to create low-cost procedures for the identification of MPs (over 50 µm) in seawater samples, which could be applied in laboratories, *in-situ* and in citizen-science projects. We have tested and optimised the digestion of seawater samples with a heavy load of organic matter using Fenton's Reagent. Collected MPs were dyed using Nile Red, observed under blue and ultraviolet light filters, allowing detection and visualization of the particles under a fluorescence microscope. A classification tree model was constructed to distinguish between polymer-based particles and other (cellulose, mineral, etc) particles. To discern particles from being plastic or not, we developed a classification tree model with the C4.5 algorithm. We validated our methodology using a quality-control sub-sample set, where all the particles were further analysed using micro Fourier Transform Infrared (µFTIR) Spectroscopy. Preliminary results show that the classification tree model has an 82 % probability of a correct classification of MPs. We anticipate that our results will enable a cost-effective and standardized methodology for identification of MPs in seawater samples, supporting the establishment of long-term monitoring programmes.

3.12P.9

Bivalves as indicators for microplastic pollution: A large-scale investigation of Nordic seas

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Microplastic (MP) contamination is ubiquitous in the environment. The ecological impact of MP pollution is still not fully understood, a gap that needs urgent attention. One important task is to identify species suitable as sentinels for monitoring in key eco-compartments, such as in coastal waters. In Norway, mussels (*Mytilus* spp.) have been monitored for hazardous contaminants through OSPAR since 1981. Adding MPs to contaminant surveys will be an important step in a European and global context. Mussels are not always common and additional sentinel species are needed if MP are to be monitored. We assessed the occurrence of MP in four other bivalve species in the Nordic marine environment in addition to *Mytilus spp*. This study investigated abundance, composition and spatial trends in MPs. One hundred sites were selected: 32 for *Mytilus* spp., 14 for *Limecola balthica,* 31 for *Abra nitida*, 20 for *Thyasira* spp. and 3 for *Hiatella artica*. The site locations included Baltic Sea and the coasts of Denmark, Norway (including Svalbard), Faroe Islands, Iceland, and Greenland (east side). Three different methods were applied to assess the abundance and composition at each site, visual identification followed by point or automated scanning µATR FT-IR and pyrolysis GC/MS. Based on these methods, four of the five species were found to contain MPs above the limit of detection (LOD): *Mytilus* spp., *L. balthica*, *A. nitida* and *Thyasira* spp. Eleven *Mytilus* sites contained MPs above the limit of detection (LOD, 2.77 MPs per individual), with the inner Oslofjord mussels containing the highest concentration of microplastics (>61 MPs per individual), as also found in 2017 and 2018. Urbanized sea areas of Skagerrak, Kattegat, Baltic Sea and the North Sea were found to have the highest levels of MPs. Black rubbery particles were dominant in *Mytilus* spp., *A. nitida* and *L. balthica*. MPs were present in *A. nitida*, *L. balthica* and *Thyasira* spp, however, no MPs larger than 63µm were detected in *Thyasira* spp. This study adds to growing evidence that bivalves living on or in sediment can be used to monitor microplastics in the range of 63 to 1000 µm. The study further shows that *Mytilus* spp. can be used for assessing the distribution and composition of MPs in most of the Nordic marine environment, and the use of *L. baltica* in the Baltic Sea and *A. nitida* along the northern Norwegian coast and parts of the North Sea can be quite beneficial to fill in the gaps.

3.12P.10

A systematic study of microplastic fiber release from 12 polyester textiles through washing

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Microplastic fibers (MPF) have been found to be a major form of microplastic in freshwaters and washing of synthetic textiles has been identified as one of the main sources. The aim of this work was to use a panel of twelve different textiles made of representative fibers and textile types to investigate the role of MPF which are already formed through the textile manufacturing process and later released during use. Using standardized washing tests, textile swatches tailored using five different cutting/sewing methods were washed up to 10 times. The MPF quantity and fiber length were determined using image analysis. The 12 textiles demonstrated a great variability in MPF release, ranging from 210 to 72'000 MPF/g textile per wash. The median MPF length ranged from 165 µm to 841 µm. The number of released MPF was significantly influenced by the cutting method (p-value < 0.001). The textiles with mechanically processed surfaces (i.e. Fleece) released significantly more (p-value < 0.001) and longer MPF (p-value = 0.011) than the textiles with unprocessed surfaces. For all textiles, the MPF release decreased with repeated wash cycles and a small continuous fiber release was observed after 5-6 washings with a slight increase in released fiber length. The decreasing trend of the quantity of released MPF is likely caused by depletion of the production-inherited MPF trapped within the threads or the textile structure. The comparison of MPF release from laser-cut samples with their sealed edges and the other cutting methods allowed us to separate the contributions of the edge- and surface- parts of the textile to the total release. On average, 84% (range 49-95%) of the MPF release originated from the edges, highlighting the importance of the edge-to-surface ratio when comparing different release studies. The large contribution of the edges to the total release offers options for technical solutions which have the possibility to control MPF formation throughout the textile manufacturing chain by using cutting methods which minimize MPF formation.

3.12P.11

Does nanosized polymethylmethacrylate toxicity dependent on the route of exposure and on the selected food items?

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It is now known that plastics are the largest source of ocean pollution. In this context, much attention is drawn to nanoplastics (NPLs), as it is recognized they can cross biological barriers and accumulate inside tissues. There is, therefore, an additional risk of trophic transfer (foodborne) aside that observed from the traditional routes of exposure (waterborne). Furthermore, most studies rely on the effects at short-term, neglecting potential long-term effects, and have been focused primarily to the most used polymers, neglecting others also ecologically relevant such as polymethylmethacrylate (PMMA). In an attempt to shed some lights in these gaps, this work aimed to assess the long-term toxicity of PMMA-NPLs on the marine primary consumer *Brachionus plicatilis*, evaluating different routes of exposure (waterborne, foodborne or both), and two different food items (foodborne route solely - the marine algae *Nannochloropsis gaditana* or *Tetraselmis chuii*). A 21-days reproduction assay was carried out considering four treatments: a) control, consisting of clean food and medium-**CTR**; b) medium contaminated (8 mg PMMA-NPLs/L) and clean algae-**MC**; c) clean medium and algae contaminated (incubated with 8 mg PMMA-NPLs/L)-**AC**; and , d) both medium and algae contaminated – **MCAC**. The total number of organisms (TN), number of egg-bearing females (fecundity- FEC) and number of dead organisms (D) were counted daily. Comparing different routes of exposure (**MC** *vs.* **AC**), using *N. gaditana* as food source, organisms from **AC** had the highest TN, FEC and lowest D comparatively to **MC**; using *T. chuii* as food item, organisms from **AC** had the highest TN but also presented the highest D. Regarding the treatment **MCAC** for the two microalgae, overall results showed that organisms performed better than the control in all endpoints. Evaluating different food items, within the **MC** treatment, overall organisms fed with *N. gaditana* had higher FEC comparatively to those fed with *T. chuii*, and similar output in the other endpoints; while in **AC** treatment, though the TN was similar, the FEC was higher and the D was lower in rotifers fed with *N. gaditana* comparatively to those fed with *T. chuii*. Results come to highlight that toxicity of PMMA may vary substantially with the type of exposure route and also with other environmental variations (as in the case of food items when studying the effects of trophic transfer). Standardization of ecotoxicity assays is therefore fundamental for the risk assessment of NPLs aiming the development of more comprehensive and consistent environmental frameworks regarding this problematic.

3.12P.12

Evidences of polymethylmethacrylate PMMA nanoplastics toxicity and teratogenicity on the freshwater cnidarian Hydra viridissima

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Scientific research has increased exponentially in what concerns risk assessment of nanoplastics (NPLs) towards biota. Notwithstanding, most studies focused on most abundant NPLs (e.g., PS, PE) and on the marine compartment, leaving a noteworthy knowledge gap on the potential toxicity of other polymers, but equally present in environmental samples, and on the toxicity of NPLs in freshwater ecosystems (e.g., polymethylmethacrylate - PMMA). In this context, the present work intended to add knowledge on the potential lethal and sublethal toxicity of PMMA-NPLs to freshwater biota, using the cnidarian *Hydra viridissima* as model species. For that, two bioassays were carried out: i) a 96-h mortality and malformation exposure to increased concentrations of 50 nm PMMA-NPLs, followed by a 30-min post-exposure feeding assay; and, ii) a 96-h regeneration assay under exposure to increased concentrations of 50 nm PMMA-NPLs, followed by a 30-min post-exposure feeding assay. All organisms died when exposed to concentrations above 80 mg PMMA-NPLs/L, the $LC_{50,96h}$ being 73.0 mg PMMA-NPLs/L. At concentrations ≤80 mg PMMA-NPLs/L, several morphological changes in the tentacles were detected. The more severe changes were observed at 80 mg PMMA-NPLs/L, where 20% of hydrants exhibited no tentacles. Moreover, morphological changes not reported in the literature (e.g., doubled tentacles, elbow-like tentacles) were registered during this 96h mortality and malformation assay. The hydras exposed to 40 mg PMMA-NPLs exhibited a slower regeneration comparatively to the hydras in the control; while at the two lowest tested concentrations (1 and 5 mg PMMA-NPLs/L), hydras seemed to regenerate faster comparatively to the control. Overall, exposure to PMMA-NPLs induced no significant changes in the feeding rates of *H. viridissima*. This research work comes to highlight the need to perform an in-depth eco(toxico)logical assessment of NPLs toxicity, especially in the freshwater compartment since no-effect concentrations already reported in the literature for some freshwater species induced adverse effects to hydras. Such differential sensitivity among species must be taken in consideration when assessing the risks of emergent contaminants. Considering the lack of information in terms of the levels of NPLs, in the water and sediments, the environmental relevance of the data cannot be ascertained. Nonetheless, data show that *Hydra* species seems to stand out as a good, cost-effective model, allowing the assessment of toxic effects in a wide range of endpoints and also as an indicator of possible teratogenic effects induced by exposure to PMMA-NPLs.

3.12P.13

Suborganismal acute responses of the aquatic midge Chironomus riparius to polyethylene microplastics

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Microplastics (synthetic polymers with less than 5 mm) are now ubiquitous and persistent in freshwater ecosystems. Once in freshwaters, microplastic particles undergo fragmentation, biofouling and sedimentation processes reaching high concentrations in sediments where they can be ingested by sediment-dwelling invertebrates. Deleterious effects of microplastics have been described at organismal level (e.g. growth, development). Notwithstanding, molecular, biochemical, cellular and physiological alterations can be used as early warning indication of sub-lethal effects providing information regarding the mechanisms of action of microplastics. This study aimed to assess and describe the potential effects of microplastics in the aquatic midge *Chironomus riparius* using biochemical responses and gene-expression tools. Fourth instar C. riparius larvae were exposed for 48 h to 0.025 and 2.5 g/kg of different size polyethylene (PE) microplastics (< 32 and 32-45 µm). Assessed biochemical endpoints included oxidative stress and damage responses (enzymatic and non-enzymatic responses, lipid peroxidation), and energy reserves and consumption (electron transport system activity) while the gene expression evaluation (Real Time PCR analysis) was developed using an array designed with 42 genes related to essential routes in invertebrates such as endocrine system, detoxification response, stress response, immune response, DNA repair and apoptosis. Results showed that short-term exposure to environmental relevant concentrations of PE microplastics impaired energy reserves, altered responses related to oxidative response, detoxification response (GST and TGSH), nervous system (AChE) and genes involved in different routes have modified their mRNA level, being able to generate important alteration in their development and response against stress situations. Such results agree with the previously reported responses at organismal level and highlight the potential adverse effects of microplastics for freshwater benthic communities considering the ecological role of *C. riparius* within these habitats

3.12P.14

Use of fluorescent-labelled nanoplastics (NPs) to demonstrate NP absorption is inconclusive without adequate controls

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Whether nanoplastics (NPs) are able to be absorbed across epithelial membranes and accumulate within internal tissues of organisms is an important determinant of their potential toxicity. Evidence of absorption and accumulation requires detection of NPs within internal tissues, and investigations with fluorescently labelled NPs have attempted to provide this information. We hypothesize that studies that do not control for the fluorescent dye leachate and/or cellular autofluorescence are inconclusive and can be misinterpreted. Our goal was to analyse previous investigations critically and conduct further research to determine if fluorescent-labelled polystyrene NPs (nanoPS) can provide conclusive evidence of absorption and internal accumulation of NPs. We exposed zebrafish embryos and larvae to NPs (500 and 1000 nm) labelled with a green or an orange fluorescent dye, to solutions resulting from nanoPS dialysis, and to Nile-Red (a fluorescent dye used as a positive control). Previous studies have claimed that NPs cross epithelia without accounting for dye leachates and/or cellular autofluorescence. Our results demonstrate that commercial fluorescent-labelled nanoPS can leach their fluorophores, and the fluorophore alone can accumulate within internal tissues of zebrafish larvae. We further observed green autofluorescence in fish larvae not exposed to any particles. Our work was pioneer in showing that previous claims of NP absorption based on observations of fluorescence in zebrafish tissues should be considered inconclusive. Schür et al 2019 have subsequently reached similar conclusions, reporting that fluorescent dyes leaching from particles can lead to misinterpretation of plastic particles translocation in *Daphnia magna*. Although the addition of purification steps and inclusion of controls for leaching of dyes are methodological improvements, the use of fluorescent nanoPS should not be considered to provide absolute conclusive evidence of particle absorption.

3.12P.15

Adverse effects induced by micronized polyethylene terephthalate microparticles (PET-µPs) to the Manila clam (Ruditapes philippinarum)

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Microplastic (µPs) contamination represents a worrisome environmental issue threatening marine ecosystems. Several monitoring studies have revealed the presence of different µPs composed by different polymers floating in the surface waters, but recently µPs have been found also in bottom sediments. For instance, because of its high density compared to the seawater, µPs made by polyethylene terephthalate (PET) are commonly found in deep sediments worldwide. Thus, this study was aimed at investigating if benthic organisms might be affected by the exposure to PET microplastic. Thus, we investigated the ingestion/egestion and the potential adverse effects induced by 7-days exposure to two concentrations (0.125 and 12.5 µg/mL) of micronized, irregular shaped PET microparticles (PET-µPs) towards the Manila clam *Ruditapes philippinarum*. In order to check the capability to ingest PET-µPs and the potential damage at clam tissues, histological analyses were performed. A suite of oxidative stress biomarkers was applied on the gills and the digestive gland dissected by clams to investigate the alteration of oxidative status and the presence of oxidative damage. Our results showed that clams were able to ingest and egest PET-µPs, while no histological alterations were induced. A significant modulation of the oxidative status was noted in the gills, leading to lipid peroxidation. In contrast, no significant effect was found in the digestive gland. Our findings suggest that PET-µPs might represent a threat for organisms living in marine bottom sediments.

3.12P.16

Evaluation of microplastic toxicity in accordance with different sizes and exposure times in the marine copepod Tigriopus japonicus

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The indiscriminate use of plastic has greatly increased microplastic contamination risk in the marine environment. Microplastics can affect all marine life via the food web, from primary producers (e.g., microalgae) to final consumers (e.g., carnivorous fish). Thus, several studies have attempted to evaluate microplastic toxicity, but information about the underlying mechanisms of their effect is limited. Therefore, in this study, we examined multiple factors that could contribute to microplastic-induced toxicity. We investigated the potential molecular effects of microplastic size and exposure time. We exposed the marine copepod *Tigriopus japonicus* to 50 nm and 10 µm polystyrene microbeads. We found that both size and exposure time increased intracellular levels of reactive oxygen species. In addition, antioxidant-related gene expression was modulated and antioxidant enzyme activities were changed significantly. The results of this study provide important insights into the molecular mechanisms of microplastic-induced toxicity in a marine organism.

3.12P.17

Do polyethylene terephtalate microplastics (PET-µPs) affect or suffer the effects by the sea urchin Paracentrotus lividus?

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Microplastic (µPs) contamination in deep marine ecosystems is of growing concern. A number of monitoring surveys have shown that µPs composed by different polymers are floating in surface waters, but many of them sink to deep sediments. However, the information on the toxicity caused by the exposure to µPs reaching deep sediments towards benthic marine organisms is still scant. The aim of this study was to assess the ingestion and the adverse effects caused by a 7-days dietary exposure to three amount (8; 80 and 800 particles/g of food) of irregular shaped polyethylene terephthalate µPs (PET-µPs) in a benthic grazer, the sea urchin *Paracentrotus lividus*. Histological analyses were carried out to evaluate the occurrence of PET-µPs within the digestive tract of sea urchins, as well as to check for potential tissue alterations to the esophagus. Oxidative stress-related effects were explored in the proximal part of the esophagus. In addition, potential alteration of PET macromolecular chain caused by the ingestion and the permanence of particles within the sea urchin digestive system were investigated. Sea urchins ingested and efficiently egested PET-µPs, which did not provoke any histological alteration to their esophagus. PET-µPs ingestion induced an overproduction of pro-oxidant molecules and modulated the activity of antioxidant enzymes. Interestingly, the time of permanence of PET-µPs within sea urchin digestive tract slightly affected the PET macromolecular chain. Our findings suggest that irregular shaped PET-µPs do not represent a hazard for the health status of a benthic grazer organism, which actually can contribute to the degradation of this polymer in marine ecosystems.

3.12P.18

Minimum reporting criteria for microplastic ecotoxicity testing - do we meet our own prerequisites?

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Since the discovery of the presence of synthetic nano- and microparticles in aquatic systems there has been an increased scientific and public interest on their occurrence and potential impact on human and environmental health. Numerous studies deal with the potential negative effects of microplastics and other particulate substances, but the methods applied for testing are mostly not sufficiently described. This is due to a number of challenges associated with the development of test procedures for specifically evaluating microparticle effects in living organisms on the one hand, and, on the other hand, a lack of standardised test protocols for handling these new test items. A common way is the use of standard methods, e.g. according to OECD or ISO. Validity of the performed tests are not only determined by the setup, performance and evaluation of the experiment, but also by the reporting of the study. Thereby, essential details on the test-setup and the properties of the test item need to be reported to guarantee reliabilty. Here we propose a list of minimum reporting criteria for ecotoxicity testing of microplastic and other particulate substances and also give specific recommendations on the methodological approaches. Reporting criteria include, but are not limited to the following aspects: particle characteristics such as porosity, surface characteristics and –charge as well as chemical identity; particle handling, including production of stock suspension (sieving, mixing, pre-wetting) and control of suspension stability; the particle behaviour in the stock suspension (aggregation, stability over time); preconditioning (ageing) procedures; the generation of exposure scenarios (matrix, dosimetry, aggregation) as well as the use of reference materials and the accounting for particle-organism interactions. Keeping in mind that analytically methods for validation of exposure concentrations and the tracking of particle behaviour in the test system are crucial to determine dose-related effects, we also critically discuss the applicability of the proposed reporting criteria and exisiting limitations. We therefore aim to raise the discussion if we are already able to meet all the prerequesites that are necessesary to evaluate particle related risks. The reporting criteria were developed within the framework of the research initiative "Plastics in the Environment – Sources, Sinks, Solutions", funded by the German Federal Ministry of Education and Research (BMBF).

3.12P.20

Uptake and interactions of nanoplastics with wheat plants

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Plastic waste arrives to soils mainly through the application of sewage sludge, biowaste digestates and through the use of plastic mulch films. Nanoplastics are likely to occur in soils from these sources and through the fragmentation of larger plastic debris. Nanoplastics are of particular concern because a recent study showed that they can be taken up by plants [1] with the potential to bioaccumulate and biomagnify through the food chain. However, there is no quantitative information about the uptake and very limited about the effects of nanoplastics on plants. One reason for this is the lack of methods for their detection in complex matrices. In the present study, wheat plants were grown in hydroponics and exposed to Pd-doped nanoplastics at various concentrations, which allowed for easier detection of the metal as a proxy for the plastic [2]. The zeta potential and agglomeration state of the nanoplastics and the pH of the nutrient solution were monitored throughout the culture. At the end of the experiment, the nutrient solution was further analysed for total organic carbon and low molecular weights organic acids (related with root exudation). Nanoplastics aggregated in the nutrient solution, which was consistent with the observed decrease in zeta potential and increase in pH and root exudation. Pd concentrations in the plant tissues were measured by ICP-MS, showing uptake and transport of nanoplastics from roots to shoots. Synchrotron micro X-ray Fluoresce (µXRF) using the Pd signal showed nanoplastics accumulation in root tips and the epidermis of the maturation region. Scanning electron microscopy (SEM) revealed that nanoplastics accumulated on the root surface were mostly present as agglomerates intimately associated with the filamentous structure of the biofilm developed on the root surface. Concerning phytotoxicity, no effects on classical parameters such as plant growth, chlorophyll and protein contents and oxidative stress were found in response to nanoplastic exposure. However, Fourier Transform Infrared Spectroscopy in Attenuated Total Reflectance (FTIR-ATR) revealed changes in the molecular environment of proteins and cell wall components in both roots and shoots of exposed plants. This study provides new insights on the fate and impacts of nanoplastics on a model crop plant using a multi-technique approach to assess several facets of uptake and effects of nanoplastics to wheat plants. [1] Lian et al. (2019). Impact of polystyrene nanoplastics (PSNPs) on seed germination andseedling growth of wheat (Triticum aestivum L.). J. Hazard. Mater. in press. [2] Mitrano, D. M. *et al*. (2019). Synthesis of metal-doped nanoplastics and their utility to investigate fate and behaviour in complex environmental systems. Nat. Nanotechnol 14(4) 362-368

3.12P.21

Toxicity of polystyrene nanobeads to the marine microalgae Rhodomonas baltica

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Plastics entering aquatic ecosystems can degrade into increasingly smaller particles that can reach the nanometer size and consequently be easily incorporated by organisms. With increasing evidence about the ocurrence of these smaller sized plastics in the environment, the need to identify the physicochemical properties influencing their toxicity has been highlighted. Polystyrene (PS) beads have been recommended as priority test material for use and development of ecotoxicological studies, in which nano-beads have been employed to investigate the impact of nanoplastics (NPLs) on marine biota. As primary producers, microalgae are essential components of aquatic ecosystems, thus being relevant biological models to assess the potential impacts of NPLs. However, very few studies have focused on the effects of plastic particles on this group of species, especially when it comes to underlying mechanisms of toxic action. With this in mind, this study aimed to evaluate the effects caused by two different surface charged polystyrene nanoplastics (negative: PS-COOH and positive: PS-NH_2, 50 nm) on the microalgae *Rhodomonas baltica* and compare them to plain PS polystyrene NPLs (50 nm). Analysed endpoints included growth rate, natural pigments content, cell size, cell complexity, cell viability, metabolic activity, reactive oxygen species (ROS) formation, mitochondrial membrane potential, lipid peroxidation (LPO) and DNA content. Additional information on the absorption, distribution and use of energy in photosynthesis was performed using PAM fluorometry. PS nanobeads were characterized in exposure media using dynamic light scattering and nanoparticle tracking analysis to understand the role of particle behaviour in the observed toxicity. Characterization data showed a different behaviour of PS NPLs in exposure media over time, with plain PS and PS-COOH forming micro-scale aggregates while PS-NH_2 maintained its nominal size range. In terms of effects at the cellular and physiological level, PS-NH_2 was found to exert the highest toxicity in *R. baltica*, followed by PS-COOH and plain PS. Results obtained allowed the identification of different mechanisms of toxicity for plain PS, PS-COOH and PS-NH_2, underlining the role of the surface chemistry and size in determining the behaviour and effects of PS NPs.

3.12P.22

Stitching the patchwork quilt; The gaps in microplastic research in freshwater biota.

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It is shown that variety of freshwater biota readily ingests microplastics (< 5mm). These microplastics (MP) are classified as an emerging contaminant and it is imperative to study their behavior in freshwater food-webs. In order to map-out the current knowledge and highlight the urgent research needs, an extensive systematic literature review was conducted to identify the current knowledge-gaps in laboratory and field-based freshwater microplastic studies. To date, the majority of the literature regarding the effects of MP uptake has been conducted in laboratory settings and has mostly focused on spherical microplastics consisting of only one (polystyrene as most common) or few polymer types that usually fall below the size detection limit that is seen in environmental sampling. Studies using environmentally relevant mixtures, varying in shape, size and polymers are lacking from the freshwater literature but these are essential to understand synergistic effects.The review confirmed that the main freshwater Animalia Vertebrata class studied in both lab and field settings is fish, and a clear bias could be seen towards this class; the most studied invertebrate is the freshwater flea (Daphnia spp.). However, as the measured endpoints for toxicity testing varied greatly between the reviewed studies, a wide variety of different conclusions were reached, even when similar polymers and size-classes were used. This calls urgently for unifying the endpoints of toxicity testing on microplastics across species, or as a minimum for inclusion of some agreed baseline end-points to enable cross-comparison between studies. Reviewed literature indicated that even if the typical toxicity endpoints, such as mortality nor growth were unaffected in the test species (i.e. *Danio rerio*), more subtle toxic effects such as elevated oxidative stress and some degree of neurotoxicity could be observed (such as decreased Acetylcholinesterase levels (AChE)); these types of sub-lethal toxic effects might induce transgenerational and population-level negative effects over the longer term and might not be visible when typical toxicity testing is carried out. For this reason, toxicity tests should be expanded to cover transgenerational boundaries in order to count for the subtle toxic effects that could have long term population-level effects.This project will aim to address the question of effects of complex mixtures and different morphologies on freshwater Diptera *Chironomus riparius* and address the possible transgenerational and population level effects that might be seen in the more environmentally realistic settings.

3.12P.23

Do microplastics affect the cosmopolitan zoanthid Zoanthus sociatus, in a short-term exposure scenario? - responses at physiological and biochemical levels

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Microplastics (< 5mm in size) are ubiquitous and persistent in marine environments, with concerning levels in the tropics due to the waste mismanagement in developing countries and intense economic activities such as tourism and fisheries. The presence of high concentrations of microplastics in tropical waters has been recently associated with increased physiological distress and promptness to diseases on corals. However, the effects of microplastics have only been described for zooxanthellae scleractinian species (Anthozoa: Hexacorallia, also known as stony corals), reporting mostly responses at the organism level (growth, feeding, microplastics uptake and photobiology). This study aimed to overcome this gap in knowledge, evaluating the potential effects of microsized particles of two widely applied polymers (low-density polyethylene, LDPE MP and polyvinyl chloride, PVC MP) in the tropical and subtropical cosmopolitan coral *Zoanthus sociatus* (order Zoantharia, Anthozoa: Hexacorallia). Organisms were exposed to two environmental relevant concentrations (1 and 10 mg L^{-1}) of both polymers during 96h and survival, behavior, photosynthetic efficiency and cellular response mechanisms (oxidative stress, detoxification capacity and energy metabolism) were evaluated. In a short-term exposure (96h), *Z. sociatus* was more sensitive to PVC MP, with no effects observed to LDPE MP treatments. Coral exposure to 10 mg L^{-1} PVC MPs caused a ten-fold higher adhesion to the coral epidermis, probably due to the higher density of this polymer that tended to precipitate and be more available at the coral level than floating LDPE MPS. PVC MP exposure increased photosynthetic efficiency, probably due to the shading effect. In spite of PVC MP did not affect energetic costs on corals, antioxidant defenses significantly change and lipid damage was registered at higher MP concentration. Although the observed physiological and biochemical effects did not compromise *Z. sociatus* survival in short-term exposure scenarios, it does not rule out potential long-term effects that could endanger this and other physiologically similar species.

3.12P.24

Oxidative stress and energy unbalance in Chironomus riparius larvae after ingestion of polyethylene microplastics

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Microplastics (MPs; synthetic polymers with < 5 mm in size) are ubiquitous and persistent in freshwater ecosystems. The deposition and persistence of plastic micro-debris in sediments (lakeshores and riverbanks) makes them long-time available for benthic species. In fact, freshwater sediments are frequently reported to contain higher number of MPs than the water column. Thus, freshwater benthic invertebrates that rely on freshwater sediments and feed on particulate organic matter are more prompt to ingest and accumulate microplastics. This study addresses the ingestion of polyethylene microplastics (PE-MPs) by *Chironomus riparius* larvae after short-term exposure (48 hours) and subsequent physiological and biochemical response mechanisms triggered to maintain organism homeostasis. Biomarkers related to energy reserves (lipids, carbohydrates and protein content) and energy consumption (electron transport system, ETS), antioxidant defence (catalase, CAT) and oxidative damage (lipid peroxidation, LPO) and detoxification response (glutathione-S-transferase, GST) were evaluated. Evidence of oxidative damage (LPO) concomitant with effects on antioxidant defences and detoxification responses, as well as, alterations on the cellular energy allocation were observed in larvae after ingestion of PE-MPs. The reported short-term effects (mainly oxidative stress and energy unbalances) along with long-term effects already reported (Silva et al., 2019) highlight the potential deleterious effect of microplastics for aquatic invertebrate populations and indirect effects on ecosystem functioning.

3.12P.25

Occurrence and effects of microplastics in tissues of coastal animals in a plastic polluted area

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Plastic pollution is ubiquitous, and global concerns are rising that increasing amounts of microplastic (MP) in the food web will eventually expose humans to ingestion of MP. Investigations of animal tissues relevant for human consumption is warranted, as is the effects in birds and mammals exposed to plastic. In this pilot study we investigated the occurrence of microplastics (MP) in selected tissues of fish, seabirds, and marine mammals from a plastic polluted coastal area near Bergen, Norway. A standardized autopsy incl. evaluation of animal condition, bacteriological and histopathological investigation and polarized light microscopy were performed in the same tissues as analyzed for MP (>10 µm) by pyrolysis Gas Chromatography/Mass Spectrometry. We analyzed stomach and intestinal wall, liver and muscle/fillet from three flatfish, three cod, three seabirds, three otters and one seal, kidneys from the seabirds, otters and the seal, and gills from the fish. No large plastic items were observed in the gastrointestinal tracts. Eight of 13 animals had MP in one or several tissues. Seven had MP in the stomach wall or intestine, while four had MP in muscle/fillet and/or liver (figure 1). No MP was found in the seal, and only in the stomach wall of one otter. The birds had MP in the intestine, the stomach and liver. The cod had the most MP, with max 3.4 µg/g ww in liver. MP was found in stomach/intestine, liver and muscle in two of three cod. In flatfish MP was found in one muscle sample. Three plastic polymers were found: polyvinylchloride (PVC)> polystyrene (PS) > polyethylene tereftalat (PET). No polyethylene, polycarbonate, polypropylene, poly metyl-metacrylat or polyamide-66/nylon (PE, PC, PP, PMMA, PA-66) were found. The concentrations were just above the limit of Quantification (LOQ: 1 µg/g ww except PMMA: 5 µg/g ww). In four parallel samples of cod muscle, MP was quantified in one sample per individual. No MP was observed by microscopy. The results indicate uneven MP distribution in tissues. Procedural blanks and air-controls showed very low MP. No adverse effects related to the presence of MP in any sample were detected. The animals were by-catch, and mostly in good condition. The study is too small to conclude on human exposure through food, but indicates that further studies are needed to confirm presence of microplastic in edible tissues and the potential for uptake and transfer through the food web.

3.12P.26

Investigating effects of Nanoplastics on the Bioavailability of Sediment-associated Toxicants by Assessment of Zebrafish Gene Expression

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Nanoplastics (NPs, ≤ 1µm), as well as microplastics (MPs, 5 mm–1 µm), can result from fragmentation of larger plastic debris released in the environment and can pose a risk to aquatic organisms. Potential effects of NPs include release of sorbed substances (co-contaminants) into organisms after particle ingestion. Although not yet effectively measured in aquatic environments, NPs may be the most abundant plastic particles present in the environment. Because the relative surface area is greater for NP than MPs, there is greater potential for co-contaminant sorption to NPs. Our group has previously shown that analytical chemistry does not accurately predict bioavailability of MP-sorbed contaminants, and that organism-based measurements can provide a unique insight into bioavailability (Sleight et al 2017). In this study we have assessed the sorption of a highly hydrophobic co-contaminant, Benzo[a]Pyrene (B[a]P), in the presence of sediment associated with nanoPolystyrene (nanoPS) particles of two size categories: 250 and 1,000 nm. The goal was to assess the contribution in the sediment of a known fraction of plastic particles of two size ranges in the sorption-desorption dynamics of a highly hydrophobic co-contaminant, using its bioavailability in the aqueous phase as a proxy for sorption. The bioavailability of B[a]P in the aqueous phase was assessed by analysing the expression of the gene cytochrome P450 1a (cyp1a) in zebrafish (*Danio rerio*) larvae (72h postfertilization). Gene expression was measured by quantitative reverse transcription PCR (RT-qPCR) after extraction of total RNA. Dose-response curves were obtained and the effective concentrations (EC_{50}; concentration that induces a gene to half-maximum for a specific compound) for each model were compared. Sorption of B[a]P to nanoPS and sediment was confirmed using analytical chemistry techniques. Preliminary data shows that the EC_{50} obtained by exposing zebrafish larvae to B[a]P (EC_{50} = 9.4 µg/L) was increased by 5.1-fold when the solution had been previously exposed to 3 g/L of sediment. The fold change increased to 26.7 (1,000 nm) and 26.9 (250 nm) when 270 mg/L of nanoPS were added to the solution in the presence of sediment. The results indicate that lower mass of plastics, compared to sediment, reduces the bioavailability of B[a]P. We anticipate that our results (ongoing data analysis) will contribute to a better understanding of the risk of NPs and their co-contaminants in freshwater systems.

3.12P.28

Does the presence of natural particles alter microplastics ingestion and effects in freshwater sediment-dwelling invertebrates?

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Sediments from freshwater ecosystems all over the globe have been identified as one of the major sinks of microplastics from inland activities. Evaluation of the effects of microplastics on freshwater benthic biota has become an urgent research priority. The few available studies addressing these effects on benthic detritivore invertebrates such as chironomids, have reported a significant MP ingestion, with consequent negative consequences on organism's behavior and life-history. However, in such studies, the use of mineral sediments or only reconstituted freshwater are predominant. Knowledge of the potential effects of MPs in the presence of natural particles, such as organic matter, is scarce. To tackle this, we conducted a 10 days bioassay, with 1st instar *C. riparius* larvae (< 24h post-hatching) to assess the effect of polyethylene microplastics (PE-MPs; 0, 1 and 5%) in sediments containing different types of organic matter (5% of alder or cellulose), and in the presence and absence of small-sized mineral particles (20% of kaolin). Results showed an increase in PE-MP larval ingestion and a decrease in larval growth with increasing concentrations of PE MP in the sediment, independent of the organic matter present. Such tendency remained similar in the presence of kaolin, despite the positive effect of this natural particle (*per se*) on larval growth. To also verify if an increment in the organic matter content in the sediments would change both ingestion and effects of PE-MPs of *C. riparius* larvae, in a second approach (which is ongoing) we exposed *C. riparius* to PE-MPs (0, 1 and 5%) in sediments containing different percentages of organic matter (0, 2.5, 5, 10% of cellulose), and in the presence and absence of kaolin. With these experiments, we will provide important insights on the role of natural particles (such as kaolin and organic matter) within sediments on the ingestion and toxicity of PE-MPs to chironomids. The presence of natural particles and their potential interaction with MPs is crucial for a proper risk assessment of these contaminants in freshwater benthic habitats.

3.12P.29

Assessment of the Degradability and Toxicity of Biobased Composites

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Biobased polymers and composites can be a sustainable and lower CO_2 emission alternative to synthetic-based plastics. In marine environments most applications currently use oil-based composites including fish, shellfish and seaweed farming, energy harvesting, shipping, and buoying elements. Due to hydromechanical forces and UV radiation, polymeric composites surfaces can undergo physical stress and degrade forming microplastics (MPs), as well as leaching chemicals to the environment. Increased concern on the toxicity and potential negative impacts to marine organisms of derivate particles (MPs) and leachates has increased the demand to create sustainable biobased composites with a lower environmental impact. The Interreg 2 Seas Mers Zeeën project SeaBioComp aims to develop and produce novel biobased composite materials as alternatives to conventional oil-based products used in the maritime industry. The project will produce analytical protocols to evaluate the long-term durability and assess the impact of biocomposites on marine organisms. On a first stage, biocomposites will be exposed to realistic temperature and UV-intensity for different durations to quantify and identify the formation of MPs and the release of leachates. To identify MPs we will employ a combination of light microscopy, scanning electron microscopy coupled to an element detection system, and infrared technology (µFTIR). The chemical analysis of the leachates will be performed applying standard methods using high performance gas and/or liquid chromatography/mass spectrometry. On a second stage, standardized ecotoxicological assays will be used to test the toxicity of the leachates to marine organisms. To do so, we will use a microalgae, *Phaeodactylum tricornutum*, growth inhibition protocol (ISO 10253, 2016), and a mussel larval development assay (ASTM E724 – 98, 2012). We anticipate that our results will contribute to assess the environmental impact of new biobased products, which can present a cost-effective and more sustainable alternative to synthetic-based composites.

3.12P.30

Modelling environmental relevant experimental data: Atlantic cod feeding on plastic

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Chemical pollutants and plastic particles are wide spread in the environment. Many experiments were conducted to show the role of plastic particles on the bioaccumulation of associated chemicals. These experiments however, used environmental unrealistic scenarios and/or concentrations. From a risk assessment perspective the question should focus on when plastic contributes to bioaccumulation and if the plastic ingestion pathway is relevant compared to other pathways e.g. food ingestion and dermal uptake. To answer these questions environmental realistic experiments were all compartments are loaded with environmental relevant concentrations and models to interpreted the data are needed. Aim of the present work was to model pathways of plastic and associated chemicals in an environmental relevant fish feeding experiment, using an integrated microplastic and contaminant accumulation model. In the experiment, four year old Atlantic cod (Gadus morhua), a commercially highly relevant fish, was fed with either a control diet or the diet mixed with plastic particles (1%). Polyethylene microplastics (PE) with a size range of 300 – 600 µm were places in permeable bags in the Oslo fjord and retrieved after four months. Fish were held in a flow through system in the indoor experimental facilities at Nofima Centre for Marine Aquaculture (Kvaløya, Tromsø). Fish were sampled during eight months. In order to know the chemical concentration in water, polyoxymethylene (POM) and polydimethylsiloxane (PDMS) were placed in the tanks. A theoretical model was used to simulate all chemical processes in the experiment and differentiate between pathways. The experiment showed that the contribution of contaminants (PCB & PBDE) from PE powder in the feeds were low compared to the level in ingredients used. After three months of feeding, 33 chemicals were measured in the fish for all treatments however patterns were not clear. Chemical concentrations were highest for PCBs 138 and 153. PCB 153 will be modelled and scenarios run. The use of environmental realistic designed experiments complemented with theoretical models help to improve both environmental and human risk assessment.

3.12P.31

Plastic Additives: How "pure" are neat polymers?

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Nowadays, the great concern about the presence of plastics in environment have led to a huge increase in research studies about polymers. Some of these studies were developed using polymers often called "pure" or neat polymers (polymer without any added additives). Nevertheless, the use of some additives are mandatory to perform the polymerization reactions used to fabricate the polymers, and for their process and improvement of properties and therefore some other substances in addition to polymer can also be found in the product. It is necessary to take into account the presence of these substances when the research experiments are performed. These substances are present in the polymers frequently not chemically bonded to them, so they can interfere in adsorption/desorption of contaminants to plastic studies or even in ecotoxycity studies. In this work physical (SEM, and size distribution) and chemical characterization (FT-IR, and analytical determinations of chemicals) of some neat conventional polymers (PA, LDPE) and biodegradable biopolymers (PHB) were performed. The polymers used are in all cases commercial materials, which were cryogenically ground and treated by sieves to select the appropriate sizes for subsequent analysis. Some alkylphenols, phthalates and volatile organic compounds were detected both in conventional and biopolymers, some of them in high concentrations (i.e. DEHP 14 µg g-1 in polyamide). Also metals were detected in relatively high concentrations (i.e. 118 µg g-1 of Br and 174 µg g-1 of Pb in polyamide). Acknowledgements: Supported by Spanish Inter-Ministerial Science and Technology Commission and European Regional Development Fund (ERDF) through ARPA-ACUA (CTM2016-77945-C3-3-R and CTM2016-77945-

C3-2-R), and the Consolidation and Structuring of Units of Competitive Investigation Program of the SUG (Xunta de Galicia) co-financed by (reference: GRC2013-047).

3.12P.32

Sorption of Chlorpyrifos on plastics and bioplastics in marine environment. Effect of temperature and weathering

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Due to its hydrophobicity, persistent organic pollutants (POPs) in marine environment trend to adsorb on MPs as well as on sediments or particles [1]. In the last decades, the presence of POPs adsorbed to plastics collected in the marine environment has been reported [2]. Chlorpyrifos is an organophosphate broad spectrum insecticide considered moderately hazardous to humans by the WHO based on its acute toxicity. Its presence in surface water is regulated by the 2013/39/EU Directive (MAC-EQS of 0.1 µg L-1). A study of the adsorption of chlorpyrifos on two petroleum based polymers (LDPE, PA) and one biopolymer (PHB) in seawater media was performed. A biodegradable plastic was included because the release of additives or adsorbed contaminants could be increased by the degradation of the plastic. The effect of temperature (20 and 4ºC) and weathering was evaluated. Artificial seawater was used to avoid interference of particulate matter on the result. The contact time was 0, 24, 48 and 96h, and 7 days. Control samples (without plastic) were also analyzed at each contact time, to ensure the stability of the pesticide during all the experiment. For each temperature assay, pristine and weathered PHB, LDPE and PA were tested simultaneously. The results shown that chlopyrifos was almost totally adsorbed to the plastics at 24h in both temperature assays, with little differences at higher contact times. Similar results were obtained at both temperatures. The highest adsorption was observed for LDPE (97% is adsorbed at 7d), then PHB and finally PA. No differences were observed between pristine and weathered plastics. That results confirm that chlorpyrifos can adsorb to plastics in a marine environment. Once adsorbed, we evaluate the possible cession of chlopyrifos to sea water by an accelerated leaching. No cession was observed for LDPE, whereas for PA and PHB the cession was negligible lower than 0.5% of the adsorbed compound. Acknowledgements Support: Spanish Inter-Ministerial Science and Technology Commission and European Regional Development Fund (ERDF) through ARPA-ACUA (CTM2016-77945-C3-3-R), and the Consolidation and Structuring of Units of Competitive Investigation Program of the SUG (Xunta de Galicia) co-financed by ERDF (reference: GRC2013-047). [1] N.B. Hartann, et al., Integr. Environ. Asses. 13 (2017) 488. [2] V.M. León, et al., Environ Pollut 236 (2018) 442.

3.12P.33

Adsorption of musk fragrances by conventional (PA) and biodegradable (PHB) microplastics in seawater

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Microplastics (< 5 mm) can be classified as primary or secondary, depending on the manner in which they are produced. Primary MPs are small plastic particles released directly into the environment via e.g. domestic and industrial effluents, or indirectly via e.g. run-off. Secondary MPs are formed as a result of gradual degradation/fragmentation of larger plastic particles, due to e.g. UV radiation, mechanical transformation and biological degradation by microorganisms [1]. For all of this, microplastics are crucial pollution which are widely distributes in the environment. Synthetic musks is an organic substance that has a similar odor as that of natural musk. It is manufactured in a large scale and implemented widely in perfumes, cosmetics, soaps, and other daily chemical products and flavor additives [2]. With the high accumulation in the environment, MPs are not only potential contaminants but they can also act as an extra compartment for the partitioning of chemicals. In this stydy, the adsorption of six synthetic musks by a conventional (polyamide) and a biodegradable polymer (polyhydroxybutyrate) was investigated. In order to carry out the analysis of the water and / or plastics, a separation of the same was done previously with 20 µm steel filters, which proved to be the one with the lowest percentage of fragrances retained (30%). The assessment of the changes and processes that MPs suffer during weathering is essential to understand their effects in the environment, and therefore the tests were made with pristine and weathered polymers and were performed at room temperature (20ºC) and at 4ºC and were collected at different hours using simulated seawater prepared with sea salt. The adsorption to the plastic is observed already at 24 hours. Moreover, adsorption capacity was affected by temperature and few differences are observed between the pristine plastic and the weathered one. PA is the plastic with the lowest adsorption capacity while many problems are observed with the PHB. References [1] de Sá L.C., Oliveira M, Ribeiro F, Rocha T.L., Futer M.N. 2018. Sci. Total Environ. 645: 1029-1039. [2] Dong X, Zheng M, Qu L, Shi L, Wang L, Zhang Y, Liu X, Qiu Y, Zhu H. 2019. Mar. Pollut. Bull 144: 129-133. Acknowledgement. Xunta de Galicia-Program for the Consolidation and Structuring of Competitive Research Units (ED431C-2017/28) and to the Ministry of Economy and Competitiveness (CTM2016-77945-C3-3-R (ARPA-ACUA)).

3.12P.34

Validation of a standardized particle toxicity testing via food

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Studies investigating the toxicity of microparticles face the difficulty that particles behave different to soluble substances in the often-used laboratory test setups. While soluble substances distribute homogeneous in the liquid phase, particles, especially micro- and nanoplastic, can form aggregates and inhomogeneous distribution. Depending on particle properties, they can be floating on the surface, sticking to test vessels, sink to the ground or even aggregate. Current standard toxicity tests are based on soluble substances and need to be adapted to particulate material. In this paper we present a reliable exposure method for benthic shredder-organisms such as crustaceans or insects with a defined number of particles homogenously distributed in the food. We used modified DECOTABs from Kampfraath et al. (2012) and developed a standardized food exposure protocol for gammarids. Food supplements were tested to optimize the nutritional value for gammarids to provide ideal test conditions. After we validated the feeding rate without particles, the food preparation was extended with fluorescent microplastic particles to validate the homogeneous distribution within the DECOTABs. We were able to quantify the ingestion by G. roeseli of the particles in relation to the consumed food. The developed method provides a more realistic and methodologically reliable uptake than from the aqueous phase and allow the quantification of the internal exposition via feeding rate. The established method is a promising tool for standardized exposure with particles via the food pathway in order to produce comparable and valid particulate toxicity results.

3.12P.35

Microplastics as possible vectors of inorganic pollutants: the study case Ofanto river

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The interaction between microplastics (MPs) and pollutants is an issue of emerging concern due to the need of evaluate the risk associated to the microplastics topic. The combination of, various kinds of polymers of different size and shape joined to the action of a large amount of additives originating from the plastics, results in a cocktail of contaminants which not only alter the nature of plastic but can leach into the air, water, food and potentially human body tissue during their use or their disposal exposing us to several chemicals together. However, to date, there is considerable lack of knowledge regard to the mechanisms of interaction between MPs and contaminants. Among the various classes of pollutants that one of heavy metals is the least studied. The goal of the present work was to identify and quantify inorganic pollutants (heavy metals) associated to different types of MPs taken from Ofanto river (Southern Italy) in order to differentiate metals naturally present as additives on MPs from elements adsorbed by the surrounding environment. To reach this aim two kinds of MPs were investigated: those one collected from the river (environmental microplastics) and the primary MPs used for the production process of plastic materials (virgin microplastics). After MPs acid digestion using temperature control microwave heating, the quantification of 28 different mineral elements was performed by mass spectrometry with an inductively coupled plasma source (ICP-MS). The determination of heavy metals highlighted the presence of different elements on the surface of the MPs. Some of them (Mg, Ca, Ba, Na, K, Rb, Cs) were found constantly present in all the samples, reporting high concentrations especially in environmental MPs. Most of these elements are applied during the production process of plastics as fillers (Calcium Carbonate, Magnesium Hydroxide), flame retardants, (Aluminum associated sodium) and thermal stabilizers (Calcium-Zinc, Barium-Zinc). Other metals, such as, Cr, Pb and Cd, used mainly as dyes based on inorganic pigments were found in high concentrations (respectively of 94.11, 418.39 and 814.61 mg / kg) above all in colored environmental MPs. Finally, metals such as V, Co, Ga, As, Se, Rb, Sb and Ba revealed significantly higher values in environmental samples than those one found in virgin microplastics suggesting thus a probable absorption from the surrounding environment.

3.12P.36

Lost in the lab: Where is all the microplastic?

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Microplastic is taken up by marine organisms from many taxa, but the fate of the particles after the ingestion as well as the consequences for the organisms are still largely unclear. Laboratory exposure experiments are a common way to investigate the effects of microplastic, but no common standards have been established for this kind of research so far. While authors usually indicate the nominal microplastic concentrations they applied in their experiments, they do not measure the exposure concentrations. Furthermore, the possibility of losing microplastic particles from the exposure system during the experiments is most often not considered. Here we show the results of a microplastic exposure experiment in which we reveal the fate of polystyrene (PS) beads (40 µm) within the system and found a reduction in food consumption in the blue mussel *Mytilus* sp. in the presence of microplastic. Two nominal microplastic concentrations (0.4 and 40 PS beads/ml) were applied and the microplastic was taken up by the test organisms from the water column into the mantle cavity without distinction between the algal cells and the microplastic. The ingestion of microplastic was shown by the presence of PS beads in the feces. However, the uptake of microplastic was not equal to the ingestion. Depuration in microplastic-free seawater for 48h reduced the number of MP that we found in the softbody of the mussels after the experiment. Food consumption, i.e. uptake of algal cells per unit time, was lower at the higher microplastic concentration. Surprisingly large differences between the intended nominal and the actual microplastic concentrations as well as high losses of PS beads during the experiment were observed. Our results show that, despite the greatest care, quantities of microplastic particles are difficult to adjust in laboratory studies. The loss of MP particles during exposure experiments should not be neglected since this could influence the interpretation of such experiments.

3.12P.37

The Hidden Microbial World of Environmental Plastic: An Initial Examination of the Interactions Between Plastic and Aquatic Microbial Communities in a Freshwater Environment.

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When plastic enters the aquatic environment, its surface is colonised by an assortment of microorganisms which form a complex three-dimensional biofilm community. Until recently however, the presence of this biofilm has been largely ignored in microplastic research. The aim of the current study was to investigate the temporal development of plastic-associated biofilms in a freshwater environment, and to assess how the presence of biofilms may alter the physical and chemical properties of plastic. A number of studies are beginning to investigate plastic-associated biofilms grown in a laboratory setting; therefore, an additional aim of this study was to assess whether biofilms grown in a controlled laboratory environment can be representative of those grown in a natural setting. Low-density-polyethylene (LDPE), polylactic acid (PLA) and glass panels were incubated in river water for twelve weeks. Samples were incubated in situ in a local river and in parallel, in a flow-through aquaria supplied continuously with fresh water from the river. Panels were removed and analysed after one, three, six and twelve weeks of incubation and a variety of endpoints were assessed. The taxonomic composition of biofilms from different treatments is presented, along with the mass and chlorophyll content of biofilms, and physicochemical changes that occurred at the surface of plastic samples. Initial results suggest that material type influenced the development of biofilms, with plastic supporting a 62 – 108 % higher biofilm mass by week six than glass from the same location. The chlorophyll and pheophytin content of biofilms from aquaria samples generally increased with biofilm age; this was not observed for samples from the river and location was found to significantly influence chlorophyll and pheophtyin levels of the biofilm. Temporal changes in the biofilm over the twelve weeks and differences between material types are apparent. The current data also suggest that biofilms grown in a laboratory setting may not be representative of those that form in the natural environment and care should therefore be taken when using artificial environments to grow and study microplastic-associated biofilms. Understanding the nature of plastic-associated biofilms is essential for advancing our knowledge of the behaviour of microplastics in the aquatic environment and their potential impact on entire ecosystems, as well as individual organisms.

3.12P.38

The effect of intrinsic properties, UV-degradation and biofilm formation on the fate of microplastic fibers in the marine environment

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Microplastic fibers (MPFs) are frequently reported as the most dominant type of microplastic (MP) found in the marine water column and sediments, as well as in waste water treatment plant (WWTP) effluents. A major source of these fibers is the use and washing of synthetic textiles. One garment can shed >1900 fibers per wash, leading to a MPF concentration of more than 100 fibers/L of WWTP effluent. Although 70% of households in the EU are connected to WWTPs, many of these are not designed to retain MPs and MPFs. In sparsely populated remote areas waste water treatment is often minimal. The use of synthetic textiles in cold-climate locations is high. In Longyearbyen, a settlement in the arctic archipelago Svalbard, waste water undergoes only coarse mechanical filtering, and MPFs are released directly into the adjacent Adventfjord. Approximately 20 000 million fibers/year are emitted directly into the Arctic marine environment. The potential for negative environmental consequences of these MPFs is highly dependent on their degradation and transformation in the environment. Intrinsic properties (polymer type, density, size) will influence environmental degradation, settling times, and ingestion of MPFs by marine organisms. Less well understood is the influence of extrinsic environmental parameters (e.g. UV radiation, microbial biofouling) on the fate of MPFs. In the current study, we compare the effect of UV exposure on the degradation and fragmentation of polyester (PES), polyamide (nylon, PA) and wool fibers (natural reference material) in laboratory- and arctic field conditions. Degradation was tested up to 10 months at accelerated laboratory conditions, and over six months in a field study. The role of biofilm formation on settling of PES, PA and wool fibers was investigated by suspending MPFs in natural seawater for up to 2 months to allow biofouling, followed by investigation of the microbial community structure and imaging of the fibers. The settling rates of fouled and pristine fibers in seawater were compared using a silhouette camera. Results showed that PES fibers degrade more rapidly when exposed to environmental UV than PA and wool fibers, demonstrated by both significant fragmentation and changes in surface morphology (e.g. pitting and fracturing). Potential ecosystem impacts discussed in the light of measured MPF emission rates.

3.12P.39

Investigation of the correlation between wastewater treatment parameters and pollutant accumulation on LDPE in the long-term deployment tests at municipal wastewater treatment plants

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The vast amount of microplastics (MP) like microbeads in personal care products, microfibers released as a results of washing of textiles as well as tire wear particles entering a municipal wastewater treatment plant (WWTP) end up in sewage sludge or in the effluent without getting completely degraded. Not only MPs but also a variety of micropollutants enter in the wastewater stream. Sorption behaviour of a pollutant on a polymer surface may differ in treatment steps operated under different conditions. Some hydrophobic persistent pollutants in the wastewater may accumulate on the surface of MPs and are released from the WWTP with MPs. Other pollutants entering the WWTP on the surface of MPs maybe be desorbed from MPs into wastewater and treated. We investigate sorption behaviours of hydrophobic organic pollutants on MPs in different wastewater treatment processes at two typical municipal WWTPs in Germany. One objective of this study is to investigate the accumulation of pollutants on low density polyethylene (LDPE) in different treatment steps of these WWTPs. Another objective is to identify wastewater treatment parameters (e.g.: temperature, pH-value and flow velocity) influencing sorption behaviour of pollutants on LDPE. The sorption experiments were carried out by deploying LDPE pellets (3 to 5 mm in diameter) at five locations, i.e. 1) at the end of grit chamber, 2) in the feed flow to the preliminary sedimentation where recycled filtrate from the sludge being added, 3) in the aeration tank of biological phosphorus elimination, 4) in the nitrification process and 5) in the secondary clarification basin. LDPE pellets are deployed at these locations in the same period of time: the duration of a deployment test is around 28 days. Pollutants were extracted from LDPE pellets and analysed with GC/MS. Treatment parameters recorded continuously, daily, weekly and monthly at each WWTP will be collected to find correlations between these parameters and pollutant accumulation on LDPE. The current result (deployment period: spring 2019) shows accumulation of polycyclic aromatic hydrocarbons (PAHs) on LDPE pellets in all five treatment steps. The pattern of PAH accumulation on LDPE varied among the different treatment processes, whereby PAH accumulation on LDPE at the end of grit chamber and in the secondary clarification basin were the highest. The project PLASTRAT is financed by the German Federal Ministry for Education and Research (BMBF).

3.12P.40

Impact of Biofilm-Formation on Microplastics' Sinking Rates

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The increased plastic production over the last centuries has led to a worldwide release of plastic waste into the environment. Thereby, freshwater systems represent an important pathway of plastic pollution to the world's oceans. When plastic debris enters the aquatic systems, it is quickly colonized by microorganisms. The consequent formation of biofilms on plastic surfaces, may modify their hydrodynamics and consequently fate, which is of possibly high relevance for microplastics (MPs) (< 5 mm). The impact of freshwater-biofilm

formation on the fate and sinking of MPs is hardly assessed. We therefore performed sinking experiments with un-plasticized polyvinyl chloride (uPVC), polyethylene terephthalate (PET) and low density polyethylene (LDPE), three of the most commonly found polymers in the environment. These polymers were assessed at a size of 250 and 300 µm. Two experimental runs were conducted with in total 250 sinking experiments and two phytoplankton communities. These communities were obtained from ponds in the vicinity of the laboratory. The involvement of two communities allowed for a first verification on whether pattern observed among different MPs are also transferable among biofilms. During the incubation of the MPs with the biofilms, the sinking velocity was assessed after 14, 17, 20 and 31 days and compared to non-aged MPs. The sinking experiments were performed using a simplified method of static light scattering (SLS). The experiments showed that biofilm growth significantly influenced the fate the polymers by altering their hydrodynamics and physical properties. Thereby, shape and surface structure played a major role on the degree of colonization and its impact on the respective sinking behavior. During both experimental iterations colonized uPVC particles tended to increased sinking rates, while the sinking rates of PET particles decreased with one of the phytoplankton communities. This patter for PET was not confirmed when using the second community as inoculum, which induced the opposite pattern in sinking rate. These partly conflicting results highlight the need to understand the drivers and traits of biofilms leading to the pattern in sinking rate as observed here.

3.12P.41

Attached And Free-Living Bacterial Communities Associated With Different Plastic Types And Sizes In Seawater And Freshwater

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Plastics are annually produced worldwide on a scale of hundreds of millions of metric tons, polyethylene (PE), polyamide (PA), polyethylene terephthalate (PET), polystyrene (PS), polyvinylchloride (PVC) and polypropylene (PP) being the ones most often found in the environment. Negative effects on ecosystems and human health have been reported, thus finding alternative biodegradable plastics is now an increasingly important area. Therefore, learning about the effects and biodegradable capacity of pristine plastics before producing functional products or releasing them into any environment is a crucial first stage. Here, we compared the differences between three types of pristine plastics: polyethylene (PE), polylactic acid (PLA), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with two sizes of granule (G) and their debris (M) in terms of their effects on natural bacterial communities in seawater and freshwater for one month in a lab-scale experiment. At first, we found a dissimilarity between attached bacteria on the plastics and free-living bacteria. Further, attached bacterial phyla were more diverse after 14 and 30 days (Planctomycetes in seawater and Acidobacteria, Cyanobacteria, Verrucomicrobia, Planctomycetes in freshwater) compared to day 0. Interestingly, the attached bacterial taxa were highly enriched alongside different plastic types and sizes after 30 days. In seawater, Polycyclovorans increased in PE_G, while Oleibacter rose in PE_M samples, Methylomgnum and Parcificibacter gained in PLA_G and PHBV_G, respectively. In freshwater, Roseomonas increased in PE_G, and Springorhabdus and Bosea in PLA_G and PLA_M, Azospirllum and Springomonas proliferated in PHBV_G and PHBV_M. Overall, we found that bacterial communities were more diverse when in contact with granules than with debris plastics. The attached bacteria were greater than free-living bacteria in both types of water.

3.12P.42

Modelling influence of seasonal variability of plankton and organic matter on distribution of microplastic in water column

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The evidence of macroscopic and/or microscopic fragments of plastic are found in all natural environments including marine systems. After entering the seawater, macroscopic fragments degrade into smaller particles (microplastics, MP), besides this MP can be delivered with coastal runoff and wastewater discharges. In addition to transport with currents and sinking, the fate of MP in the seawater is heavily related to biota: density of MP particles (that are typically neutrally buoyant) can be changed due to biofouling, or growth of algae on them, which changes their density and can lead to sedimentation or MP simply can be eaten by zooplankton and packed into faecal pellets with increased sinking rate. We hypothesise that seasonality of production and destruction of organic matter and corresponding changes in the development of the plankton ecosystem should affect the fate of MP in the water column. The fate of MP can be simulated with the help of coupled hydrodynamical- biogeochemical model, that provides a baseline scenario of the seasonal changes in the planktic ecosystem and changes in the availability of particulate and dissolved organic matter. In this work, we use a simplified biogeochemical model OxyDep (Yakushev et al., 2014) that simulates seasonal changes of phytoplankton (PHY), zooplankton (HET), dissolved organic matter (DOM) and detritus (POM). A specifically designed MP module considers MP particles as free particles (MPF), particles with biofouling (MPB), particles consumed by zooplankton (MPH) and particles in detritus, including faecal pellets (MPP). A 2D coupled benthic-pelagic vertical transport model 2DBP was used to study the effect of the seasonality of plankton and organic matter development on lateral transport of MP and its burying in marine sediments. OxyDep and MP module of the model were coupled with 2DBP using Framework for Aquatic Biogeochemical Modelling (FABM), that is a modern standard for coupling of biogeochemical and hydrodynamical models (Bruggeman, Bolding, 2014). The results of calculations demonstrate that the “biological pump” can be one of the important driving processes controlling the quantity and the distribution of MP in the water column. It has been shown that the biological pump can deplete MP from the surface water and accelerate MP burying in summer period compared to the winter. The elaborated modules will be used with the FABM compatible 3D models FVCOM and ROMS to study the MP fate in the Arctic seas.

New Approaches to Characterising the Global Chemical Exposome (P)

3.13P.1

Poly- and perfluoroalkyl substances (PFASs) in surface waters of Western Europe, North America, and the global oceans. A review of spatial and temporal trends

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Poly- and perfluoroalkyl substances (PFASs) are aliphatic organofluorine molecules with highly fluorinated carbon chain “tails,” and a hydrophilic “head” groups of carboxylates, sulfonate, or phosphonates/phosphinates. Many are degradation products of more complex precursors. It has been well established that they are transported in wastewater and rivers to the oceans which serve as a terminal sink. In this study our goal was to provide an updated overview of spatial trends, and where possible, temporal trends of PFASs in the open oceans globally and in freshwater, estuarine and nearshore marine environments of Western Europe and North America. The broader objective is to assess the effectiveness of national and global bans on PFOS, PFHxS, and PFOA and provide a baseline for future work. We used results from lakes, rivers and estuaries that included two or more sampling sites per river reach and that were identified by the study authors as being upstream or remote from urban wastewater effluents. On large rivers only data from mainstream river sampling sites were selected rather than tributaries. For marine environments we used results from open ocean cruises. We found that the spatial coverage of PFAS measurements in freshwater was dominated by information for rivers in western Europe and eastern USA, as well as in the Great Lakes in North America. For open oceans measurements in the North Atlantic and the Arctic Ocean predominated. Results showed that ice and snow melt inputs of PFAS are likely to be important during spring melt events and need to be considered in timing of monitoring programs in polar areas. The presence of PFOS and PFOA in remote sectors of the southern Ocean, the Arctic Ocean and the mid-North Atlantic, including in deep waters (>1000 m), illustrated the long range ocean transport of these PFASs and their value as tracers of ocean circulation. We compared concentrations reported pre-2010 with those reported from 2010–2018. Temporal trend information has improved over the past 10 years due to repeat sampling of some locations, especially for the Eastern North Atlantic, and the first evidence is emerging of declining PFOS and PFOA concentrations inland, in coastal areas and in deep waters as well as declining PFHxS in marine environments. Data for PFHxS in rivers and lakes indicate a possible increase in Western Europe and a stagnation in Northern America between 2000–2009 and 2010–2018.

3.13P.2

Screening for persistent and bioaccumulative chemicals in China's industrial chemical inventory

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The global chemical industry is very large with sales of €3,475x109 in 2017 and it has been growing rapidly at about 8% per year over the period 2007 to 2017. China’s share of global chemical manufacturing and sales has risen from 15% to 37% in 2017. The rapid development and large volume of China’s chemical industry has led to calls for effective management of the chemicals produced and used. China’s Ministry of Environmental Protection has identified a lack of data related to environmental and health issues related to chemical substances. Determining which chemical substances pose the highest risks is challenging given limited data on uses, emissions, physicochemical properties and toxicity of most industrial chemicals in commerce. Over the past 12 years *in silico* approaches have been applied to large lists of substances in the chemical inventories of Europe and North America with the objective of identifying chemicals that constitute environmental and/or human exposure concerns. Our goal in this study, was to identify chemicals with POP-like environmental properties including persistence, bioaccumulation, and long range transport potential that are unique to China. We compared chemicals listed on the Chemical Inventory of Existing Chemical Substances of China (IECSC) with the North American and European inventories, compiled chemical structures, and predicted physicochemical properties. We calculated two indicators of environmental hazard, overall persistence (Pov) and transfer efficiency (TE) using the OECD tool and bioaccumulation factors using the US EPA EpiSuite program. Of the 37080 substances with CASRNs in the IECSC, 18748 (51%) overlap with the both European and North American chemical inventories; 6916 (19%) are unique in the IECSC. A large number of organofluorines (929) were among the unique chemicals and, of those, 43 had 4 or more fluorines. Besides organofluorines, substances classified as organosilicons, organophosphates and reaction products were unique to the IECSC. Of all the 19092 chemicals in the IECSC, 779 (4%), 2993 (16%) and 6714 (35%) had Pov, logBAF and TE values (700-3000 d, 3.3-6.6, 0.15-52%, respectively) within the range of PCBs. This initial screening of substances in the IECSC has identified POP-like chemicals that are unique to the Chinese inventory. The results of *in silico* screening and prioritization can be used as a guide for further assessment on the global environmental fate processes and impact of these chemicals.

3.13P.3

Results of the German Environmental Survey for Children and Adolescents 2014-2017: PCB and OCP in blood plasma

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Polychlorinated biphenyls (PCB) and organochlorine pesticides (OCP) are persistent organic pollutants and show slow degradation rates, long range transport and accumulation in the environment and in humans. These properties and their impact on health lead to restrictions according to the Stockholm convention. Due to the persistence of these substances, environmental and human biomonitoring is still necessary to investigate the effectiveness of measures taken. Therefore, these compounds have been included in the 5th German Environmental Survey (GerES V), which investigates the current internal exposure of children and adolescents (age 3-17 years) for a population-representative sub-sample of 1,135 participants. Blood plasma samples were collected from 2014-2017 and analyzed for seven PCB (PCB 28, PCB 52, PCB 101, PCB 118 PCB 138, PCB 153 and PCB 180) and OCP (hexachlorobenzene, three hexachlorocyclohexane isomers, 4,4'-DDT, 4,4'-DDD and 4,4'-DDE). Concentrations were determined after protein precipitation with gas chromatography coupled with a mass spectrometer using internal calibration with ^{13}C-labelled standards. To gain insight into possible exposure factors such as food consumption, exposure-relevant behaviors and aspects of the residential environment were collected via computer assisted personal interviews and self-administered questionnaires. Highest geometric mean plasma concentrations were measured for 4,4'-DDE (0.158 µg/L), followed by PCB 138 (0.049 µg/L), PCB 153 (0.066 µg/L) and PCB 180 (0.032 µg/L). Plasma concentrations for PCB were significantly higher in former West Germany, while for DDE the plasma concentrations are higher for children living in former East Germany. These differences reflect the different application patterns in both parts of Germany before reunification. Concentrations also differed statistically significantly by age group, sex, status of breastfeeding, and fish consumption. Comparison with the results of GerES IV (2003-2006) show that differences between former East and West Germany are decreasing and confirm the globally decreasing trend in blood plasma concentrations also observed in other countries.

3.13P.4

Results of the German Environmental Survey for Children and Adolescents (GerES 2014-2017): Per- and polyfluoroalkyl substances in blood plasma

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Per- and polyfluoroalkyl substances (PFAS) are extremely persistent chemicals and have been detected in humans and in all environmental compartments in the last decade. They have an impact on health caused by endocrine disruption, carcinogenicity, toxicity to reproduction, effects on the immune system, and on lipid metabolism. In the EU, several PFAS are classified as carcinogenic and/or toxic for reproduction, and identified as substances of very high concern according to Art 57 REACH. Due to these properties and effects, PFAS were included for the first time in the German Environmental Survey (GerES) aiming to investigate the internal exposure of children and adolescence (age 3 to 17 years) in Germany in a population representative large-scale sample. From 2014 to 2017 1,109 blood samples were collected and analyzed for 12 PFAS including perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonic acid (PFHxS). Concentrations were determined after protein precipitation with liquid chromatography coupled with a mass spectrometer using internal calibration with ^{13}C-labelled standards. To gain insight into possible exposure factors such as food consumption, exposure-relevant behaviors and aspects of the residential environment were collected via computer assisted personal interviews and self-administered questionnaires. Highest geometric mean concentrations were measured for PFOS (2.49 ng/ml), PFOA (1.12 ng/ml) and PFHxS (0.36 ng/ml). These substances were detected in more than 70 % of the samples. The detection frequencies of perfluoronanoic acid (PFNA) and perfluoro-decanoic acid (PFDA) were significantly lower. Almost all concentrations of the other PFAS measured in GerES 2014-2017 were below LOQ (depending on individual compound 0.25 ng/ml up to 1.0 ng/ml). Stratification of results show age and sex related differences, but no clear pattern for associations with diet. For health assessment, measurements are compared with health based guidance values (HBM values), derived by the German Human Biomonitoring Commission considering the current knowledge on adverse health effects linked to these compounds.

New Methods in Environmental Analytical Chemistry: From Interrogation of Complex Matrices to Innovative Tools for Monitoring (P)

3.14P.1

The Total Oxidizable Precursor (TOP) Assay as a Tool to Detect Unknown Per- and Polyfluoroalkyl Substances in German Rivers

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The group of perfluoroalkyl and polyfluoroalkyl substances (PFAS) comprises several thousand known and unknown chemicals which are used in a variety of industrial applications and consumer products. In the past years, monitoring programs for PFAS were performed worldwide. However, these programs are usually limited to the analysis of perfluoroalkyl acids (PFAAs) and a relatively small spectrum of other known PFAS. In this study, we used the total oxidizable precursor (TOP) assay to quantify the presence of PFAA precursor substances in German Rivers by comparison of PFAA concentrations before and after oxidation. Solid particulate matter (SPM), bream liver, bream filet, and zebra mussel samples were obtained from the German environmental specimen bank. For target analysis, more than 30 PFAS (i.a. perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids, fluorotelomer sulfonic acids, diesters of fluorotelomer phosphates, sulfonamide-based PFAS, GenX, ADONA, F-53B) were extracted with an ion pair extraction method prior to analysis via UPLC coupled to a high-resolution mass spectrometer. The TOP assay approach was modified by fully oxidizing a small amount of sample with an increased amount of oxidation agent and subsequent SPE cleanup. Target analysis showed the highest PFAS concentrations in bream liver, followed by bream filet, SPM, and zebra mussel. The most abundant PFAS were perfluorooctane sulfonic acid (PFOS), long-chain PFCAs (C9 to C14), and perfluorohexane sulfonic acid (PFHxS). Notably, low or non-quantifiable concentrations were found for short and medium chain PFCAs and the analyzed PFAA precursor substances. In many samples, PFCA concentrations showed a substantial increase after the TOP assay was applied. The highest absolute increase in ∑PFCA concentrations was determined in bream liver from the Elbe River, i.e. from 12.3 µg/kg ww before to 526.5 µg/kg ww after oxidation. The spectrum of formed PFCAs ranged from C4 to C14 indicating a broad spectrum of present PFAA precursors, including precursors of PFOA which is regulated under REACH. The results of this study demonstrate the wide distribution of substantial amounts of PFAA precursors, which were not detected by target analysis and thus remain unknown. The presence of unknown PFAS in environmental samples emphasizes the need for new approaches in PFAS monitoring and also in regulation.

3.14P.2

High sensitive detection of hormones (E1, E2, EE2) according to the requirements of the EU Water Framework Directive

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The watch-list established by the European Union as part of the Water Framework Directive, includes the estrogen active substances estrone (E1), 17β-estradiol (E2) and 17α-ethynylestradiol (EE2) (EU 2018/840). For water monitoring, detection limits (LODs) of 400 pg/L for E1 as well as E2 and 35 pg/L for EE2 were set. A recent status report by the Joint Research Center (JRC) of the EU showed the progress of some laboratories, especially in the detection of EE2. So far, only very few laboratories have been able to achieve a LOD of 35 pg/L in surface water

(Loos et al. 2018). The challenge to develop a robust method, which allows quantification of estrogens in the ultra-trace range, lies in the targeted separation of interfering matrix components (Itzel et al. 2019). In this study, we developed a highly selective sample pre-treatment as well as detection method based on offline solid-phase extraction (SPE) and a silica clean up combined with a further online-SPE and detection by high-performance liquid chromatography (HPLC) coupled to a triple quadrupole mass spectrometer (MS/MS). Parallel to the online-SPE HPLC-MS/MS, a second method based on GC-MS/MS was established.

3.14P.3

From niche to necessity: GC

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Environmental forensics is concerned with the source, fate and transport of contaminants. Legislation such as the EU Environmental Liabilities Directive 2004/35/EC, which promotes the 'polluters pay' policy, has encouraged the development of accurate and robust scientific methods for the identification of contaminants. Analytical instrumentation is constantly evolving, thus new protocols to trace the origin of contamination must also be developed to utilise these technological advances. Two-dimensional gas chromatography (GC×GC) is one such example which has the potential to aid source identification. GC×GC offers enhanced separation capacity over conventional one-dimensional chromatography, allowing complex samples to be resolved and sample preparation to be minimised. While GC×GC has been heavily adopted in research and development labs, it has been less common in routine analyses, with many analysts believing it to be too complicated, fragile and/or expensive. Furthermore, the thermal modulation devices which have previously dominated GC×GC literature require liquid cryogen to modulate volatiles in the C_4-C_8 range – meaning additional running costs and the extra hassle associated with health and safety management of the laboratory. Here we explore the use of flow modulation, as a simple, yet effective, method of gaining all the benefits of GC×GC without incurring any of the historical cost or hassle. Flow modulation does not exhibit the same volatility restrictions as thermal devices, meaning that very volatile to semi-volatile organic compounds (VVOCs - SVOCs) can be efficiently modulated– expanding the application range that can be tackled by GC×GC. Here, we use reverse fill/flush flow modulation for robust, repeatable and consumable-free GC×GC - making it well-suited to routine environmental investigations. In this poster, we will demonstrate the enhanced separation of GC×GC across a broad range of real-world applications, including analysis of chlorinated paraffins, emerging contaminants in river water and total petroleum hydrocarbons in soil.

3.14P.4

Development of a Solid Phase Microextraction Method for Fast Analysis of Cyclic Volatile Methylsiloxanes in Water

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Cyclic volatile methylsiloxanes (cVMS) are organic compounds with alternating silicon−oxygen [Si-O] atomicunits backbones, and with methyl groups on each silicon atom. As a result of their widespread production and comprehensive used, cVMS has been found in various environmental matrices including water, atmosphere, sediment, as well as biota. In some countries, these compounds have been classified as persistent (P), bioaccumulative (B) and inherently toxic to the environment (iT_E) and categorized as priority chemicals for environmental risk assessment. The European Union implemented a ban on the use of D4 and D5 in wash off cosmetic products in 2019. Because of its ubiquitous nature that could introduce high background contamination, there are challenges in the analysis of cVMS. The current study introduces a sample preparation method based on solid phase microextraction (SPME) for monitoring the cVMS in waters. Efforts were made to eliminate the background contamination during sample preparation and instrument analysis. A laboratory prepared MIL-101 coating was prepared using polysulfoneas to avoid the contamination from commercial fibers that contained polydimethylsiloxane. The sampling conditions of the MIL-101 fiber was optimized and the extraction performance was evaluated. The optimized sampling time and temperature were 60 min and 40 ºC, respectively. The method quantification limits of MIL-101 fiber for octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecylcyclohexasiloxane (D6) in water were 0.15 ng mL^{-1}, 0.14 ng mL^{-1}, and 0.27 ng mL^{-1}, respectively. The extraction efficiency of the proposed MIL-101 fiber was comparable to the commercial polydimethylsiloxane/divinylbenzene fiber. The developed method was applied to analyze the cVMS in wastewater treatment plant and the concentrations in the influent and in the aeration tank ranged from 0.73 to 3.3 ng mL^{-1}, 7.74 to 85.1 ng mL^{-1}, respectively. The MIL-101 fibers was also applied to study photo-degradation of the cVMS in water. Our preliminary results showed the cVMS can be degraded under the light and the D6 showed highest degradation rate compared to D4 and D5.

3.14P.5

MOBILITY OF MICRONIZED MATERIALS IN GROUNDWATER BY USING ON-LINE ABSORPTION SPECTROMETRY

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Engineered micro and nanoparticles are being used for in-situ remediation of groundwater. A key point for success of the application is the mobility and the radius of influence of the particles in the saturated zone. The present study shows the results of a setup focused on the micronized calcite and commercial iron(III) mobility at lab scale of hydroxide as a possible alternative for in-situ elimination of contaminants as metals or arsenic. The studied particles were micronized calcite (0.1-10 µm), slurry calcite (0.1-40 µm) and iron(III) hydroxide with two sizes (1-40 µm) (1-100 µm). The particle suspensions disperse the intensity of visible light and this attenuation is measured by using molecular absorption devices. Some coloured particles (e.g. iron hydroxide) could also absorb part of visible light. In both cases, there is a range of concentration (between 0 and 500 mg/l in the case of the studied solids) where the measured absorbance is linear with concentration. The setup consists in a glass column of 1 cm of diameter and 10 cm of length filled with silica sand (1 to 1.2 mm grain size) to mimic the porous media. The column output is connected to a Z-shape cell of 1 cm pathlength that allows to study the attenuation of visible light (400 to 800 nm). An optical fiber circuit allows the comparison of intensity before and after passing the suspension of particles through the column. An acquisition system (Ocean View^{TM}) brings a continuous signal of absorbance, that is proportional to de concentration of particles. Another effect is that with higher sizes of particles there is a decreasing of specific absorbance. First, the characterization of the column by using a tracer (nitrate solutions) and the monitoring in the UV range are performed. Results show no tracer retention in the column. Afterwards, calcite and iron hydroxide suspensions experiments by using different sizes and concentrations are performed. Finally, from the on-line experimental data obtained was used to model the interaction of particles with the sand by using the simulation software MNMs 2018. In conclusion, the increase in particle's size for the studied micronized solids shows straining mechanisms that could retain higher particles during injection phase. Blocking effects (saturation of particle retention in porous media) is another possible effect that is observed for the solids.

3.14P.6

Uncertainty analysis in synchrotron hard X-ray chemical imaging of nano CuO in digested sewage sludge

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Bulk X-ray absorption spectroscopy (XAS) has been used to determine the speciation of trace metals in environmental samples for many decades. Advanced light sources and beamlines that focus X-rays onto micrometre sized spots allow users to rapidly record multiple X-ray fluorescence (XRF) maps around the absorption edge of a target element. Crude XAS spectra can be extracted from these maps for every individual pixel. Using linear combination fitting (LCF) "chemical images" containing spatially resolved chemical information can be extracted. In contrast to bulk-XAS, the uncertainty in individual XAS spectra derived from multiple x-ray maps is considerably larger. Therefore, we propose a model, derived from first principles to quantify the uncertainty associated with the LCF based construction of chemical images. The model describes the measured X-ray absorption coefficient ($\mu_{measured}$) as samples from a normal distribution (N) around the "true" X-ray absorption (μ_{true}) with a standard deviation (σ) ($\mu_{measured} \sim N(\mu_{true}, \sigma)$). Bayesian inference is used to recover and determine the σ of synthetic and experimental datasets, respectively. Two benchmarking criteria (score and the Correct largest Spectral Component Identified (CSCI)) were introduced to assess the LCF quality. Synthetic datasets comparable to the measured data with known σ can be prepared and used for benchmarkin. We recorded chemical images (size: 500 x 500 μm^2, resolution: 3 x 3 μm^2) of digested sewage sludge spiked with nano CuO (SLG NP) or dissolved Cu (SLG AQ) around the Cu K-edge. The number of pixels included was a compromise between reducing the uncertainty and including as much pixels as possible. Both experimental images were associated with an uncertainty of σ = 0.18 indicating an average score of 1.51 and a CSCI of 68%. The images indicated that in most of the pixels Cu was associated with S. In SLG NP, round objects (d ≈ 40µm) of Cu(II) associated with O were observed. Depending on the local Cu concentration derived from XRF, these objects represented untransformed nano CuO resulting from either agglomeration or preservation due to locally limited sulphide availability. Comparable objects were not observed in SLG AQ.

3.14P.7

Hyphenated Laser ablation-single particle ICP-MS to quantify size and concentration of engineered nanomaterials in environmental solid samples without prior extraction

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Analysis of inorganic engineered nanomaterials (ENMs) in environmental samples has been revolutionized mostly because of fast development of single-particle ICP-MS (spICP-MS), but the experimental options to analyse them without sample pretreatment are much more limited. Laser ablation was therefore investigated as an alternative introduction system into single particle ICP-MS (LA-spICP-MS), to make use of the superior detection limits of spICP-MS but avoiding artefact-prone extraction methodologies. Soils, wastewater treatment sludge, fly ash, road dust and a food sample spiked with a variety of different ENMs were analysed using LA-sp(HR)ICP-MS and a calibration technique using dissolved elemental standards was applied. It was found that corresponding spherical sizes expected based on parallel measurements could be reproduced in most cases but for the smallest nanomaterials and mass recovery of nanomaterials was often higher than 80 %. Detection limits are, however, likely higher than for aquatic samples. Further development of this approach may present a more convenient method to analyse realistic cocnentrations in solid samples.

Non-target Analysis in Environmental Sciences: The State of the Art and Future Perspectives (P)

3.15P.1

Suspect and non target analysis of emerging polar xenobiotics in water: workflows relevance

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The analysis of the miriads of xenobiotics present in the environment stand for one of the more complex chalenges for scientists. Improvements of analytical instrumentation such as the development of different screening techniques in high resolution mass spectrometry points a promissing path for this purpose. The three main techniques include target analysis, supported by analytical standards confirmation but limited to few available compounds. Suspect and non target techniques are able to analyse a much broader range of compounds and degradation products, but the conditions in which they are applied may compromise higly the results reported. One factor that affects the number of identified compounds is the suspect lists used or the molecular formula restrictions included in order to reduce the number of annotated features. To enlighten this, 3 different suspect/non target workflows were applied to water from the Nerbioi-Ibaizabal (Biscay, Spain) sampled in May, July, September 2018. The analysis of the samples was performed on positive or negative mode in Full MS ddMS2 discovery acquisition mode in a Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific Q Exactive quadrupole-Orbitrap mass spectrometer equipped with a heated ESI source (Thermo-Fisher Scientific, CA, USA) and the datatreatment was performed using the Compound Discoverer 2.1. In two of the workflows two different suspect lists were used, one containing around 9000 xenobiotics included in the STOFIDENT list from the NORMAN network and the other including up to 40,000 suspects included in the same network. Finally, a typical non-target workflow was applied that limited the compounds annotated to those containing C, H, O, N, S, P, F or Cl. For xenobiotics annotated at a level 1 confidence, there was no difference among the three workflows studied and 8 compounds were identified. In the case of level 2a identification, a similar number of compounds were annotated (from 6 to 7). The major difference was in the number of compounds annotated in levels 2b, 3 and 4, where with the nontarget workflow were 36 compounds, while only 11 with the NORMAN and Stoffident suspect lists. This result could be predictable due to the absence of a suspect list in the nontarget approch. For the river water studied in the present work, there were no differences for the compounds annotated using either Stoffident and NORMAN.

3.15P.2

Detection and prioritization of unknown compounds in water extracts from drinking water production

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The goal of this study is to enhance the identification of unknown compounds in LC chromatograms of untreated raw water used for drinking water production by (1) preconcentration of samples and (2) prioritization of the unknown peaks based on their bioactivity. Preconcentration was done by solid phase extraction (SPE) of 3L large volume (LV) groundwater samples or by 6-week passive sampler (PS) deployment in the groundwater, using divinylbenzene as active phase. Bioactivity of the samples was tested as a decrease in bioluminescence in the *Allivibrio fischeri* bioassay, which was chosen as a broad-scale bioanalytical tool responding to many different types of pollutants. Samples were collected at three different drinking water production stations with low or high degree of anthropogenic influence. At two stations, different groundwater inlets were sampled. At one station, samples were taken at different stages in the drinking water production process. SPE and PS extracts were used for target analysis and non-target screening, and for testing in the bioassay before and after high-resolution fractionation. More target compounds were detected in the concentrated LV and PS extracts than in a 1 mL direct injection of the water. As expected least compounds and lowest bioassay responses were detected in the raw water from the station with least anthropogenic influence. PS extracts gave much higher bioassay responses than the LV extracts. Both chemical and bioassay analysis of samples collected during subsequent steps in the drinking water production process confirmed the efficient removal of (bioactive) contaminants. In addition to the 4 peaks detected in the bioassay chromatogram of the reference station, the more anthropogenically influenced stations showed additional peaks indicative for the presence of anthropogenic substances. Identification of suspect compounds responsible for these additional peaks will proceed according to a three-step procedure, i.e. based on (1) exact mass and isotopic pattern of compounds available in different suspect lists, (2) fragment ions assigned to a precursor available in spectral libraries, and (3) estimated elemental composition. Reference standards will be obtained for the suspect compounds to confirm their retention time and bioactivity. Results from the identification process are available by the end of May 2020, and will be presented at the SETAC Europe conference.

3.15P.3

Ion mobility separations in environmental analysis: the additional value to current high resolution mass spectrometry screening strategies

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The screening of a large number of known and unknown contaminants potentially present in the aquatic environment pose many analytical challenges. High resolution mass spectrometry (HRMS) is currently one of the most adopted instruments to monitor organic pollutants in environmental samples. In the last decade, huge steps forward have been made to develop and combine comprehensive target, suspect and non-target HRMS screening strategies. In this context, ion mobility separation (IMS) combined with HRMS instruments (IMS-HRMS) introduces a new dimension to the separation, which allows to further improve the identification process, making use of the drift time of an ion (i.e., the time an ion takes to travel through the mobility cell) in addition to the obtained retention time and accurate mass. The collision cross section (CCS) of an ion is a value unique to IMS, which can be derived from the drift time and provides information about the shape and size of an ionized molecule. The use of CCS values to identify compounds, alongside retention time and accurate mass, is of special interest to analytical chemist since this parameter is known to be unaffected by the matrix or chromatographic separation. The creation of empirical CCS databases is, however, pivotal for an enhanced and robust screening strategy. In this work, a CCS library developed for hundreds of pharmaceuticals, hormones, mycotoxins, pesticides, illicit drugs and new psychoactive substances, in both positive and negative ionization modes using electrospray ionization, will be presented for its application to monitor aquatic samples using IMS-HRMS instruments. Attention will also be paid to the much cleaner and higher-quality spectra obtained after drift time alignment of (de)protonated molecules and their fragment ions and the use of prediction tools to improve the identification capabilities in high-complex samples. This will be supported by some illustrative examples of contaminants found in environmental samples. Finally, the information and expertise gained during the study is used to propose the inclusion of CCS values/ion mobility as additional parameter in widely adopted confidence levels for the identification of compounds in comprehensive screening strategies. It is expected that ion mobility will be incorporated as an additional criterion for reliable identification in different areas of analytical research, in the near future.

3.15P.4

Prediction of the removal efficiency of organic contaminants in wastewater treatment plants by QSAR

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The removal efficiency (RE) of organic contaminants in wastewater treatment plants (WWTPs) is a major determinant of the environmental impact of these chemicals which are constantly discharged from WWTPs into surface waters. In a recent study by Li et al. non-target screening analysis was applied to quantify the removal efficiency (i.e. RE%) of over 300 polar contaminants, by analyzing influent and effluent samples from a Swedish WWTP with direct injection UHPLC-Orbitrap-MS/MS. The method generated reliable estimates of RE% for large numbers of contaminants with comparatively low effort and is foreseen to be particularly useful in applications where information on a large number of chemicals is needed. In this poster we focus on the development of quantitative structure–property relationships (QSARs) starting from RE% data measured and estimated by Li et al. to predict this parameter from the chemical structures. QSAR represents another possible solution to support hazard and risk assessment when experimental data are scarce or absent.Several QSAR models have been generated starting from 31 compounds for which reference standards and measured RE% values were available, and then expanding the dataset using all the remaining available RE% data which were calculated from the ratio of the peak areas in the influent and the effluent from the non-target analysis. For a correct parametrization of the linear regression models the available RE% data expressed as complementary values (100-RE%) were scaled in a 0-1 interval. Multiple modelling strategies were used to address the heterogeneity of the available dataset as a whole, or by studying smaller sets of chemicals grouped according to structural similarity. The QSARs presented here were realized by beta regression based on the theoretical molecular descriptors calculated by the freely available software PaDEL-Descriptor. The best models were selected by a genetic algorithm-based procedure and validated using in-house R scripts. The quality of the QSAR relationships identified in this study is promising and validates the data estimated using the non-target analysis mentioned above.

3.15P.5

Multi-target analysis and suspect screening of emerging polar xenobiotics in milk

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Multi-target analysis of polar xenobiotics in fatty matrices such as milk is a challenge due to the high matrix effect that the co-extracted lipids and proteins cause during the detection step. The evolution of high-resolution tandem mass spectrometry renders the challenge to apply the methods developed and validated for a few hundreds of analytes to thousands of contaminants. Within this context, the objective of the present work was to optimize and validate a method for the multi-residue analysis of up to 200 polar xenobiotics in milk that was further applied to a suspect screening of thousand of compounds. The compounds used for method optimization and validation were chosen in order to cover a wide range of polarities and different applications, including pharmaceuticals, pesticides or industrial chemicals, among others. During sample preparation, we tested the influence of different solvents and solvent mixtures for analyte extraction and protein precipitation (i.e., acetonitrile and methanol, the latter in combination with both trifluoroacetic acid and trichloroacetic acid). The protein precipitation was aided with a cooling step and specific cartridges were also tested for an improved removal of proteins and lipids. Besides, the addition of ethylenediaminotetraacetic acid (EDTA) was also added in other to improve the recovery of compounds such as fluoroquinolones that form chelates with metals, such as the abundant Ca^{2+} present in samples like milk. In order to eliminate the water added during EDTA treatment and to preconcentrate the compounds, we studied the conditions used in reverse phase solid phase extraction (SPE) to recover the maximum number of xenobiotics. The analysis of the extracts was performed using liquid chromatography coupled with a high resolution mass spectrometer on a Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific™ Q Exactive™ Focus quadrupole-Orbitrap mass spectrometer equipped with a heated ESI source (Thermo, CA, USA). Measurements were performed in the Full scan-data dependant MS2 (ddMS2) discovery mode both in the positive (pH=3) and negative (pH=7) modes. The validated method was further applied to the suspect screening of compounds from the NORMAN list (Network of reference laboratories, research centers and related organizations for monitoring of emerging environmental substances). ACKNOWLEDGEMETS: This work was financially supported by the Spanish Ministry of Science and Innovation through the CTM2017-84763-C3-1-R project.

3.15P.6

Non-target screening approach applied to groundwater samples can lead to a better characterization of the territory

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The mass production of chemicals inevitably affects the aquatic environment, including the groundwater (GW). GW protection acquires an additional value when used as drinking water resource, as a chemical contamination poses a risk for environment and human health. The preservation of these resources is a priority to the European member states, the national agencies and the water managers, nevertheless the good quality status is actually defined only by few chemicals parameters, indicated in the European Water Framework Directive and in the Directive 98/83/EC for drinking water. Determining the risks with such parameters and targeted analysis means to underestimate the wide variety of pollutants that threats the water quality. In this study, a non-target screening approach has been applied to GW samples collected in Lombardy region, located in the northern part of Italy. This area is one of the most impacted of the country, because densely inhabited and industrialized, but even characterized, in the southern flat part, by intensive agriculture. GW samples were analyzed by high-resolution mass spectrometry to investigate the presence of unknown compounds. The samples were directly injected in the instrument, avoiding a pre-treatment phase. The chromatographic conditions was set up with acetonitrile 0.1% formic acid and ammonium formate buffer and ESI+ ionization mode was used. A full scan acquisition, with m/z range between 75 and 950, was performed to extract an inclusion list of exact masses detected in the samples, after blank subtraction (ratio Sample/Blank ≥ 2) and a visually peak checked prioritization. All the workflow was carried out with Compound Discoverer 3.1 (Thermo Fisher). A secondary step of FS and data-dependent analysis was performed to obtain the fragmentation of the selected masses; the MS/MS information helped to identify the unknowns and to assign them a molecular name. Our final aim is to match the list of compounds related to the corresponding sampling site with the environmental stressors and pressure on the territory; finding a relationship between the pollution of GW and the anthropic pressures, like pesticides and an agriculturally rich area, can lead to a better understanding of the territory and to adopt preventive measures.

3.15P.7

Fractionation of wastewater effluent for the characterization of organic micropollutants by high-resolution mass spectrometry

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Non-target screening has gained interest in recent years for environmental monitoring purposes because it enables the analysis of a greater number of pollutants, including still unknown ones. In this study, we divided a wastewater effluent sample into eleven various fractions using SPE cartridges and macroporous resins. All fractions were analysed with three chromatographic columns (C18, hypercarb, HILIC). Each fraction exhibited specific peaks and differences were also observed between a single fraction analysed by different chromatographic techniques. The chlorination of the wastewater effluent sample showed an increase of the number of peaks for most fractions, especially most polar compounds (as analysed with an Hypercarb column). Another set of experiments is being conducted with another oxidation process (UV photolysis) to compare the impact of different oxidation techniques on the distribution of organic compounds in each fraction. Differences in terms of number and intensity of peaks in each fraction will be presented and compared between each oxidation processes. Overall results confirm that the use of multiple sample preparation techniques and analytical methods provides complementary information about the diversity of compounds (especially for polar ones) present in a sample.

3.15P.8

Suspect screening and non-target analysis in human biomonitoring: an overview of new challenges and perspectives

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Biomonitoring of environmental contaminants in human biological samples (HBS; e.g. urine, blood, breast milk) is vital for assessing exposure to chemicals with potential adverse health effects. Human biomonitoring is a fundamental tool for prioritising chemicals of known concern, detecting contaminants of emerging concern (CECs), identifying populations at risk and generating evidence to inform environmental policy. In recent years, many studies have focused on the development of more holistic analytical methods seeking to undertake ‘suspect’ (i.e. tentative identification without reference standards) and ‘non-target’ screening (i.e. detection of unknowns), alongside target analysis (confirmed identification and quantification using reference standards) of organic chemicals in complex matrices. The most commonly applied approach has been liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) operating in full-scan acquisition mode. Following successful application in routine metabolomics profiling, minimal and rapid sample preparation methods have been widely adopted for ‘suspect’ and ‘non-target’ human biomonitoring. However, little attention has been paid to the limitations that full-scan MS acquisition and minimal sample preparation procedures impose on measurement capabilities. Critical feasibility assessments of such approaches when applied

within the framework of human biomonitoring are lacking. As such, we objectively highlight the difficulties facing routine measurement of environmental contaminants in human biological samples and potential solutions.

Occurrence, Fate, Transport and Reactivity of Emerging Micropollutants in Aquatic Systems (P)

3.16P.1

Sources and occurrence of pharmaceuticals in Irish surface waters.

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At each stage of the pharmaceutical lifecycle, there is a significant risk of environmental exposure. For this reason, it is imperative to implement both source directed and end of pipe control measures to mitigate hazards to the environment and humans. The ever-increasing use 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. The stability and biological activity of these “micro-pollutants” can lead to chronic environmental exposure, with ensuing behavioural and health-related effects. 8 pharmaceuticals have been incorporated into the updated surface water “Watch List”, indicating that insufficient data exists to assess their potential impact. This project will assess the measured environmental concentrations of Watch List pharmaceuticals, in addition to pharmaceuticals commonly found in EU surface waters have a low removal efficiency in conventional activated sludge type wastewater treatment plants (trimethoprim, sulfamethoxazole, carbamazepine, gemfibrozil and diclofenac). Furthermore, performing a life cycle assessment will provide an holistic view of how the pharmaceuticals are manufactured, prescribed, used and disposed can be achieved. This paper will show the development of a comprehensive prioritisation framework and a risk-based assessment by calculating the risk quotient for each pharmaceutical chosen. Outcomes from this research will aim to improve education surrounding appropriate use, disposal and waste management of pharmaceutical products.

3.16P.2

A year-long study of pharmaceutical and personal care products in Irish urban and rural water samples using SPE-LC-MS/MS

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Contaminants of emerging concern (CECs) are increasingly being shown to occur in surface waters at ng/L to µg/L concentrations and their risks in the environment require further investigation. The consumption of such compounds is increasing and therefore reliable analytical methods for identification and their determination need to be constantly re-developed [1,2]. In this study 12 low level pharmaceuticals and personal care products (PCPs) were selected from literature review to be monitored and risk assessed in the environment. For this purpose, samples were collected monthly for a year (October 2018 to September 2019) in a rural and an urban influenced area in Ireland for surface waters and wastewater samples (both influent and effluent) from wastewater treatment plants (WWTPs). A comparison between both areas is presented with their respected concentrations to identify the contaminants that persist in the environment and which persisted following treatment. Sample concentrations were enriched using solid-phase extraction (SPE) prior to analysis using liquid-chromatography using a reversed-phase C_{18} analytical column, coupled to mass spectrometry (LC-MS/MS). The method was validated for 8 pharmaceutical compounds and 4 PCPs including: hormones (17β-estradiol, 17α-ethinylestradiol, estrone), antibiotics (erythromycin, clarithromycin, azithromycin, amoxicillin, ciprofloxacin), an anti-inflammatory (diclofenac), UV-filters (octinoxate, octocrylene, benzophenone-4) and an antibacterial (triclosan), where quantitative analysis was carried out for these target compounds. Validation parameters such as limits of detection (LODs) and limits of quantification (LOQs), method precision and accuracy, range, recovery and matrix effects were evaluated using a composite sample matrix to provide a robust analytical method for the three different matrices. Overall, the monitoring of CECs in water will inform the levels of these species in Irish water bodies and support the development and optimisation of strategies for the efficient removal of identified CECs and to minimise the potential risk in surface waters. [1] Kung, T. A., Lee S. H., Yang, T. C., Wang, W. H.; Survey of selected personal care products in surface water coral reefs in Kenting National Park, Taiwan (2018) Science of the Total Environment, 635, pp. 1302 – 1307 [2] Munro, K., Miller, T.H., Martins, C.P.B., Edge, A.M., Cowan, D.A., Barron, L.P.; Artificial neural network modelling of pharmaceutical residue retention times in wastewater extracts using gradient liquid chromatography-high resolution mass spectrometry data (2015) Journal of Chromatography A, 1396, pp. 34-44.

3.16P.3

Pharmaceuticals and personal care products - An anthropogenic fingerprint in the Pearl River Estuary and South China Sea

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In the Pearl River Estuary (PRE), a megacity is currently developed by merging the cities Macau-Guangzhou-Shenzhen-Hongkong into one large city. This merger influences the ecosystems of the Pearl River and the adjusting northern South China Sea (SCS). The aim of this study was to define which pharmaceuticals and personal care products in the PRE and SCS could be of major interest to analyze the anthropogenic fingerprint of the megacity in the aquatic environment. In the PRE, 4 human pharmaceuticals, 5 antibiotics (veterinary and human) and 5 UV-filters were detected. Only 4 of them were also detected in the near shore surface waters of the northern SCS. The human pharmaceuticals caffeine, carbamazepine, diclofenac and metoprolol can be used as indicators of anthropogenic input as they were detected along the river (frequency 50-100%). Their concentrations (e.g. caffeine 152 ng/L) decrease from the city center along the river as the salinity increases, displaying a dilution curve. Only caffeine and metoprolol could be detected in the SCS at low ng/L concentrations, which are similar to the detected concentrations at the PRE mouth. As the PRE is subjected to a large livestock and aquaculture industry, which use sulfonamide antibiotics (sulfamethoxazole, sulfamerazine, sulfamethazine, sulfamethoxypyridazine) and trimethoprim. They occur at lower concentration range than the human pharmaceuticals and display a slightly different distribution pattern. The concentrations increase slightly from the city center along the river and then decrease towards the South China Sea. The livestock industry is probable a source for the input for the antibiotics. None of the antibiotics were found in the coastal waters of the South China Sea. The UV-filter pattern differs to both the human and veterinary pharmaceutical patterns. The UV-filters (benzophenone-1, benzophenone-3, octocrylene (OC), phenylbenzimidazole sulfonic acid (PBSA)) and the metabolite (4-DHB) were also detected at low ng/L concentrations along the river. PBSA and OC occur at higher concentrations near the SCS than at the city center. Thus, they are not diluted from the city center towards the northern SCS. Both UV-filters were also detected along the coast line of the northern SCS at similar concentrations as at PRE mouth. Hence, they probably originate from diffused source or industrial waste water within the estuary mouth region.

3.16P.4

Mycotoxins as Micropollutants of Emerging Concern in Freshwater Systems.

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Natural toxins are commonly overlooked as a group of micropollutants. Those toxins originating from fungi, termed mycotoxins, are being detected in numerous freshwater samples. The levels of which are highly variable, with main sources indicated as wastewater treatment plants and agricultural run-off. The later varying in impact depending on levels and species of fungi present in crops combined with changes in weather conditions. We have considered the potential impact of two major mycotoxins, deoxynivalenol and zearalenone, on various freshwater invertebrates to consider the need for further investigation into the aquatic occurrence of mycotoxins. Organisms selected for the acute studies were: *Brachionus calyciflorus, Chironomus riparius, Daphnia magna, Hydra vulgaris, Lymnaea stagnalis, Thamnocephalus platyurus* and *Tetrahymena thermophila*. Results highlighted some invertebrates as more sensitive, based upon EC50 values generated, than the commonly studied zebrafish which is the main source of toxicity data for mycotoxins currently. Crustaceans, *Daphnia magna* and *Thamnocephalus platyurus*, were most sensitive to deoxynivalenol with survival EC50 values of 0.13 and 0.4 mg/L respectively. For zearalenone it was the embryos of *Lymnaea stagnalis* which were the most sensitive with a 7 d survival EC50 of 0.59 mg/L. The effects seen in acute studies here for freshwater invertebrates highlights the value of a multi species approach. Previously deoxynivalenol may have been considered of little concern based upon the absence of effects in zebrafish embryo exposures. The acute 5 d survival EC50 reported of 0.89 mg/L for zearalenone in zebrafish embryos shows a similar sensitivity to the *Lymnaea* embryos tested in this study. From here, it is important to develop a better understanding of the temporal and spatial variety of mycotoxin levels in the environment. Highly elevated peaks are often seen in environmental sampling data throughout the year, but the absence of frequent sampling means the length of exposure to potentially concerning levels is still unknown. The lack of thorough freshwater occurrence and toxicity data so far has caused an absence of adequate risk assessments for these micropollutants of emerging concern. However, the novel invertebrate data here along with recently published freshwater plant data will hopefully lead to a better understanding of their potential impact in freshwaters.

3.16P.5

Development and validation of a high-throughput method for biomonitoring exposure of invertebrates to contaminants in aquatic systems.

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Many contaminants have been detected in rivers across the UK, including a range of pharmaceuticals, some of which have been placed on an EU 'Watch List'. However, the risk associated with long-term exposure of aquatic biota to these compounds is not well understood. To improve understanding of the risk it is necessary to characterise the exposure of these organisms. However, determination of emerging contaminants from biota is analytically challenging and has significant costs in terms of time and other resources. The aim of this investigation was to develop and validate a simple high throughput targeted analytical method to rapidly determine different pharmaceutical classes in the freshwater invertebrate, *Gammarus pulex*. Sample preparation was developed using pulverised liquid extraction and 96-well plate micro-elution solid phase extraction (µSPE) for preconcentration and clean-up. Subsequent analysis was performed using liquid chromatography tandem mass spectrometry (LC-MS/MS) in under 12 min. Methods performed very well for 67 pharmaceuticals, pesticides and illicit drugs in *G. pulex* with linearity of $R^2 \geq 0.98$ for the majority of compounds, and lower limits of quantitation in the low to sub ng g^{-1} concentration level. Samples from the field were collected from the River Cray in London (UK) and analysed. The high-throughput method significantly reduced time and associated costs in comparison to standard analytical approaches and enabled of up to 96 injections in 24 hours (including time for extraction of ~90 samples). The new validated method was used to quantify contaminants in field-collected animals, including EU Watch List compounds, and showed widespread exposure of these organisms to different contaminant classes. Overall, the method provided rapid and reliable determination of several classes of contaminants in a freshwater invertebrate that could be extended to other species to enable faster and better characterisation of the exposome in aquatic fauna at reduced cost compared to standard analytical approaches.

3.16P.6

Competitive adsorption and desorption of three tetracycline antibiotics on bio-sorbent materials in binary systems

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Batch-type experiments were used to study competitive adsorption/desorption for the tetracycline antibiotics tetracycline (TC); oxytetracycline (OTC), and chlortetracycline (CTC), onto two forest by-products (oak ash and pine bark) and one from the food industry (mussel shell). Binary competitive systems were performed by setting the dose of one antibiotic t 200 µmol L-1, and varying the concentration of another one from 50 to 600 µmol L-1. For the concentration of 200 µmol L-1, the results were also compared with those obtained in parallel experiments using simple and ternary systems. The results indicated that pine bark can adsorb most of the antibiotics added, while desorption was less than 5% in most cases. Oak ash showed high adsorption for all three antibiotics in the simple system (100% CTC, 90% TC, and 80% OTC), but clearly decreased in the binary systems (up to values below 40%), especially for higher concentrations, although desorption was generally less than 5%. Mussel shell showed adsorption results lesser than 25% for OTC and CT in the simple system, while increased up to 65% in binary systems in which CTC was present at high concentrations, but desorption was generally very high. CTC was the antibiotic with the highest adsorption on all three by-products, and the one showing less decrease for its adsorption in the binary systems. Overall, the smallest differences among the various competitive systems were obtained when pine bark was used as an adsorbent, and especially for the CTC antibiotic. These results could be an aid to develop management practices based on the use of low-cost bio-sorbents that would decrease pollution risks due to tetracycline antibiotics spread in the environment.

3.16P.7

Evaluation of enrofloxacin adsorption/desorption in soils using a stirred flow chamber

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Enrofloxacin (ENR) is a fluoroquinolone antibiotic commonly used in veterinary medicine. After ENR administration to cattle, a significant proportion of this antibiotic is excreted via animals' dejections. Therefore, ENR may reach soils when amended with cattle manures or slurries. Once in the soil, this compound may undergo different processes, both physicochemical and/or biological. Among the most important is adsorption/desorption onto soil components, since it affects ERN availability, and has subsequent effects on soil organisms and/or absorption by plants. Also, the adsorption and desorption processes may highly influence ENR transport in soils and the subsequent potential contamination of water bodies. In this work we used stirred flow chamber experiments to study ENR adsorption/desorption processes in 6 soils devoted to agricultural use. The soils were sandy loam or sandy clay loam in texture, presented an organic carbon content ranging from 0.3 to 19.9%, a pH in water from 4.2 to 7.0, and an effective cation exchange capacity from 4.1 to 27.7 $cmol_c\ kg^{-1}$. The amount of Fe and Al oxides was also variable in the soils; Fe oxides associated to the organic matter ranged from 68 to 2910 mg kg^{-1}, inorganic amorphous Fe oxides from 0 to 1630 mg kg^{-1}, Al oxides associated to organic matter ranged from 427 to 3830 mg kg^{-1}, whereas inorganic amorphous Al oxides from 0 to 535 mg kg^{-1}. The ENR adsorbed at the end of the experiment ranged from 4.9 to 10.4 mmol kg^{-1} for the six studied soils. However, no significant correlation was found between ENR adsorption and soil properties, although a high negative correlation value was found between ENR adsorption and inorganic amorphous Fe oxides. The desorption of ENR taking place after the adsorption experiment was highly variable, ranging from 4 to 100% of the previously adsorbed ENR. ERN desorption was significantly and negatively correlated with the previously adsorbed ENR, but also with inorganic amorphous Fe oxides, suggesting that the presence of inorganic Fe oxides in the soil hindered ENR adsorption and favored its desorption from soils.

3.16P.8

Measuring the dissipation half-life of PPCPs in the Brisbane River estuary using a mass balance model and benchmarking

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Organic pollutants such as pharmaceuticals and personal care products (PPCPs) and the perfluoroalkyl substances (PFASs) are compounds of emerging concern. These compounds have an anthropogenic origin and are continuously discharged into the environment. Some of these compounds, such as PFASs are persistent and belong to the persistent organic pollutants (POPs). Their environmental persistence is one of the factors that establishes if they will spread and reach other environments. This in turn influences their risk for humans, flora and fauna, etc. The persistence of a compound can be represented by its half-life, which will change depending on environmental factors such as light intensity, humidity, temperature, etc. These factors can be fixed in a controlled environment in order to study the behaviour of the compounds, but real environments possess variable and unique characteristics that are hard to reproduce under controlled conditions. In the present study, surface water from the Brisbane River estuary (Queensland, Australia), was sampled between March and December 2017. The hypothesis was that the rainfall from a tropical storm in late March would flush PPCPs and PFASs present in the water out of the estuary, allowing to observe how the contaminants accumulate again in the estuary over time. The samples were extracted using solid phase extraction (SPE) and analysed via high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Concentration trends between March and December of a total of 15 PFASs and 41 PPCPs were investigated using a mass balance model and chemical benchmarking [1] to determine the dissipation half-lives of the compounds. Chemical benchmarking was previously been applied in lakes [1, 2], but this application to an estuary is novel. The method used in this study can effectively determine the persistence of organic contaminants and may be applied to other environments. **Acknowledgements** Rodrigo Álvarez-Ruiz acknowledges the Spanish Ministry of Economy, Industry and Competitiveness (MINECO) and the ERDF (European Regional Development Fund) for his FPI grant BES-2016-078612. 1. Zou, H., et al., *Using Chemical Benchmarking to Determine the Persistence of Chemicals in a Swedish Lake.* Environmental Science & Technology, 2015. **49**(3): p. 1646-1653. 2. Zou, H., M. MacLeod, and M.S. McLachlan, *Evaluation of the potential of benchmarking to facilitate the measurement of chemical persistence in lakes.* Chemosphere, 2014. **95**: p. 301-309.

3.16P.9

Polycyclic Aromatic Hydrocarbons (PAH) in alpine surface water of the European Alps

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Polycyclic Aromatic Hydrocarbons (PAH) are ubiquitous contaminants in the environment. They are mostly generated by incomplete combustion of organic material, such as wood, coal or oil. In natural areas PAH usually originate from anthropogenic atmospheric sources. From deposition studies it is well known that even at high altitudes PAH are deposited and stored in alpine soils with remarkable masses (e.g. Schiedek et al. 2019). But, yet little is known about the occurrence of PAH in alpine surface waters. The aim of this study was to investigate the PAH concentration in surface water in two alpine valleys with well known PAH deposition. In the valleys Sarntal and Martelltal (Südtirol/Aldo Adige, Italy) ca. 30 water samples were taken from creeks and lakes using a solid-phase extraction techniques with IRA amberlite resin (Grathwohl&Schiedek, 1997). With this technique up to 5 liters of water were extracted at each location directly in the field. Thus, the PAH were pre-concentrated on the resin and a sample alteration during transport and storage was minimized. The analyses of 20 PAH was performed with a GC-MS system after extraction with acetone and c-hexane. Results showed that the sum of 20 PAH usually ranged between ca. 12 – 70 ng/L in all samples (highest value 397 ng/L, valley Martelltal), which is in the same order like results from investigations in surface waters in China. In general, valley Martelltal showed higher water concentrations (median: 38 ng/l) compared to valley Sarntal (median:18 ng/l). This finding is in contrary to the deposition of PAH in the studied valleys, where in Martelltal PAH deposition was much lower compared with Sarntal. In general, the PAH concentrations were higher in the lakes at high altitudes compared to the creeks. Schiedek, T.; Nersissian, L.; Birk, S.; Herndl, M. (2019): Mass Balance of Atmospheric PAH at Alpine Lysimeter, SETAC Europe, 29th Annual Meeting, 26‒30 May 2019, Helsinki, Finland. Grathwohl, P.; Schiedek, T. (1997): Passive Samplers as a Long-Term Monitoring System for Hydrophobic Organic Contaminants.- in: Gottlieb, J. et al. (eds.), Field Screening Europe, S. 33-36, Kluwer Academic Publishers.

3.16P.10

Emerging and legacy contaminants across land-use gradients and the risk to aquatic ecosystems

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The objective of this study was to assess the occurrence, concentration, and distribution of emerging contaminants across a gradient of land-use intensity (undeveloped< agriculture< urban residential< urban industrial) in Victoria, Australia. The study concludes that urban land uses are important sources of emerging contaminants, including synthetic pyrethroids such as bifenthrin, per- and poly-fluoroalkyl substances (PFAS), polybrominated biphenyl ethers (PBDEs) and phthalates. Twenty-five sites were sampled for surface water and sediment (total = 50 samples). A total of 218 chemicals were analysed: pesticides (current use and legacy organochlorine pesticides), PFAS, polybrominated biphenyls (PBBs), PBDEs, short-chain chlorinated paraffins (SCCPs), and phthalates. The risk these chemicals posed to the aquatic environment was measured using hazard quotients (HQs) which were calculated by dividing the maximum measured environmental concentration by a predicted no-effect concentration for each chemical. A HQ>1 was considered to indicate a high risk of adverse effects from the given chemical. A total of 66 chemicals (28 PBB/PBDEs, 19 pesticides, 17 PFAS, 1 phthalate, and 1 SCCP) were identified at concentrations above their respective limit of reporting. Of these, 18 had a HQ>1, suggesting an increased risk of adverse effects in aquatic ecosystems at the most polluted sites. Most chemicals with a HQ>1 occurred in sediments and were restricted in spatial distribution to the high intensity agriculture or industrial sites in Melbourne, the largest population centre in this study. The exception to this was PFOS, which had a HQ>1 in water samples across all land uses (except the undeveloped sites), indicating the potential for increased exposure. The results of this study improve our understanding of the magnitude and spatial variation of emerging contaminants and will contribute to better assessments of exposure and risk to aquatic ecosystems.

3.16P.11

Estimation of Trophic Magnification Factor (TMF) in a real trophic web: a case study of Lake Mergozzo (Northern Italy)

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Trophic magnification factor (TMF) is one of the strategies available to evaluate the potential biomagnification of a chemical. This field-based metric considers the diet as the major route of exposure to a contaminant, whose bioaccumulation is directly related to the trophic positions of the organisms. For this reason, TMF is calculated using the slope of the linear regression between logarithmic concentrations of a chemical found in sampled organisms and their trophic positions. In this context, the degree of bioaccumulation of mercury, organochlorine compounds (OCs), such as PCBs and DDTs, and perfluoroalkyl substances (PFASs) was evaluated in the trophic web of a small and deep Italian lake. Necessary data were obtained by analysing zooplankton samples and fish of thirteen different species collected from Lake Mergozzo, located in the north-west of Italy. Recorded concentrations, expressed as a sum of congeners, ranged from 0.4 to 60.2 µg kg^{-1} ww for PFASs and from 15.9 to 14778 µg kg^{-1} lw for OCs while Hg values were between 20 and 501.1 µg kg^{-1} ww. Other than biota contamination levels, these results highlighted that the chemical status assessment of the investigated lake is highly variable and depends on the species and the organism feeding behaviour. For this reason, biota trophic levels were experimentally calculated by the analysis of stable isotopes ^{13}C and ^{15}N in order to derive trophic magnification factors. Data estimated in Lake Mergozzo showed clearly that biomagnification occurs for Hg and organochlorines, with TMFs always greater than 2. Interestingly, different situation occurred for PFASs and PFOS was an example. Indeed, its TMF was equal to 3 only if complete dataset was considered. On the contrary, when regression was performed by excluding zooplankton data, TMF for PFOS were not significant and lower than 1, suggesting that biomagnification in fish is not an effective process for PFASs in this environment. This should lead to new studies for a better understanding of PFASs accumulation in fish and underlines the importance to include the analysis of compounds such as Hg in the experimental design, which serve as benchmarks for assessing the bioaccumulation potential of other contaminants of concern.

3.16P.12

The influence of perfluoroalkyl acid (PFAA) concentrations on the enrichment of PFAAs in sea spray aerosols (SSA) in a laboratory study

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Perfluoroalkyl acids (PFAAs) are persistent organic pollutants that have been detected in the global oceans. Previous laboratory studies have demonstrated that PFAAs could be highly enriched in sea spray aerosols (SSA), suggesting SSA might be an important source of these substances to the atmosphere. In order to further the understanding of the enrichment process and reduce the uncertainty when evaluating the water-to-air flux of PFAAs, the current study used a laboratory sea spray chamber coupled with a 14-stage cascade impactor (0.015 – 9.91µm) to investigate the influence of PFAAs concentration in water on their enrichment in different sizes of SSA. The results showed that though the enrichment factors (EF) of PFAAs increased with decreasing particle size in general, the patterns were different between film droplets (< 1µm) and jet droplets (>1µm). No significant correlation was observed between the EFs and PFAA concentrations with the investigated range (10-1 ng L-1 ~ 102 ng L-1), implying little influence of the concentrations on the enrichment process. However, high levels of PFAAs might change the properties of the air-water interface and potentially affect SSA production and thus indirectly affect the water-to-air transfer of PFAAs.

3.16P.13

A Temporal Study of Pyrethroid Pesticides in Irish Surface Water Environments

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Pyrethroid pesticides are extensively used in the forestry and agricultural industries to control for insects both in Ireland and worldwide. It is estimated that one quarter of the world insecticide market consists of pyrethroid type pesticides (1). Due to their non-polar nature and high degree of lipophilicity, pyrethroids tend to sorb to solid particulates in waters. Following rainfall events, agricultural run-off can cause solid particulates to enter water bodies, making pyrethroid contamination a cause of concern in surface waters (2). It has been found that although the majority (≤90 %) of the pyrethroid concentration can be removed with secondary treatment, the approximate 10 % that remains in water can have a high enough concentration to be acutely toxic to certain sensitive species, making monitoring their concentrations in natural waters vital for preserving biodiversity (3). Previous monitoring studies in Ireland have shown occurrence of pyrethroids in surface waters, indicating further investigation is required (4). Gas Chromatography-Mass Spectrometry (GC-MS) with electron impact (EI) ionization has traditionally been the most popular technique for analysis of volatile non-polar compounds. However, hard ionization techniques such as EI can produce excessive fragmentation of certain analytes resulting in reduced sensitivity and selectivity (5). Pyrethroid pesticides are one such group of compounds that exhibit this excessive fragmentation (6), making reaching the Limits of Detection (LOD) required to comply with Environmental Quality Standards (EQS) difficult unless very large sample volumes are processed. Softer forms of ionization, such as Chemical Ionization (CI), yield improved sensitivity for this class of compounds (6). In this study, a GC-CI-MS method was investigated for the quantitative determination of pyrethroid pesticides in a variety of wastewater matrices. An urban and a rural catchment water body were sampled monthly from October 2018 to September 2019. Samples were concentrated using solid phase extraction (SPE) prior to analysis. Parameters including LODs, Limit of Quantitation (LOQs), matrix effects, range, recovery, precision and accuracy are presented. The results of this year-long study establish a better awareness of pyrethroid contamination within an Irish context and encourage further investigation into water management practices. W. Liu, J. J. Gan, S. Lee and I. Werner, J. Agric. Food Chem., 2004, 52, 6233–6238.J. Gan, S. Lee, W. Liu, D. Haver and J. Kabashima, Journal of Environment Quality, 2005, 34, 836.F. Regan, L. Jones, J. Ronan, D. Crowley, E. McGovern and B. McHugh, EPA Research Report 2012-W-MS-11, 2018, ISBN: 978-1-84095-767-9. D. P. Weston, H. L. Ramil and M. J. Lydy, Environ. Toxicol. Chem., 2013, 32, 2460–2468T. Portolés, J. Mol, J. Sancho and F. Hernández, Analytical Chemistry, 2012, 84, 9802-9810.C. Shen, X. Cao, W. Shen, Y. Jiang, Z. Zhao, B. Wu, K. Yu, H. Liu and H. Lian, Talanta, 2011, 84, 141-14

3.16P.14

Occurrence of antifouling biocides in areas under influence of different naval activities

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Tributyltin (TBT) was used as active ingredient in antifouling paints for more than four decades and, due to its intensive use and high toxicity, was banned by the International Maritime Organization on January 1, 2008. As a consequence, organic or organometallic (booster) biocides of different chemical classes are concurrently used in these commercial products in order to improve the biocidal action of copper and zinc metals. However, the release of these compounds to aquatic environments cause toxicity to non-target organisms. In the present study, levels of TBT and its metabolites (MBT – monobutyltin and DBT – dibutyltin), and booster biocides (Diuron, Irgarol, Chlorothalonil, Dichlofluanid and DCOIT) were assessed in 30 sediments and antifouling paint particles (APPs) of Santos-São Vicente Estuarine System (Southeastern Brazil), covering areas under the influence of the largest Latin American port, marinas, boat traffic and ship/boat maintenance facilities. Results from sediments showed a contamination directly related to the profile of local maritime activities. In general, the highest concentrations of BTs (ΣBTs >700 ng Sn g^{-1}) were found along areas under the influence of small fishing boats and boat yards, adjacent to the main navigation channel. The relatively high levels of TBT (>140 ng Sn g^{-1}) suggest that a combination of fresh and old (chronic) inputs were taking place in at least 11 sites located in those areas. On the other hand, marinas of recreational boats (expensive boats) showed higher levels of booster biocides, including DCOIT (3.2 to 75 ng g^{-1}) and diuron (< 1 to 9.9 ng g^{-1}), while the main navigation channel (under the influence of the main port) showed higher levels of DCOIT (< 0.2 to 64 ng g^{-1}) and chlorothalonil (< 0.1 to 9.2 ng g^{-1}). The occurrence of APPs in sediments and the correspondent levels of BTs and booster biocides, which confirmed APPs as a relevant secondary source of contamination from antifouling compounds, seemed to be related to the type of boat/ship maintenance activities and to the paint removal method used. These results highlighted the urgent need for more attention to the inputs related to maritime activities areas.

3.16P.15

Occurrence of organic antifouling biocides in Danish marinas

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Antifouling paints containing biocides are often applied on underwater structures, including boat hulls, to prevent the growth of (micro) organisms. There is a high risk of leaching for these biocides, since the paints are in direct contact with surface waters. Currently, there are seven organic biocides that are registered under the biocidal product regulation (BPR, Regulation (EU) No 528/2012) and thus, may be found in the environment. From these seven, there are three that were previously found to hydrolyze rapidly. In a comprehensive monitoring study, the occurrence of these seven organic antifouling biocides and the three hydrolysis products in Danish marinas was investigated.For this study, we collected water and sediment from 13 different marinas across Denmark in order to assess which biocides are found at what concentrations in the environment. Two additional studies were performed in two of the marinas (Roskilde and Brøndby). In these two marinas, samples were taken on a monthly basis, over the course of a year, in order to track seasonal changes in antifouling biocide concentrations. Moreover, a transect study out to open sea was performed in order to identify whether there is a dilution effect from the source. Water samples were extracted using solid phase extraction and sediments were extracted using accelerated solvent extraction. Hereafter, analysis was performed by HPLC-MS/MS. Detection limits down to the ng/L range were obtained using these methods. The results show that there are two antifouling biocides that are commonly found across the selected marinas in Denmark. These two biocides are Irgarol (Cybutryne) and *N,N*-dimethyl-*N'*-phenylsulfamide (DMSA). Irgarol has lost approval status in 2016 and has not been used in paints since. DMSA is the hydrolysis product of Dichlofluanid, which is currently approved for use in antifouling products. None of the biocides were found in concentrations exceeding 25 ng/L. In the seasonal study these two biocides were found before the onset of the sailing season, and gradually increased in concentration for the duration of the sailing season. The biocides were found to rapidly dilute with distance; to background concentrations after 200 meters away from the source. Finally, a market survey was conducted to identify the use of antifouling biocides in paints. The findings in the market survey correlate to the results of the monitoring studies.

3.16P.16

Determination Of Marine Toxins In Ebro Delta By HPLC-HRMS

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Frequency of harmful algal blooms (HABs) has increased worldwide promoted by the eutrophication of waters and the global warming situation. These phenomena cause potential risks for the ecosystems as well as for human health. During HABs, marine toxins (MTs) can be produced by determined phytoplankton species and can bioaccumulate along the food chain, producing intoxication when are consumed via seafood [1]. Since toxin-producer species are ubiquitous, coastal areas are sensitive to suffer toxic events, and especially intensive harvesting areas of seafood. In Ebro delta is placed the most important shellfish harvesting area in the region of Catalonia. The presence of MTs had been previously reported there, with the consequent enclosure of the facilities [2]. These economic losses, joint with the risk of intoxication, have encouraged the necessity of an exhaustive monitoring of MTs by selective and sensitive methods. In this work is presented the determination and quantification of MTs in Ebro delta waters during different seasons. The analysis was performed by high performance liquid chromatography (HPLC) coupled to high resolution mass spectrometry (HRMS) by electrospray ionisation (ESI). The acquisition mode employed was data dependent scan in the range of 120-1700 m/z. Full scan was recorded at 70000 FWHM units and MS/MS spectra of each toxin at 35000 FWHM of resolution. From all analysed samples, the 97% showed the presence of MTs. These toxins were OA, DTX-1, PTX-2 and DA, at concentration levels of ng/L. DA and OA were both present in the 87% of the samples, being DA at the highest concentrations. Contamination of MTs was present during all sampling campaigns, being higher during the summer months. This fact proves the constant presence of MTs even that al trace level concentrations and emphasize the need of monitoring the marine ecosystems by sensitive and reliable methods. **References** [1] Luckas B., Dahlmann, J., Erler K., Gerdts G., Wasmund N., Hummert C., Hansen PD. 2005. Overview of key phytoplankton toxins and their recent occurrence in the North and Baltic Seas. Environ toxicol 20(1): 1-17 [2] Busch JA., Andree K., Diogene J., Fernandez-Teixidor M., Toebe K., Uwe J., Krock B., Tillmann U., Cembella AD. 2016. Toxigenic algae and associated phycotoxins in two coastal embayments in the Ebro Delta (NW Mediterranean). Harmful Algae 55: 191-201. *Acknowledgement* - The authors thank the project PLAS-MED (CTM2017-89701C3-1-R).

3.16P.17

Technology-critical element in marine organisms - analytical challenges, toxicology, and environmental aspects

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With the ongoing changes in resource use and technological progression, Technology-Critical Elements (TCEs) undergo major disturbance of their geochemical cycles. These trace elements are naturally scarce at the Earth surface but have a great economical interest since they offer peculiar characteristics applied to modern technologies. Platinum Group Elements (PGE), and particularly platinum (Pt) are part of this group. Coastal environments, as final recipients of land-based human activities, show increasing Pt concentrations over the last decades due to various applications (car catalytic converters, anticancer drugs, industries). Despite growing concern on Pt occurrence, reactivity and fate, little is known on its interaction with marine organisms. The lack of information reflects the analytical challenges in Pt quantification in marine systems and biota, due to relatively low environmental concentrations, complex biological matrices and the absence of certified reference material for quality control. The present work addresses Pt concentrations in marine biota in terms of: (i) analytical methods, through the successful application of a pre-concentration step, an inter-comparison using two independent methods, and resolving potential signal interference; (ii) toxicological effects, through an exposure experiment on oysters using isotopically labeled Pt (194Pt) spiked at various concentrations; and (iii) environmental aspects, through field sampling in contrasting coastal systems. Oysters exposed to relatively high Pt concentrations (10,000 ng.L-1) display adverse physiological effects at cellular, tissue, and organism levels. Under environmentally-relevant levels (50 and 100 ng.L-1), Pt accumulation in oysters rapidly reached steady state, supporting their potential to serve as environmental biomonitors of Pt contamination. The first data on Pt in marine phytoplankton, i.e., the first level in the marine trophic chain, suggest relatively high bioconcentration factors (BCF; 103-104) in both phytoplankton and marine bivalves, whereas no biomagnification occurred. Therefore, monitoring Pt in marine biota, integrating and amplifying the environmental signal, may provide unique information on spatial (pollution source assessment) and temporal (diel cycles, historical pollution) trends of Pt distribution in coastal systems. Especially, oysters and other sedentary marine bivalves are promising sentinel species for TCE contamination in seawater.

3.16P.18

Anthropogenic anomalies of Gadolinium in waste-water influenced creeks in the Rhine-Main area, Germany

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Rare earth elements (REE) represent the 14 lanthanide elements (atomic numbers from 57 (La) to 71 (Lu)). There is a growing economic concern in rare earth elements since they are of great relevance in high tech products and applications, such as superconductors, super magnets, catalysts, medical treatment etc. This is inevitably associated with an increased release of anthropogenic REE into the environment. Since the mid-1990s anomalously high concentrations of the REE Gadolinium (Gd) were detected worldwide in rivers and streams within densely populated catchments in countries with highly developed healthcare systems (Bau et al., 2006). Magnetic resonance imaging (MRI) diagnostics are considered to be the major source of anthropogenic Gd in aquatic systems (Bau & Dulski, 1996), because Gd is not restrained in waste water treatment plants (WWTP). Shale normalized (SN) REE distribution patterns of pristine surface waters often show smooth REE pattern with slightly higher relative concentrations of heavy REE compared to light REE (Ebrahimi, 2019). An anomalous concentration of a single REE can therefore be easily recognized on a Shale-Normalized REE pattern. In this study we investigated the REE-pattern of water samples from several small creeks (receiving treated wastewater from several WWTPs) in the Rhine-Main-Region, one of the most densely populated areas in Germany. 14 REE were analyzed directly, without pre-concentration by ICP-MS (Plasma Quant MS Elite-Analytik Jena). Results were normalized against Post Archean Australian Shale (PAAS) and the Gd excess was determined by (Gd/Gd*) = GdSN / (0.33 SmSN + 0.67TbSN). Almost all samples showed anthropogenic Gd. The anthropogenic Gd loads of the samples were normalized to the discharges of the creeks and varied between 168 and 982 µg/s (highly contaminated creek) and 8 and 574 µg/s at lower contaminated tributary creeks. The most significant Gd-sources in the study area are probably the WWTP of the city Darmstadt and of a big company. The anthropogenic Gd-load at one creek close to the mouth of the Rhine river was calculated to 22 kg per year which equals to MRI checks of 4.7% of the residents in the catchment. References: Bau, M., & Dulski, P. (1996). Anthropogenic origin of positive gadolinium anomalies in river waters. Earth and Planetary Science Letters, 143(1-4), 245-255. Bau, M., Knappe, A., & Dulski, P. (2006). Anthropogenic gadolinium as a micropollutant in river waters in Pennsylvania and in Lake Erie, northeastern United States. Chemie der erde-Geochemistry, 66(2), 143-152. Ebrahimi, P., & Barbieri, M. (2019). Gadolinium as an Emerging Microcontaminant in Water Resources: Threats and Opportunities. Geosciences, 9(2), 93.

3.16P.19

Assessment pollution for heavy metals in sediments of irrigation canals of Ramada, Cundinamarca, Colombia.

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Western savannah of Cundinamarca has been one of the areas production vegetables of Colombian Capital. However, during last decades near to these areas there has been a growth in industrial activities and urban area expansion, and is known of discharges on bodies water. These activities have been the cause of environmental degradation of soils and water streams mainly in Bogotá river. The irrigation of water used in this zone to agricultural production come from streams of the district irrigation the Ramada where the mainly supplier is the Bogotá river. Exist reports of pollution water Bogotá river like pharmaceutical compounds (Hernández, 2015) and heavy metals (INGEOMINAS, 2004) and heavy metals in sediments (Bustos, 1999). Based on this background, it was established as the objective of the work to establish whether there were differences in the presence and concentration of heavy metals in the sediments from channels affected by the different activities carried out in the surrounding areas (agricultural, urban and industrial-urban). The samples were taken of surface sediments from each site chosen, the samples were transported to the laboratory at 4°C and then were dried sieved. Sediment samples were analyzed using digestion acid through method USEPA 3050B. These extracts were used for analyzing heavy metals (Cd, Cr, Pb, Zn, Cu, Ni, Hg and As to help of atomic absorption spectrometry (UNICAM 929 AA) and hydride generator (UNICAM VP 90) respectively. The pollution in these sediments were evaluated using several indices, such as: pollution load index (PLI) (Tomilson et al. 1980), contamination factor (CF) (Hakanson 1980), geoaccumulation index (Igeo) (Müller, 1694) and enrichment factor (EF) (Abrahim and Parker, 2008). The analysis of metals in sediments showed the following trend Zn>Cu>Cr>Pb>Ni>As>Cd>Hg. The PLI values were above one in all areas studied, which indicated polluted conditions. The IGEO index and the factors FC and FE showed that for the agricultural zone a moderate contamination with Cd was observed, in the urban zone a moderate contamination with Zn and Cr, while in the industrial zone the contamination varied from severe to moderate with Cu, Cr and Pb. Hg and Ni were detected in all areas. The results obtained indicate a worrying panorama of contamination by heavy metals of anthropogenic origin, which can constitute a potential danger for both human health and the ecosystem.

3.16P.20

Screening Emerging Contaminants of Wastewater Origin in Boreal Sediments and Surface Waters

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Emerging contaminants are chemicals that have not previously been discovered in the environment and are typically not monitored and regulated under environmental laws. These chemicals have the potential to cause known or unknown environmental effects. Emerging contaminants can enter the aquatic environment through agricultural and urban runoff, as well as through municipal wastewater processing. Sources of these chemicals can be common household and hygiene products, medication for both humans and animals, industrial processes and pesticides from agriculture. The type of these chemicals can range from different pharmaceuticals and ingredients of personal care products (PCPs) to ingredients of plastic materials. In this study, chemicals from municipal wastewater treatment plant effluents in Finland were under investigation. Selected chemicals were screened from both sediment and water samples from different lakes, rivers and streams located in Western, Eastern and Southern Finland. The sampling sites were chosen according to their location in relation to active or decommissioned municipal wastewater treatment plants. The list of compounds to be screened comprised of 31 chemicals, mainly consisting of pharmaceuticals and personal care product ingredients, with the addition of for example Caffeine and Bisphenol A (BPA). The sediment samples underwent Liquid Phase Extraction (LPE) followed by Solid Phase Extraction (SPE). For water samples only SPE was performed after initial filtration. The samples were analyzed with High Performance Liquid Chromatography (HPLC) coupled with Tandem Mass Spectrometry (MS/MS). Out of the 31 screened chemicals 20 were detected, ranging from antibiotics, nonsteroidal anti-inflammatory drugs, cardiovascular disease medication, opiates and analgesics to PCP ingredients. The data gathered from this screening is valuable in developing current ecotoxicological testing to work better with new emerging contaminants and in enhancing the capabilities of tools used in risk assessment.

3.16P.21

Urban wastewater streams differ in CEC composition: results from a screening study in Nijmegen (NL)

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An increasing number of chemical compounds enter the market each day. Via different pathways, many chemicals of emerging concern (CECs) enter the environment and put pressure on the quality of our resources - especially on water. In an urban context, CECs refer to compounds like pharmaceuticals, fire retardants, or plasticizers and are mainly discharged via toilets, sinks and other outflows into the sewer system reaching ultimately the local wastewater treatment plant (WWTP). Depending on the WWTP characteristics, some CECs are removed almost completely during the treatment process, while other compounds are hardly reduced. Consequently, WWTPs can be seen as point sources releasing complex CEC mixtures into receiving surface waters. With this poster we would like to present our results [currently still pending] regarding a field study conducted in September/October 2019 in the city of Nijmegen (177 000 inhabitants) located in the Netherlands. The aim of this study is to better understand the links between urban CEC sources and their emission pathways and to lay the groundwork for a subsequent emission estimation study. Therefore, we screened four different urban wastewater types for their CEC composition. Passive samplers (Speedisks®) were deployed in a domestic sewer, an industrial sewer, a separated rainwater sewer and into the treated effluent of the respective WWTP. Each measuring location was screened for the semi-quantitative concentrations of 350+ compounds, including pharmaceuticals, opioids, illicit drugs, consumer products, pesticides as well as industrial chemicals. Sampling locations were selected carefully to contain as much as possible "pure" wastewater from either households, industry or surface run-off but avoiding combinations of them. Furthermore, attention was paid to avoid other disturbances such as effluents form hospitals, universities or office buildings.

Persistent (P) and PMT/vPvM Substances in the Environment: Improving Experimental and Weight of Evidence Assessment

Methods, Providing Solutions, and Informing Regulations (P)

3.17P.1
Temperature dependency of biodegradation kinetics in environmental surface waters across Europe
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Biodegradation kinetic data are keystone in persistency and risk assessment of chemicals. To account for temperature dependency, biodegradation tests are conducted at standard temperatures of either 12°C or 20°C (ECHA, 2017). Current practice in compensating for differences in test and environmental temperature is to extrapolate biodegradation rates from one to another temperature using the Arrhenius equation (ECHA, 2017). However, the applicability of the Arrhenius equation to biological systems is debated (Bagi, 2013; Matthies and Beulke, 2017). The aims of this study were 1) to separate the impact of inoculum origin temperature and test temperature on the biodegradation kinetics, 2) to investigate if the temperature effects are compound specific and 3) to assess the suitability of the Arrhenius equation for extrapolation between temperatures. Natural inoculum from the rivers Gudenaaen (Denmark) and Danube (Austria) was used untreated within 24 hours after sampling. Biodegradation kinetics of >40 petroleum hydrocarbons (HCs) were determined in composed mixtures at environmental realistic low concentrations. Tests were performed in airtight vials incubated at ambient and standard test temperatures: 2.7ºC (only Gudenaaen), 12ºC and 20ºC. After 0-28 days of incubation, triplicate test systems were analyzed by SPME-GC-MS, and the ratio between the signal responses of single substances in biotic test systems relative to the abiotic controls were used to determine the biodegradation kinetics (Birch et al 2018). Changes in microbial community induced by different test temperatures were characterized by DNA sequencing. The isolation and the quantification of the effect of test temperature on biodegradation kinetics was successful. Clear test temperature effects on biodegradation kinetics were observed for most HCs. The test temperature effect on biodegradation half-lives, lag-phases and half-times (lag-phase + half-life) will be presented and discussed. Arrhenius predictions will be assessed against the observed temperature effect. Finally, the sequence of biodegradation between locations and temperatures will be compared for the tested chemicals. The effect of the inoculum temperature on biodegradation kinetics remains confounded by differences of microbial communities between sites, and seems thus impossible to be fully isolated.

3.17P.2
Demonstration of Phenanthrene ready biodegradability and enhancements of OECD 301 testing using bioavailability improvement methods.
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Biodegradation is a natural process by which organic matter is converted to simpler compounds and ultimately mineralised to inorganic end-products or incorporated into biomass. It is an important environmental fate process that influences the exposure of chemicals both to humans and environmental receptors. Assessment of chemical biodegradability is usually performed experimentally with initial investigation involving a relatively stringent screening test of ready biodegradability corresponding to OECD 301 guidelines. Ready biodegradability tests are used as an indicator of rapid biodegradation of chemicals in all compartments (water, soil, sediment) under most environmental conditions. Difficulties are regularly encountered in estimating the biodegradation of poorly water-soluble chemicals which are often linked to the limited bioavailability of the test substance to microorganisms. This limitation can lead to lower than expected biodegradation measurements and possible false negatives in ready biodegradability tests, which can trigger further unnecessary, higher tier, testing. The biodegradability of phenanthrene, a poorly water-soluble, crystalline, polycyclic aromatic hydrocarbon (PAH), is impacted by low bioavailability in ready biodegradability tests. A study was performed to assess the effects of several bioavailability improvement methods (BIM) on phenanthrene biodegradation in standard OECD 301F tests. These methods, undertaken according to ISO 10634 guidelines and good laboratory practice (GLP), included (i) ultrasonic dispersion, (ii) adsorption onto silica gel, (iii) dispersion with silicone oil, and (iv) heating alongside direct addition. The threshold for ready biodegradability in the context of persistence assessment (≥60% biodegradation at day 28, not necessarily meeting the 10-day window) was achieved using all BIMs, highlighting the high intrinsic biodegradability of phenanthrene. In addition, silicone oil appeared to be the most promising BIM, with phenanthrene achieving ≥60% biodegradation within the 10-day window in this test. Based on these results, an approach for evaluating poorly water-soluble chemicals in ready biodegradation tests is described.

3.17P.3
Compartment specific half-life factors of hydrocarbons in water, soil and sediments.
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Environmental risk and hazard assessments for chemicals rely on compartment specific degradation half-lives (DT50) for water, soil and sediment. Although priority is given to data generated by laboratory simulation studies, experimental degradation data for all compartments is rarely available, and assessments rely on a combination of standard testing, non-standard information and QSAR information. This issue is exacerbated for complex, so-called UVCB substances, where a large number of potential constituents require assessment. An alternative approach is often taken, whereby compartment-specific biodegradation half-lives are derived through extrapolation from another compartment based on a constant factor. In this work, a database of 2014 experimental half-lives for hydrocarbons and heterocycles has been compiled across water, soil and sediment compartments. Analysis of this database provides new insights in to the carbon number dependency of DT50 data for various hydrocarbon classes in sediment, soil and water compartments, as well as the ratios between compartments. Overall analysis of bulk DT50 values for all data, as well as detailed analysis for the n-alkanes and aromatics classes, support a general 'rule of thumb' ratio of 3:2:1 for sediment:soil:water data, respectively. When considering individual carbon numbers, this ratio holds well up to C20 for both n-alkanes and aromatics. Further analysis of data for hydrocarbons present below saturation concentration vs hydrocarbons present at above saturation concentrations in water and soil suggests opposite effects on carbon number vs half-life for water and soil compartments. We hypothesise this is due to the contribution from free phase material having a contrasting influence on the bioavailability of hydrocarbons in each scenario. Comparison of empirical soil DT50s to those extrapolated from water data based on equilibrium partitioning demonstrates that this approach grossly overestimates soil half-lives. This work demonstrates the applicability of fixed half-life factors to extrapolate from water to soil and sediment for hydrocarbons and supports their use in environmental hazard and risk assessments.

3.17P.4
Biodegradation simulation testing of hydrocarbons in activated sludge systems
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Water treatment is an effective means of removing a wide range of chemicals from both industrial and municipal wastewaters. Hydrocarbons released from a variety of human activities will often undergo some form of water treatment. Biodegradation in wastewater treatment systems is typically assessed using the OECD 314 test guideline, with 314B (Biodegradation in activated sludge) intended to simulate the most common secondary wastewater treatment process. A series of five hydrocarbons have been tested under OECD 314B to assess their biodegradation in wastewater treatment systems. These tests employed radiolabelled test compounds in order to provide detailed information on parent degradation, mineralisation, metabolite formation and degradation, post-extraction residues radioactivity, and to provide a complete mass balance of the radioactivity in the system. Due to the difficult-to-test properties of the tested compounds, a number of modifications to the experimental design were implemented in order to prevent significant abiotic losses through volatilisation and sorption. Results demonstrated rapid degradation of all hydrocarbons tested, indicating effective removal during wastewater treatment and a high overall potential for biodegradation. Additionally, the modifications to the test apparatus were successful in minimising abiotic losses and obtaining complete mass balance. Formation of post-extraction residues was significant in all experiments, but this was mostly concurrent with mineralisation and not evident in the corresponding sterile test systems. This provides strong evidence that the post-extraction residues formed in these experiments were incorporated into microbial biomass. Further, the information on mass balance in these studies may provide insights as to the performance of these compounds in other biodegradation tests where results are determined based on non-specific parameters such as CO_2 formation.

3.17P.5
Optimization of closed setup for biodegradation testing of volatile chemicals in water-sediment systems (OECD 308)

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Standard OECD tests are used to generate biodegradation data for hazard and risk assessment of chemicals. OECD 308 is a higher tier test used to simulate biodegradation of chemicals in water-sediment systems. Normally, a flow through setup is used but a closed test setup is necessary for testing volatile chemicals. For hydrophobic chemicals, application of the test chemical is not possible using only aqueous solutions and the use of a co-solvent is essential. In our recent studies, closed setups were developed for OECD 308 tests for a range of volatile hydrocarbons, which were applied using acetone:water mixtures (≤ 0.25% acetone v/v). Such closed test setups were key for obtaining complete mass balances for these test chemicals. However, the test conditions within these setups were affected during the test by 1) biofilm formation due co-solvent application 2) significant depletion in the oxygen concentration in the water phase during the test. In this current study, we aim to further optimize this closed setup in line with OECD 308 guideline to 1) improve the aerobic conditions in the water phase, and 2) to check for a suitable co-solvent application technique. The use of larger water-sediment interfacial area and agitation of the water phase can improve aerobic conditions in these OECD 308 tests. As an improvement to the previous closed test setups (500mL Cylindrical flask ; Ø=5.5cm), two new test setups (both 500mL) with larger system geometries (Cylindrical; Ø=7.5cm and Conical; Ø=10cm) will be used. In contrast to the stagnant system in previous studies, an agitated water layer will be established using either overhead stirring or orbital shaking. At first, the effect of co-solvent on oxygen saturation in both headspace and water phase will be monitored during the test. Additionally, turbidity and DOC in the water phase will be monitored to check for sediment resuspension and solvent degradation, respectively. As these test setups need to be aerated regularly for maintainance of aerobic conditions, a comparison of manual vs. automatic aeration in this test setup will also be performed. Thus, based on the results an appropriate test setup and optimized aeration technique will be selected. Finally, the selected test setup will be used to conduct a preliminary biodegradation test using ^{14}C labelled volatile test chemical with different co-solvent application techniques to check the influence of applied solvent on the degradation of test chemicals.

3.17P.6
Scientific Concepts and Methods for Moving persistence (P) assessments into the 21st Century
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Persistence is a fundamental, key parameter in chemical assessments to determine environmental exposure, which is used to estimate the risk of harmful effects to biota. Persistence is defined as the ability of a chemical to remain in the environment in an unchanged form, being a function of both intrinsic chemical properties and environmental conditions. In regulatory persistence assessments, it is legally defined using threshold compartment-specific half-life criteria determined primarily through laboratory simulation tests (i.e. OECD 307, 308, 309), after undergoing initial screening using ready biodegradability tests (OECD 301, 310). Current persistence assessments evolved around tests that were originally developed >15 years ago based on the scientific evidence at the time. Both the persistence criteria[1] and tests in which they are judged were based on experience with a narrow range of chemical properties (e.g. soluble and non-volatile substances) delivered as single constituent chemicals. This does not adequately represent the wide range of manufactured chemical substances or the environmental conditions in which they are released and can lead to uncertainties in chemical half-life estimates. In 2019, an ECETOC task force (http://www.ecetoc.org/taskforce/moving-persistence-p-assessments-into-the-21st-century/) was set up to address the conceptual and methodological limitations facing the current persistence assessment process and the tests involved. The task force has identified several major challenges that fall under three main categories: Substrate (e.g. bioavailability issues represented by particles, poorly soluble substances, and non-extractable residues of single chemicals, unknown, variable composition or biological substances, and mixtures). Microbial (e.g. representation of microbial variability and adaptation within and between different compartments). Testing format and abiotic factors (e.g. high throughput data generation and the influence of abiotic processes and temperature effects that better represent environmental conditions). The task force will present an evaluation of recent scientific evidence that specifically addresses these challenges and further contemporary state-of-the art advances in chemical analysis, microbial ecology and modelling that may inform and transform future persistence assessments in the context of improved regulation. Matthies, M; *et al. Environ. Sci.: Processes Impacts*, **2016**, 18, 1114.

3.17P.7
Conceptual framework for moving persistence (P) assessments into the 21st Century
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A cornerstone of hazard-based environmental protection is to avoid accumulation of anthropogenic substances which may lead to unpredictable hazardous effects, potential irreversible contamination, and increased possibility for long range transport. Regulatory-mandated screening for Persistent, Bioaccumulative, and Toxic (PBT) substances relies on biodegradation testing to flag substances that may be persistent. For substances that do not pass a ready biodegradation test (OECD 301, 310), persistence is assessed by compartment-specific biodegradation simulation tests (OECD 307,308,309). The measured half-life of the substance is compared against regulatory criteria for persistence, e.g., >40 days in estuarine or freshwater, >120 days in soil. However, the use of half-lives based on biodegradation and the values for the half-life criteria are based on dated scientific concepts that reflect a narrow range of chemistry, primarily neutral, non-polar organics e.g. 'dirty dozen' POPs. Many substances, such as poorly soluble materials, UVCBs (substances of unknown or variable composition, complex reaction products, and biological origin), polymers, and particulates, fall ouside of the applicability domain of current persistence frameworks. An ECETOC task force was launched in mid-2019 partly to re-evaluate the manner in which persistence is defined and assessed (http://www.ecetoc.org/taskforce/moving-persistence-p-assessments-into-the-21st-century/). The task force is building upon the existing practices to develop a persistence assessment framework that uses a weight of evidence approach1 in a multimedia fate model, with data on biotic and abiotic loss mechanisms that is informed by the use of the substance, to estimate overall persistence in the environment (Pov)2. Three case studies will be presented, spanning the range of degradability, to demonstrate the functionality of the weight of evidence approach. These illustrate the importance of varied degradation processes in a multimedia context. Other lines of evidence, such as known metabolic pathways and field data, are also used to support the assessment. These three case studies will help to illustrate how a holistic and transparent approach for estimating fate of a substance can generate persistence assessments that are more relevant for environmental protection objectives. 1. Solomon, K., et al. Environ. Sci. Eur. 2013, 25 (1), 10. 2. Fenner, K., et al. Environ. Sci. Technol. 2005, 39 (7), 1932-1942.

3.17P.8
Seeking Alternatives to the Proposed 'M' criteria in the PMT Concept
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The current proposed PMT criteria consider that any substance fulfils the "mobile" criterion (M) if its log KOC value is ≤4.0. It is notable, also, that the commonly used Bioaccumulation (B) screening criteria is log KOW >4.5. Since log KOC co-varies with log KOW for most nonionic chemicals, this essentially means nearly every non-readily biodegradable chemical, natural or manufactured, is caught by the screening tools. Seeking alternatives to the proposed 'M' criteria is the priority to improve the realism and effectiveness of screening criteria. To get a better understanding of the influence of substance properties on occurrence in groundwater, we evaluated available European monitoring datasets to correlate

the absolute concentrations in the groundwater, and relative changes between river and groundwater against log KOC, and other physicochemical properties relative to common reporting thresholds. The chemicals were further benchmarked against available ready biodegradation test data. The analysis indicates the presence of some chemicals in ground water and surface water was not correlated with log Koc or with the biodegradation results. This indicates that log Koc-based M criterion, or even the P screening criteria, are not necessarily predictive of exposures through drinking water. This is likely due to the prevailing importance of exposure scenarios (amount, pathways) over the importance of the intrinsic properties of the substance as well as additional processes and mechanisms acting on the fate of chemical substances under natural environmental conditions.

3.17P.9

Consideration of Relevant Metabolites in the Context of the PMT Concept and Protection of Water Resources

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The PMT/vPvM-concept is proposed to be applied to REACh registered substances and its transformation product(s). For metabolites, the relevance threshold was set as 0.1% (w/w) of the parent (similar to PBT assessments). The applicability of this threshold was reviewed in light of the existing data requirements for plant protection products, the OECD 307, 308 and 309 TGs, and, taking into account relevant ECHA/Board of Appeal decisions. Furthermore, the logic behind increased mobility of substances/metabolites and the possibility of non-extractable residue (NER) formation were evaluated. Experience with plant protection products indicates that metabolites are considered as relevant if they are ≥10% (w/w) at any time during the study, or any that appeared twice at ≥ 5%, or, if the concentration is increasing towards the test end and the metabolite level is ≥5%. The OECD 307, 308 and 309 TGs recommend using environmentally relevant concentrations to derive more realistic biodegradation kinetic data. For industrial chemicals, predicted environmental concentrations (PECs) are typically in the range of 0.01 to 10 µg/L, which corresponds to a relevant metabolite concentration of 0.01 to 10 ng/L, which is consistent with the prioritisation exercise of the EU Water Framework Directive. An evaluation threshold of 0.1% is analytically very challenging and may not always be possible. The low concentrations result in relatively low risks from metabolites, especially since for industrial chemicals they have a relatively lower contribution to risk than the parent itself. Additionally, the measurement of non-extractable residues (NER) depends on the test conditions and the extraction methods, although the risks from these NERs remains unclear due to limitations in bioavailability. For example, formation of biomass through metabolism should not be part of a persistence assessment. However, the speciation of NER (e.g., bound vs biomass) is difficult to achieve experimentally and difficult to interpret mechanistically due to variable test methods that have undergone limited validation. Due to these uncertainties, the ECETOC recommendations on NER include estimates of bioavailability to quantify their contribution to risk. By their very nature, risks due to NER are lower than the extractable material. To conclude, setting a threshold of 0.1% (w/w) for relevant metabolites, is not applicable to most of the industrial chemicals on the EU market and the relevance of NERs is low.

3.17P.10

Review of predictive risk assessment approaches for the transport of chemicals to groundwater. A proposal for an improved tiered approach for use in Regulation

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The management of drinking water resources aims at providing water in sufficient quantity and quality and, as a consequence this objective is reflected in European regulations (EU Drinking and Ground Water directive). Acknowledging the different sources and release patterns of chemicals in the environment, regulations aiming at the proactive protection of humans and the environment from chemicals, such as the Plant Protection Product Regulation or the REACH Regulation, include groundwater in their assessment frameworks. Several risk assessment tools have been developed to model and predict the leaching of chemicals to groundwater for the purpose of risk assessment in order to proactively protect drinking water. Information typically needed to support the assessment include the substances properties, such as the physical-chemical and fate properties, the characterization of the environmental compartment(s) of release and the associated rates of emissions. Several risk assessment models were reviewed with a focus on routes of exposure addressed, key metrics supporting the exposure assessment and their level of conservatism. Independently of the model, the application rate to soil or dose applied to land is an important driver of the magnitude of exposure. The sensitivity analysis performed with fictional substance with a range of half-lives in soil and partitioning to organic carbon (Koc) provides contrasting results illustrating the important of the route of exposure to soil. For chemicals with direct application to soil, the potential to reach groundwater is greater for substances with a slow degradation in soil and low Koc. In contrast, chemicals released down the drain may have exposure in soil as consequence of sludge application to land, however a prerequisite of such exposure is adsorption to sewage sludge. Substance with slow degradation thus also have a greater potential for exposure in soil, but the magnitude of such exposure is greater for substance having a high binding affinity to sludge. A tiered risk assessment approach is proposed by the ECETOC task force on “Persistent chemicals and water resources protection” to support an improved screening assessment of potential groundwater contaminant, and as a risk-based alternative to PMT/vPvM criteria.

3.17P.12

Scientific and regulatory challenges to deal with "mobile" substances.

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Protection of drinking water remains a priority for industry. The recently proposed Persistence, Mobil, and Toxic (PMT) prioritization scheme is being debated as a possible tool to support the maintenance of high quality drinking water. Identification of Mobile and Persistent substances is not a straight forward exercise. Several parameters have to be considered to address the scientific and regulatory complexity. For example, monitoring data, use patterns, and appropriate physicochemical and degradation properties can influence the exposure profile of chemicals in drinking water. Implementation of incomplete prioritization schemes can result in mis-direction of resources with limited, and inefficient impacts on our goals to protect drinking water resources. The presentation will focus on some food for thought on scientific and regulatory challenges encountered to identify these chemicals as well as indication on challenges and potential way forward to address them in the regulatory framework.

3.17P.13

Necessity of Joint Consideration of Persistence and Mobility for Determining the Leaching Risk of Chemicals

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In the UBA proposal, P/vP and M/vM are assessed separately. Those chemicals which fulfil the UBA definition of both M/vM and P/vP, are taken into consideration for PMT/vPvM categorisation (with exceedance of the T threshold also being required for attainment of PMT status). This approach produces on the one hand many false positives, substances that are classified PMT/vPvM although they do not reach the groundwater used for drinking water in a considerable amount. On the other hand, it is not excluded that substances are classified false negative and are excluded from further assessments although they may pose a risk to reach groundwater. Despite the need of a combined consideration of soil degradation and sorption, the metrics proposed by UBA may be deemed adequate only for preliminary screening of a large dataset of chemicals, but they present scientific shortcomings that preclude them from being used for any definitive regulatory classification. The main limitations are: • The Koc concept used for the M classification is not valid for soils with low organic carbon content as well as for polar or ionised substances. • Non-consideration of non-linear of sorption. Concentration dependent sorption is mostly observed for chemicals resulting in non-linear isotherms showing increasing sorption with decreasing concentrations. • Non-consideration of slow kinetic sorption also called “aged sorption”. This process causing the well-known phenomenon of hysteretic sorption (desorption deviate from adsorption isotherms) is the expression of considerable increase of chemical sorption with time and must not be ignored. • Competitive sorption which is not considered in the Koc concept can lead to displacement of a sorbed species by another. • Immobilisation through formation of NER Due to the inadequacy seen with the proposed concepts to identify the leaching risk, alternative approaches are discussed: • Leaching indices combining the main chemical characteristics such as degradation and sorption. • Ranking approach as used by the CIS Working Group to setup the Groundwater Watch List. • Risk assessment approach based on modelling of the leaching potential using models that consider the essential processes and taking into account relevant pedo-climatic scenarios.

Potential Roles for Diffusive Gradient in Thin Film (DGT) Passive Samplers in Investigative and Regulatory Monitoring (P)

3.18P.1

High frequency measurement of metals: steps towards the acceptance of passive samplers for regulatory monitoring

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Diffusive Gradient in Thin Films (DGT), and passive samplers (PS), in general, are already widely used in investigative monitoring and there is an increasing interest in their use for the environmental assessment of water bodies, within

European policies requirements. In the regulatory context of the European Water Framework Directive (WFD) (2000/60/EC), the main barrier for the acceptance of PS is the lack of appropriate Environmental Quality Standards (EQSs). Regulatory EQSs for metals in water are defined in the dissolved fraction, preventing the use of DGT-labile concentrations for the establishment of the chemical status of water bodies. The specific objectives of this study are: to investigate the relationship between dissolved metal concentrations from spot sampling with DGT-labile metal concentrations, to evaluate the reliability of the techniques for the measurement of metal concentrations in estuaries and to provide recommendations for the use of DGTs to develop environmental guidelines. In November 2019, five samplings were carried out in the Oiartzun estuary (Basque Country, Bay of Biscay), an area with high presence of industrial and port activities. DGTs (triplicates) were deployed and retrieved after 12 hours. During that period, hourly spot water samples were taken, at the same depth than DGTs. Water samples were filtered for the posterior analysis of metals, dissolved organic carbon and the quantification of suspended particulate matter (SPM). Priority metals (Cd, Ni, Pb) and other specific metals (Al, Ag, Cu, Cr, Co, Fe, Mn, Zn) were analysed in the 2 defined fractions (dissolved and DGT fraction) by ICP-MS. Hydrographic variables were measured at each sampling time. The relationships between dissolved and labile metal concentrations and the environmental factors influencing such correlations were studied. The results show that dissolved Ni and Cd can appear mostly labile and available for uptake by DGTs, while most of the dissolved Cu, Pb and Zn can be forming organic complexes no detected by DGTs. Statistical analysis highlighted the relationships between metal concentrations in DGT and in spot water samples, together with the variability in relation to the hydrographic variables. *Acknowledgement* – Monitool project (https://www.monitoolproject.eu) (nº contract: EAPA_565/2016) is co-financed by the European Regional Development Fund through the Interreg Atlantic Area Programme and by the Basque Water Agency.

3.18P.2

Impact of temperature, seasonality and metal concentration on DGT membrane biofouling in marine envrionments

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Heavy metals as environmental contaminants are of particular concern due to their toxic effects when accumulated within organisms. Under Directive 2013/39/EU, cadmium, nickel, and lead were identified as priority metals of ecological concern in the field of water policy. At present, Environmental Quality Standards (EQS) for these metals only include biota sampling, and therefore the development of new in situ solution sampling methods are a priority. Passive sampling devices (PSDs) allow for the accumulation of an analyte over time, providing a time-weighted average of concentration of the analyte. Their low cost and ease of use offers benefits over traditional point grab sampling or biota sampling. For heavy metals in solution, the most prominent PSD is the Diffusive Gradient in Thin Film (DGT) device. DGT devices are composed of an ion-exchange resin, separated from solution by a diffusive ion-permeable gel layer. DGTs measure a labile metal fraction, often considered a bioavailable fraction. In this research, multiple factors were examined to determine their impact on the application of DGT passive sampling devices in a marine environment. Biofouling on DGT devices deployed in marine waters along the Atlantic coast was examined, studying speciation and extent of fouling, particularly in relation to temperature induced effects, and an examination of potential methods to mitigate fouling through the use of alternative membrane filters. Results will be presented here with explore multiple strategies for biofouling impacts to be minimised during DGT deployment.

3.18P.3

Unravelling dynamic characteristics of metal species in mixtures from Diffusive Gradients in Thin films (DGT) measurements

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Dynamic speciation is an important issue to understand and to prevent toxic effects in natural systems. When Diffusive Gradients in Thin films devices (DGT) are used to measure metal availability, all metal species can contribute to the accumulation depending on the respective diffusivities and labilities. Although many efforts have been devoted to the study of simple systems with only one ligand (single ligand system, SLS)[1], the influence of the system composition on the lability of the species is largely unknown. Thus, a series of experiments with DGT (Diffusion Gradients in Thin films) devices [2] were carried out to measure nickel (Ni) accumulations in laboratory solutions containing either Nitrilotriacetic acid (NTA), Ethylenediamine (EN) or mixtures of both ligands. It is shown that NiEN and NiNTA can become more labile and inert (respectively) in the mixture than in the corresponding SLS. As variations in the lability degrees of NiNTA and NiEN arising in the mixture tend to cancel out, the summation of partial fluxes calculated from lability degrees obtained in SLS yields a reasonable estimate of the DGT performance in the mixture [3]. These results pave the way to obtain dynamic characteristics of mixtures from DGT devices with different thickness of the resin or of the diffusive disc[4]. References: 1 Uribe, R.; Mongin, S.; Puy, J.; Cecilia, J.; Galceran, J.; Zhang, H.; Davison, W. Contribution of partially labile complexes to the DGT metal flux. Environ. Sci. Technol. 45 (2011) 5317-5322. 2 Interpreting the DGT measurement: speciation and dynamics. In Diffusive Gradients in Thin-Films for environmental measurements, Davison, W., Ed.; Cambridge University Press: Cambridge, 2016. 3. Altier, A.; Jimenez-Piedrahita, M.; Uribe, R.; Rey-Castro, C.; Cecilia, J.; Galceran, J.; Puy, J. Effects of a mixture of ligands on metal accumulation in diffusive gradients in thin films (DGT). Environ. Chem. 15 (2018) 183-193. 4. Baeyens, W.; Gao, Y.; Davison, W.; Galceran, J.; Leermakers, M.; Puy, J.; Superville, P.; Beguery, L In situ measurements of micronutrient dynamics in open seawater show that complex dissociation rates may limit diatom growth. Scientific Reports, 8 (2018) 16125 The authors gratefully acknowledge financial support from the Spanish Ministry MINECO (Project CTM2016-78798).

3.18P.4

A New Polar Organic Chemical Integrative Sampler (POCIS) for Linear Alkylbenzene Sulfonate (LAS)

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This study aimed to use the Polar Organic Chemical Integrative Sampler (POCIS) under non-steady-state conditions resulting from natural disasters and environmental accidents. In such conditions, it is expected that a chemical which is stored in large amounts will leak to the aquatic environment easily, and its concentration in the aquatic environment will increase. Therefore, the priority to be prepared should not be decided only for the chemical's toxicity but also the stored amounts.Linear Alkylbenzene Sulfonates (LAS) were widely used as a kind of an anionic surface-active agent in the world. Products including the LAS have been used as household detergents such as laundry powders, laundry liquids, and all-purpose cleaners. However, the LAS are extremely toxic to fishes and water fleas and has been detected in the aquatic environment. In addition, the amount of the LAS stored for industrial use is expected to be huge. For example, the largest stored amount of the LAS in an industry in Osaka, Japan is about 320 tons. Therefore, the LAS were selected as the target chemicals in this study. The peak concentration and a half-life of the LAS in a river contaminated by chemical leak from an industry were estimated by a model. The POCIS is one of the most widely used passive sampler for aquatic environment. Compared to grab sampling, passive sampling has an advantage to measure time weighted average of the target concentration. In short, the passive sampling can measure a short time peak which would be slipped over by the grab sampling.The POCIS which is a strong tool for monitoring hydrophilic organic matter; $0 \leqq \log P_{ow} \leqq 5$; has been applied for pesticides, pharmaceuticals, perfluorinated compounds, munitions, and drugs. Thus, the POCIS should be suitable for the monitoring of LAS which its $\log P_{ow}$ is 3.32 (calculated as $C_{11.6}$).We developed a POCIS optimized for the LAS via batch experiments and recovery experiments. Oasis WAX, a mix-mode, Weak Anion-eXchange, reversed-phase, water-wettable polymer was chosen as the best receiving phase for the new POCIS from eleven adsorbent candidates including polymer type adsorbents and active carbon type adsorbents.The developed POCIS was validated in a chamber which replicated the non-steady-state condition following to the model results. The results of the chamber experiment indicated that the POCIS was a good tool to be used in emergency.

3.18P.5

Experimental calibration of the copolymer ethylene vinyl acetate (EVA) passive sampler for emerging contaminants in freshwater.

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Monitoring and quantification of emerging contaminants in freshwater needs the development of new methodological approaches for a detection improvement and in a cost-effective way. Currently, there are various methods of monitoring the occurrence of pollutants such as grab, continuous, automatic and passive sampling. In recent years, the effectiveness of contaminant monitoring has been observed through the use of passive sampling. These devices allow combining the sampling, selectivity and pre-concentration of chemical substances in a single step, using a minimum quantity of solvents in the whole process of extraction and deployment in the field. In this work we present an experimental calibration of the copolymer ethylene vinyl acetate sampler (EVA) by using a calibration system designed in the laboratory. Here, 5 chemicals, with a wide range of Log K_{OW} (from 0 to 5) were considered for the experimental calibration (namely, caffeine, chloridazon, atrazine, ibuprofen and triclosan). The partitioning coefficient between EVA and water ($K_{eva\text{-}w}$) and the sampling rates (Rs) were determined under the experimental flow rate (range 0.05 to 0.25 m/s) and temperature conditions (range 8 to 14 °C). The chemical analyses were conducted both in water and the EVA sampler by HPLC-DAD. The results show that EVA sampler had a good selectivity and adsorption for all chemicals, including the less hydrophobic contaminants. A directly proportional relationship between the values of Log K_{ow} and Log K_{eva-w} were observed (r^2=0.71, p< 0.068). Due to thin-film of EVA (~10 µm) the equilibrium was reached for all tested chemicals in an average time from 1 to 2 days, after a total of 14 days exposed in freshwater. The sampling rates ranged from 0.053 (caffeine) to 2.17 (triclosan) L/d. These results make EVA samplers a promising and cost effective tool for monitoring contaminants in aquatic ecosystems. Supported by CONICYT/FONDECYT 1180063; CONICYT/FONDECYT/3180159 and CONICYT/FONDAP/15130015.

3.18P.6

Field testing of novel passive samplers to detect PFASs in aquatic environments

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Poly- and perfluoroalkyl substances (PFASs) are of growing concern worldwide, due to their ubiquitous presence, and adverse health effects in humans and the environment. Surface waters in the northeastern United States in particular have displayed elevated concentrations of PFASs. Here we utilize microporous polyethylene (PE) tube passive samplers to gain a better understanding of the sources and spread of these contaminants. Two sampling campaigns were conducted in the fall of 2017 and summer 2018, deploying a total of seventy-two PE tube passive samplers (containing Hydrophilic-Lipophilic-Balanced sorbent) across nine sites in Narragansett Bay (RI, USA) and two wastewater treatment plant effluents. 25 PFAS compounds were measured across all sites in the passive samplers, as well as analogous water and sediment samples. In the estuary, the PE tube samplers accumulated a sum PFASs of 2 to 15 ng $sampler^{-1}$, and in the waste water treatment plant effluent 60 to 74 ng $sampler^{-1}$. These results demonstrate that the passive sampler PFASs concentration are consistent with what is observed in the water grab samples. *In situ* sampling rates, which are essential when needed to calculate the contaminant concentrations in water, were characterized using a first order kinetic model, yielding sampling rates of 10-50 mL day^{-1}. By using passive samplers to analyze the spatial and temporal trends of these contaminants we can better assess their longevity in water and connected environmental compartments.

3.18P.7

Mixed polymer passive sampler development for large spectrum organic chemical sampling from different matrices in combination with passive dosing of complex mixtures

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Passive sampling is an increasingly applied method to detect organic chemicals in different environmental matrices. It is a cheap method with a wide range of possible applications, delivering time weighted averages of chemical concentrations. Passive dosing utilizes a polymer (e.g., silicone) loaded with test compounds or mixtures to control their exposure by continuous equilibrium partitioning to the test medium. In this way, losses resulting from, e.g., sorption, degradation, or evaporation are efficiently buffered. A restraint of many available samplers is the limited spectrum of collected chemicals. Commonly used samplers such as polar organic chemical integrative samplers (POCIS) on the basis of Oasis HLB sorbent, or silicone samplers often only cover either rather hydrophilic or hydrophobic compounds, respectively, and therefore are not sufficient for comprehensive screening on their own. We aim to develop passive samplers to cover a broader spectrum from hydrophobic to hydrophilic organic chemicals within a single system. During the first stage a polymer (e.g., silicone) will be mixed homogeneously with sorbent to widen the spectrum of compounds to be sampled. Once a well-suited sampler mix has been established, its sorption kinetics will be investigated. Additionally, different techniques will be tested to bring the samplers into different three-dimensional shapes that allow for highly specific areas of application (e.g., adaptations to different surroundings) and the regulation of sorption kinetics through the adjustment of the surface area to volume ratio. Passive dosing will be applied to test different fuel distillates with regard to their ability to promote the formation of biofilms in storage tanks and further, to reduce these biofilms without the use of biocides. Biofilm formation in fuel storage tanks is problematic both because it leads to clogging of drainpipes and degradation of fuel components. To understand the underlying processes of biofilm formation and to identify the major biofilm-promoting compounds of fuels, the developed mixed polymer samplers will be applied to the aqueous phase, providing freely dissolved concentrations and release kinetics from fuel distillates to assess the bioavailability for microorganisms. Chemical analysis by means of GC-MS and LC-MS/MS will be applied. We will present the current state of the project and the overview of the applied methods, dosing, sampling, as well as analytical systems.

Siloxanes and Related Chemicals: Environmental Monitoring and Behavior, Ecosystem Exposure and Energetic Efficiency (P)

3.19P.1

Analytical instrumentation as a source of contamination in the analysis of siloxanes

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Silicones have highly beneficial properties making their use in a multitude of analytical instrument applications attractive, especially gas chromatographs, from O-ring seals and septa to column films and packings. The use of silicones in these components improve the function of these instruments. However, trace level migration or thermal degradation from these instrument components can cause false positives when analyzing for oligomeric siloxanes. Examples of specific instrument components as a source for siloxane contamination are presented here based on the experience gained by silicone industry chemists over multiple decades. Practical applications using alternative components are demonstrated to identify and minimize contamination. Evaluations of non-silicone-containing inlet septa, sample vial septa, inlet liners and analytical columns are presented as support for their recommended use in the analysis of siloxanes.

Acknowledgements: Methods development research was funded by the Global Silicones Council.

3.19P.2

Sample Processing and Laboratory Supplies as Sources of Contamination in the Trace Analysis of Cyclic Volatile Methyl Siloxanes

J.A. Durham, Dow Chemical Corporation / Toxicology Environmental Research and Consulting; D. McNett, The Dow Chemical Company / Toxicology Environmental Research and Consulting; T. Boehmer, C. Mund, Evonik Nutrition & Care GmbH

When analyzing for siloxanes, careful consideration of potential contamination and/or analyte loss associated with processing procedures is critical to achieving accurate results. Due to the inert properties of Silicon containing materials they are often used in analytical equipment and supplies which can lead to contamination of samples, it is critical to evaluate these supplies and procedures to ensure accurate results. Additionally, cyclic volatile methyl siloxanes D5 and D6 may be present in personal care products that can inadvertently contaminate samples, supplies, and equipment. This contamination is not only possible from the point of collections and the analytical laboratories performing the analysis, but also unknowingly from the manufacturing of equipment and supplies. In this presentation, various collection and processing procedures, as well as supplies, are critically reviewed to demonstrate sources of contamination and loss. This includes evaluation of samples contaminated when personal care products are inadvertently used near sample processing, the loss and contamination during processing of soils via freeze drying techniques, along with evaluation of contamination due to manufacturing of supplies used in processing. Incorporation of a robust QC program allows for successful isolation and identification of sources of analytical bias. Interactions and mass transfer of siloxane can also occur during storage over time. Therefore, a strong QC program utilizing matrix-matched blanks, individual and combined spiked samples, and replications are critical when evaluating environmental and biological samples for concentrations of siloxanes. Methods for reducing or eliminating analytical bias during sample collection, processing, and analysis are recommended.

3.19P.3

Chemical Transformation as a Source of Analytical Bias in the Trace Analysis of Cyclic Volatile Methyl Siloxanes

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The propensity for chemical transformations is one of the outstanding characteristics of polyorganosiloxane compounds (“silicones”). This ability is a readily accessible source of unintended formation of volatile cyclic siloxanes (cVMS) such as hexamethylcyclotrisiloxane (D3), octamethyl-cyclo-tetra-siloxane (D4), decamethylcyclopentasiloxane (D5) or dodecamethyl-cyclo-hexasiloxane (D6). One corresponding molecular mechanism is known as "back-biting", where cVMS are generated from the thermal degradation of higher molecular weight siloxanes. In this process the siloxane chain reaches back on itself and reacts with another Si atom, resulting in the formation of a cyclic siloxane. When analysing for cVMS by GC or GCMS, these characteristics must be taken into account. In addition to the analytical system, the sample processing and upstream steps such as sampling, storage and transport, the analytical matrix itself often is not adequately considered. The sample itself both can import siloxanes and/or deploy components such as acids, bases, salts or simply water, promoting catalytically the back-biting mechanism. In combination with carry-over effects and cross-contamination, the effect becomes unpredictable to some extent and difficult to control as its appearance may be facilitated systematically or may be supported by random coincidence. In this presentation, various cVMS analysis experiments are explained demonstrating the potential for bias introduced by matrices and/or sample processing procedures. This includes examples from analyses of consumer products as well as approaches used for the evaluation of environmental samples.

Additionally, common sample preparation procedures, as well as proposals for "non-target" screening analyses are critically reviewed.

3.19P.4

Siloxanes in Global Air and UV Filters in Urban Air

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In the 2017 sampling year of the Global Atmospheric Passive Sampling (GAPS) Network, sorbent impregnated polyurethane foam disk (SIP disk) samplers were deployed at all sites to investigate the spatial distribution and patterns of linear and cyclic volatile methyl siloxanes (LVMS and CVMS, respectively). LVMS, on an average, contributed < 5% of the total concentrations, so the spatial profile was mainly driven by CVMS. Σ_4CVMS concentrations in air were highest at urban sites, where mean concentrations (n=4) were 350 ng/m^3; followed by rural sites (100 ng/m^3, n=6), background sites (50 ng/m^3, n=24) and finally, polar sites (40 ng/m^3, n=4). Octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) were the predominant CVMS constituting >70% of the total. The results reflect the importance of the "human emission signature" of siloxanes from urban areas due to use of personal care products and industrial products (e.g. sealants, polymers) that contain siloxanes as well as emissions from the waste sector (waste water treatment plants (WWTPs), landfills). Similar to siloxanes, Ultraviolet (UV) filters are another class of chemicals used widely in personal care products, such as sunscreens and lotions, as well as in some industrial applications such as paints and polymers. UV filters have been shown to be strongly associated with emissions to air from WWTPs and are gaining importance as emerging pollutants. To address the limited information on UV filters in air in Canada, a retrospective analysis was conducted using high volume air samples (n=70) collected over a 1-year period in Toronto during 2010-2011. Samples were collected using a sampling train comprising glass fibre filters for particle sampling, followed by a PUF/XAD resin cartridge for trapping gas-phase chemicals. UV filters were detected in both the gas- and particle-phase but dominant in the gas-phase (>90%). Concentrations in air showed a strong seasonality (Clausius-Capeyron relationship) with peak concentrations during the warmest months. Geometric mean concentrations (pg/m^3, n=70) of the dominant UV filters were: 87 for HMS (homosalate), 71 for EHS (2-ethylhexyl salicylate), 41 for E-EHMC (2-ethylhexyl trans-4-methoxycinnamate), 33 for Z-EHMC (2-ethylhexyl cis-4-methoxycinnamate), 12 for 4-MBC (3-(4-methylbenzylidene) camphor) and 8.7 for BP-3 (benzophenone-3). These data contribute to chemical assessment and highlight the importance of urban areas as emission sources to air.

3.19P.5

Deposition of airborne volatile methylsiloxanes is negligible in the surface media of remote regions

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Persistent organic pollutants (POPs) can travel long distances from source regions and redeposit to the surface media of remote environments. The atmospheric long-range transport and redeposition of POPs have been established by both monitoring and model simulations. In contrast, airborne volatile methylsiloxanes (VMS) have shown different behaviors from POPs in that redeposition of VMS in remote regions are not likely due to their high volatility compared with organic chemicals with similar hydrophobicity. Since Henry's Law constants (or equivalently dimensionless air-water partition coefficients) of VMS are several orders-of-magnitude greater than those of POPs, negligible deposition is predicted by dynamic (Level IV) multimedia models such as SimpleBox (version 4.0) and GloboPOP. Quantitative deposition metrics of the transfer efficiency (TE) and the Arctic Contamination Potential (eACP) show that the TE and eACP of VMS were >5 orders-of-magnitude smaller than those of POPs. The model predictions indicate that deposition of airborne cVMS in remote areas including Polar Regions is negligible. In addition, monitoring data of VMS also support that negligible deposition to the surface media is occurring near the Arctic region. VMS that were detected in samples collected in the region were traced to local sources of emissions rather than atmospheric long-range transport followed by deposition. Furthermore, the hypothesis of snow scavenging of airborne VMS was recently tested in a snow chamber. The results showed that almost all VMS sorbed by the snowpack was lost through re-volatilization and hydrolysis during the snow melting process without contributing to deposition to the surface media in cold remote regions.

3.19P.6

Siloxanes as biogas impurities: sampling, analysis and occurrence in landfills and WWTPs

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Biogas is a promising substitute to fossil fuels since it is carbon neutral and may be obtained from residues. In fact, its use is already extended as supplementary power in landfills and wastewater treatment plants (WWTP). Still, biogas obtained in these facilities presents several impurities (*e.g.* H_2S, NH_3 or VOCs) that compromise its calorific performance and may corrode the burner systems. Among these impurities, siloxanes present great concern. Even in concentrations below 1 mg/m^3 they can form SiO_2 precipitates along the tubing and/or engines, affecting dramatically the performance of the process. But currently there are no standardized methodologies to sample and analyse siloxanes in biogas from WWTPs and landfills. Consequently, the main objective of the present study was to review those studies reporting sampling, analysis, and results of siloxanes gathered in these two facilities worldwide. After searching different scientific databases, 40 studies fulfilled our criteria. Being the oldest from 1999, 27 were published from 2011 onwards. The most extensive sampling techniques were the use of sampling bags (made of aluminium, PTFE or PVF) and sorbent tubes (mostly containing synthetic or carbon-based adsorbents). Regarding analyses, gas chromatography coupled to mass spectrometry (GC/MS) was the most popular technique, used in more than 80% of the studies. Reported concentrations of siloxanes in biogas are very wide ranged, spanning from 10^4 to 10^{-1} µg/m^3, being octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) the dominant compounds. Levels of different siloxane congeners in landfills and WWTPs are alike, except for trimethylsilanol (TMS) and hexamethyldisiloxane (L2), which are significantly higher in landfills, and D5, which is predominant in WWTPs. To reduce the concentration of siloxanes in biogas, the most extensively reported technique is the adsorption in different types of sorbents (mainly carbon and silicate based materials), although other technologies (e.g. photochemical reactions, membranes, biological reactors) seem to be promising alternatives in the future. Acknowledgements: This work was financially supported by: (i) Project POCI-01-0145-FEDER-006939 (LEPABE UID /EQU/00511/2013), funded by the European Regional Development Fund (ERDF), through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds, through FCT - Fundação para a Ciência e a Tecnologia; (ii) Project NORTE-01-0145-FEDER-000005 – LEPABE-2-ECO-INNOVATION, supported by North Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the ERDF; (iii) Project LANSILOT (Ref. PTDC/CTA-AMB/32084/2017; POCI-01-0145-FEDER-032084), funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES

3.19P.7

Impact of volatile methylsiloxanes in the solid line of an urban wastewater treatment plant

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Volatile methylsiloxanes (VMSs) are organic pollutants that are widely used in daily life products due to their unique characteristics (e.g. resistance to high temperatures and to chemical reactions, stability, biocompatibility, lubricant properties, low water solubility, high vapor pressures). These compounds are usually discharged with down-the-drain practices, being received in wastewater treatment plants (WWTPs). Conventional plants are not able to degrade them, so they are discharged into the environment through effluents and specially sewage sludge, due to their lipophilic nature. These compounds are suspected of being toxic, persistent and bioaccumulative and, therefore, it is important to understand the levels at which they are present in WWTPs. Special attention should be given to the sewage sludge, which may be use in agriculture as fertiliser due to the high content of important nutrients. In this work, the presence of seven volatile methylsiloxanes (VMSs: L3-L5 and D3-D6) was assessed in the sludge line from an urban WWTP. Sewage sludge samples were collected from the primary settler, gravitational/mechanical thickening, biological reactor, anaerobic digester and dehydration centrifuges. 98% of the VMSs detected in all sludge samples were cyclic compounds, being D5 the predominant compound. Higher total concentrations were achieved in the biological reactor (up to 8059 ng/g dw). The digested sludge registered a decrease on the concentrations (up to 6791 ng/g dw), which may be explained by the biogas formation, which promotes the volatilization of VMSs. **Acknowledgments** This work was financially supported by: (i) Project UID/EQU/00511/2019 - Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE funded by national funds through FCT/MCTES (PIDDAC), (ii) Projects PTDC/ASP-PLA/29425/2017 - POCI-01-0145-FEDER-029425 and PTDC/CTA-AMB/32084/2017 - POCI-01-0145-FEDER-032084 funded by FEDER funds through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES.

3.19P.8

The presence of emerging contaminants in urban sewage sludge - results of a Portuguese snapshot

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The reuse of sewage sludge in agricultural fields as organic correctives/fertilizers has raised concern, particularly with the possible migration of poorly biodegradable organic pollutants and potentially pathogenic microorganisms present in sludge to soil and crops. However, this residue contains a high content in organic matter and nutrients extremely valuable for soils. Therefore, the European Commission (EC) is committed to encourage the use of sewage sludge in agriculture, promoting a circular economy model. So, EC has been working on the establishment of a new regulation to prevent harmful effects related to this practice. However, the existing studies, namely those focused on emerging pollutants, are still insufficient to prove that there are no real environmental and human health risks involved. In this work, a monitoring plan in Portuguese wastewater treatment plants (WWTPs) was established and sewage sludge samples were collected and analysed for four representative classes of organic pollutants - polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), synthetic musk compounds (SMCs) and volatile methylsiloxanes (VMSs) – heavy metals and other parameters considered in national legislation (microorganisms, organic matter, pH, nutrients). This is essential to determine the pollutants' levels, physicochemical/agronomic characteristics of these residues and the variation of sewage sludge quality according to their origin, sociodemographic aspects and seasonality. First results suggest compliance with legal heavy metals concentrations as well as PAHs and PCBs levels, but high concentrations of some VMSs (namely D5) and SMCs (HHCB) suggest the need for greater attention to these emergent pollutants. **Acknowledgments** This work was financially supported by: (i) Project UID/EQU/00511/2019 - Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE funded by national funds through FCT/MCTES (PIDDAC) and (ii) Project POCI-01-0145-FEDER-029425 - AGRONAUT - Agronomic Impact of Sludge Amendment Using a Comprehensive Exposure Viewpoint, funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES.

3.19P.9

Long-term Monitoring and Trend Analysis of Cyclic Volatile Methylsiloxanes (cVMS) in Aquatic Environments Receiving Wastewater Effluent

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Cyclic volatile methylsiloxanes (cVMS) are organosilicon substances with unique combinations of physical-chemical properties. These substances are key intermediates for the manufacture of siloxane polymers and are also used in a variety of consumer applications. Effluent from wastewater treatment plants represents the major post-use disposal route for cVMS. Thus, the Silicone Industry Associations in North America, Europe, and Japan have been conducting a global monitoring program of predominant cVMS oligomers, including octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) in surface sediments and aquatic biota. Aquatic environments that receive wastewater effluents were selected as study areas for the monitoring program, and included Lake Pepin (USA), Lake Ontario (on the border between Canada and USA), Oslofjord (Norway), and Tokyo Bay (Japan). The target objective of the monitoring program was to determine if concentrations of cVMS were stable or changing, which is defined as a statistically significant (α=0.05) annual rate of change of ±6% per year (net change of -27% to 34%) detected with 80% power (β=0.20). Target matrices for each study area include surface sediments, benthic invertebrates near the base of the food chain, low trophic level forage fish, and high trophic level piscivorous fish. Over the course of the monitoring period (2011 to 2016), concentrations of cVMS in both surface sediment and biota exhibited stable or decreasing trends; however, the statistical power was relatively weak in many cases. In an effort to increase the statistical power to identify temporal trends in concentrations of cVMS in these ecosystems, monitoring campaigns were conducted again in 2018. Trend analyses by location and species, updated to include the results of the 2018 monitoring campaign, will be presented.

3.19P.10

Multimedia environmental fate of volatile methylsiloxanes modeled in the Tokyo Bay catchment basin

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We investigated the multimedia fate of decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6) in the catchment basin of Tokyo Bay, Japan, by using a georeferenced multimedia model, G-CIEMS. We assumed the daily per person consumption rate of these compounds in Japan according to literature and used this consumption as the only emission source to the environment. Emissions to the atmosphere were estimated to account for almost all of the emissions of these compounds to the environment. Most of these compounds was predicted to be distributed in the atmosphere (about 60%) and sediment (about 40%). The advective flows in and out of the atmosphere over the Tokyo Bay catchment basin dominated the flows of these compounds among environment compartments. The sewerage systems contributed considerably to the transport and fate of D5 and D6 in water. They transported these compounds from households to discharge outlets of sewage treatment plants (STPs), which in turn accounted for approximately one quarter of the emission of these compounds to surface water and to Tokyo Bay. The STPs also effectively removed these compounds from the wastewater. The overall persistence of D5 and D6 in the catchment basin was estimated to be 3.8–9.5 days. The horizontal distributions of both compounds were similar among environmental compartments; high concentrations were generally observed in populated areas, which was mainly caused by an assumption that distribution of the consumption proportional to population. A sensitivity analysis by a one-at-a-time method demonstrated that the D5 inflow rate to Tokyo Bay via rivers and the D5 mass in the river compartment were most sensitive to changes of the organic-carbon-water partition coefficient. Comparison with the concentrations in river water measured recently in the target area showed that the model captured overall trends of low to high concentrations in rivers. However, there was some variability and a bias toward underprediction. The model provided a better fit to measurements for D5 than for D6. One potential factor contributing to the bias toward underprediction was underestimation of the consumption rates.

3.19P.11

Presence of volatile methylsiloxanes (VMSs) in beach sand

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Volatile methylsiloxanes (VMSs) are anthropogenic molecules that have a structural unit of alternating Si-O bond with organic side chains and are employed in a myriad of personal care and industrial applications. Given their widespread nature and massive production, VMSs represent a potential source of exposure for humans and the environment, as they were found in various environmental matrices. Coastal areas can be potential hotspots for their presence, particularly in coiuntries where tourists introduce a huge impact in terms of seasonal population due to the search for beach areas. Sunscreens are among the products that have commonly VMSs in their formulations and typically, the use of these and other cosmetics increases in the summer. Sand was never reported in literature as an indicator of VMSs in coastal areas, and so this study intended to assess the presence, levels and trends of three linear (L3-L5) and four cyclic (D3-D6) VMSs in beach sand from several sampling sites in the south of Europe, using a QuEChERS extraction protocol with GC-MS quantification. Overall, the cyclic VMSs were predominant in sand samples, being D5 and D6 the congeners with the highest concentrations. were the predominant compounds. As expected, the highest concentrations were found in the summer months, in line with the presence of tourists. This suggests that coastal areas need to be more thoroughly studied, as they may embody patterns that are different from other areas. Also, in Europe the trends in the south may also differ from those in the north, due to the changing climatic patterns in both latitude ranges. *Acknowledgements*: This work was financially supported by: (i) Project POCI-01-0145-FEDER-006939 (LEPABE UID /EQU/00511/2013), funded by the European Regional Development Fund (ERDF), through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds, through FCT - Fundação para a Ciência e a Tecnologia; (ii) Project NORTE-01-0145-FEDER-000005 – LEPABE-2-ECO-INNOVATION, supported by North Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the ERDF. Funding was also provided by Project ECoMarE (ANR-11-LABX-0061), funded by Labex OT-Med, French Government "Investissements d'Avenir" (ANR) (France). The authors wish to thank all parties that helped in the collection of the samples.

3.19P.12

Reducing uncertainty in predicting trophic magnification factors for cyclic and linear volatile methylsiloxanes

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Assessment of trophic magnification factors (TMFs) of chemicals is a way to evaluate the bioaccumulation potential through dietary exposure pathways. However, there has been a wide range of field TMFs measured for certain hydrophobic compounds. This is due to the fact that TMFs are influenced by multiple factors including spatial variations in exposure concentrations, migration of organisms, and variations in biotransformation rate constants and dietary assimilation efficiencies among species. The objective of the current study is to apply the Multibox-AQUAWEB (MBAW) model to (i) better characterize the influence of various factors on the determination of TMFs and (ii) to derive TMFs

that better represent the bioaccumulation potential of cyclic and linear volatile methylsiloxanes (cVMS & lVMS) in real-world food-webs. We selected six aquatic food-webs including Lake Erie, USA; False Creek, Canada; Lake Pepin, USA, Lake Ontario, USA/Canada; Oslofjord, Norway; Tokyo Bay, Japan. For the selected food-webs, all predicted TMFs were shown to be less than 1, indicating trophic dilution of volatile methylsiloxanes (VMS) through dietary exposure pathways. The model predictions were generally in agreement with field-measured TMFs for VMS. Model calculations also revealed that spatial differences in exposure concentrations can have a substantial effect on TMFs; in some cases, causing apparent TMFs to be substantially greater than the TMFs in the absence of spatial concentration gradients. We conclude that in order to determine TMFs that accurately describe the bioaccumulation behavior of VMS in food-webs, it is important to investigate spatial concentration gradients at study sites and to use proper models to correct TMFs determined in locations that are subject to spatial concentration gradients.

3.19P.13

Toxicity assessment of siloxanes and human exposure by inhalation in a WWTP within the project LaNSiloT

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Siloxanes are a growing concern for our environment and have a particularly important presence and effect in wastewater treatment plants (WWTP). The domestic and industrial effluents carry a significant load of these widely used chemicals into these facilities, promoting an extended partition between the water line, the sludge line, and the surrounding air. Being predominantly lipophilic and volatile, siloxanes tend to prefer the latter two matrices, creating a false assumption that the conventional treatments are efficient in their removal, as the treated effluent concentrations are usually low. In fact, they hide in sludge and disperse in the air, creating other problems that urge for effective solutions. Their release into the atmosphere affects firstly the workers of the WWTPs and then the neighbouring and even more remote areas due to the transport of the air masses. Despite recent reports suggested that siloxanes can cause toxic effects in exposed animals, toxicity assessments of siloxanes are still scarce and the implications to human health significantly unravelled. Therefore, one of the aims of the project LaNSiloT is to estimate human exposure via inhalation to siloxanes in a Portuguese WWTP (including workers and surrounding population), complemented with toxicological tests in pulmonary cells applied to standard siloxanes and field air samples. For that, siloxanes will be continuously measured in the indoor air of a Portuguese WWTP (individual office, meeting room and maintenance), as well as in the surrounding outdoor air. Concomitantly, MTT and Comet Assays, both using human lung epithelial cells A549 as target cells (available commercially), will be applied to assess the cytotoxicity and genotoxicity of individual and mixed siloxanes' standards and air samples collected in the WWTP. Results will allow establishing a toxicity potential for siloxanes which, complemented with the indoor and outdoor air measurements, will provide an estimation of human exposure.Acknowledgements: This work was financially supported by: (i) Project POCI-01-0145-FEDER-006939 (LEPABE UID /EQU/00511/2013), funded by the European Regional Development Fund (ERDF), through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds, through FCT - Fundação para a Ciência e a Tecnologia; (ii) Project NORTE-01-0145-FEDER-000005 – LEPABE-2-ECO-INNOVATION, supported by North Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the ERDF; (iii) Project LANSILOT (Ref. PTDC/CTA-AMB/32084/2017; POCI-01-0145-FEDER-032084), funded by FEDER funds through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; (iv) Investigator FCT contract IF/01101/2014 (Nuno Ratola); (v) FCT-MCTES (SFRH/BPD/109382/2015) (Sofia Augusto).

Sources, Fate and Effects of Metals in the Environment: Advances in Risk Assessment, Regulatory Guidance, and Remaining Gaps (P)

3.20P.1

Aquatic hazard assessment of vanadium for the protection of organisms in European freshwaters based on a species sensitivity distribution approach

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Vanadium is a metallic element and widely distributed and abundant in the earth's crust resulting in natural background levels of European freshwaters from < 0.05 to 19.5 µg/L, with a median value of 0.46 µg/L (FOREGS database). However, it is of relevance to investigate the influence of anthropogenic releases of vanadium on environmental vanadium levels. A toxicity threshold for vanadium in freshwater environments was derived based on reliable chronic toxicity data available for relevant taxonomic groups of freshwater organisms. Long-term EC10 and NOEC values for the aquatic toxicity of vanadium are available both from a research programme and peer-reviewed literature for 17 different species, including algae, plants, invertebrates (Annelida, Crustacea, Rotifera) and fish. The species-sensitivity-distribution (SSD) approach was applied to derive the hazardous concentration at which 95 % of the species of the community are protected (HC_5). Chronic toxicity data were fitted to different distribution functions, and the log-normal distribution was selected as the best fit. The median estimate of the 5th percentile of this distribution (HC_{5-50}), protective of 95 % of the species with 50 % confidence, was determined at 51.3 µg V/L. To derive the Predicted No-Effect Concentration (PNEC) for vanadium in freshwater, an assessment factor of 3 was selected to account for uncertainties associated with the HC_{5-50} in accordance with current European Guidance resulting in a $PNEC_{freshwater}$ of 17.1 µg/L based on dissolved vanadium concentrations. This PNEC provides a robust, ecologically relevant and protective threshold for vanadium in European freshwaters.

3.20P.2

Water solubility testing of metals and metal compounds

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Water solubility testing according to OECD Guideline No. 105 (1995) provides prerequisites for effect and fate studies. The water solubility of a substance is specified by its saturation mass concentration in solution. To estimate the solubility and the loading often a preliminary test is required. For the main test, in a straightforward procedure a specified loading of the substance is agitated at a constant temperature, and filtrates of samples are analyzed for their metal(s) concentrations, e.g., by ICP-OES/ICP-MS. The OECD-Guideline 105 describes two methods to cover the whole range of water soluble substances: column elution method for low soluble chemicals and flask method for substances with higher solubility. An objective of the column elution method is to increase the surface of the test item to improve the contact with the eluent. This is mostly applicable for organic compounds. In case of metals or metal compounds, the surface often cannot be increased by using the column elution method. Thus, mostly the flask method is applied to determine the water solubility of inorganics. Care has to be taken regarding the differentiation between dissolved and suspended test item, as in Chapter R.7a of the "Endpoint specific guidance" from ECHA (2017) is stated: "Different definitions for the dissolved fraction exist. In the range of 0.01 - 0.45 µm colloid inert particles that remain suspended may exist." This contribution presents a straightforward and elaborated approach to determine the water solubility of metals or metal compounds. If required, two fractions can be obtained by membrane filtration (< 0.2 µm) and by applying centrifugal filtration (< 3 kDa ≈ 2 nm, fraction operationally defined as truly dissolved). However, only assessing the concentration in 0.2 µm filtered samples will cover a worst case. Water solubility may be influenced by reactions of the dissolved metals, e.g., with oxygen or carbon dioxide. Formed metal oxides or carbonates can result in different water solubilities and may lead to an incorrect value. Therefore, testing under protective argon gas to optimally exclude gases from the atmosphere was established. The procedures (e.g. exact loadings, monitoring of temperature, analytical quantification of the amount of metal) can be performed according to the requirements of good laboratory practice (GLP).

3.20P.3

Transformation/Dissolution testing of metals and metal compounds and determination of dissolved ions

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Transformation/dissolution testing of metals and metal compounds according to OECD Chemicals Testing Monograph No. 29 (2001) provides data for the environmental risk assessments of metals and sparingly soluble metal compounds. The test is intended to determine the rate and extent to which metals compounds can produce soluble bioavailable ionic and other metal-bearing species in aqueous media. Straightforward testing is performed under a set of standard laboratory conditions representing environmental conditions, for example, use of reconstituted water based on ISO 6341 adjusted to a pH range of 5.5 to 8.5. The environmental fate of inorganic substances is different to organic material. According to ECHA Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a, inorganics usually transform through dissolution to the dissolved form. The OECD guidance document recommends 0.2 µm hydrophilic polyethersulphone membrane filters for separation of solids from the aqueous phase. However, sometimes in these filtrates from one replicate vessel an increased standard deviation of quantified metal concentration is observed as compared to the other replicates. The same effect can also be seen by consecutively analyzing the same filtrate after different time periods and points at an inhomogeneous distribution of the metal compounds in the solution after 0.2

µm filtration. Probably, in these cases colloidal/particular metal species are present. To also assess the truly dissolved ionic species, the experimental approach is modified by applying centrifugal filtration tubes with a 3 kDa polyethersulphone membrane which only particles of approximately < 2 nm can pass. Thus, the metal species in the filtrate are operationally defined as truly dissolved. The methods (e.g. assurance of exact loadings, temperature monitoring, dissolved metals determinations) can be performed according to the requirements of good laboratory practice (GLP). Quantification of dissolved metal ions are conducted by ICP spectrometry with validated protocols (measurements of certified reference materials, recalibration and fortified samples, reagent and method blanks). The feasibility of the approach will be discussed on basis of the experiences from tests with several compounds.

3.20P.4

A Tiered Compliance Assessment of Zinc Exposures in France Using a Nationally Derived Environmental Quality Standard

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A bioavailability-based metal freshwater Environmental Quality Standard (EQS) for zinc has been derived for France. The French Zn EQS is based on the hazardous concentration at the 5th percentile (HC5) that is protective of 95% of the waters for the region most sensitive to exposures of zinc in France. The dataset utilised for its derivation covered 4,565 sites, encompassing all 96 departments and 13 regions of France. The lowest 5th percentile HC5 concentration for the 13 regions was determined to be 11.3 µg L^{-1} for the Bretagne region and this value was used as the compliance metric. Of the 4,565 sites used for determination of the EQS, 2697 sites had measured dissolved Zn concentrations, and these sites were assessed against the EQS of 11.26 µg L^{-1} using a tiered approach. At Tier 1, all sites were screened against the derived EQS without taking bioavailability into account with all sites with measured concentrations < 11.26 removed as requiring no further action at this tier. Of 2697 sites assessed, 162 sites were not removed at this tier, equivalent to 6 % of the original datasets. The sites were assessed at Tier 2 taking into account bioavailability, through the use of the user-friendly biotic ligand model (BLM) tool “bio-met”. At this tier, a further 106 sites were removed resulting in 56 sites progressing to Tier 3. At Tier 3, the full ZnBLM was utilised for assessing exceedances, and one further site (in Occitaine) was removed at this tier. This resulted in 55 sites demonstrating exceedances at Tier 3, without accounting for natural background concentrations, equivalent to 2.0 % of the starting dataset. According to recent EU Technical Guidance Documents, the significance of (local) natural background concentration at these sites can also be assessed at Tier 3. This was, however, not yet done in the present analysis. The geographical distribution of these failures is not even across France; with the majority of exceedances in the south-west of the country in the vicinity of Toulouse, with some isolated exceedances located in the north of France. The significance of local zinc background could be checked in these regions. Two regions in this dataset did not exhibit any exceedances. The potential for identification of exceedances in central France was hampered by the lack of measured dissolved Zn concentrations; however, it was possible to perform local HC5 calculations for sites in this area where pH, DOC and Ca data was available.

3.20P.5

Extending the application range of the nickel biotic ligand models: the influence upon compliance assessment in France

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According to the EU EQS Technical Guidance, a bioavailability-based metal Environmental Quality Standard (EQS) is derived as hazardous concentration at the 5th percentile (HC5) that is protective of 95% of the waters for the region most sensitive to exposures. The current EQS for nickel of 4.0 µg L^{-1} was derived in 2010 based on the monitoring datasets available at the time. Since the derivation of this value there has been an update to the nickel biotic ligand model (NiBLM) which has extended the upper pH range from 8.2 to 8.7. This change therefore captures more of the waters that are likely to be sensitive to potential nickel exposures. Freshwater monitoring data from across France of 4,565 sites, from all 96 departments and 13 regions were processed using the updated BLM. The 5th percentile HC5 for the most sensitive region was 2.21 µg Ni L^{-1}. The number of sites included in the compliance assessment was greatly increased when utilising the extended pH range of 8.7, compared to 8.2. 2,699 sites had measured Ni concentrations and a pH of ≥6.5 and ≤ 8.2, an additional 455 sites had measured Ni concentrations with a pH of > 8.2 and ≤ 8.7; an increase of 17 % for a total of 3,154 sites. The number of exceedances, after taken into account bioavailability at Tier 3, for sites with a pH of ≥6.5 and ≤ 8.2 was 15 when compared against the current EQS of 4.0 µg $L^{-1.}$ When the extended pH range dataset was assessed, 26 sites out of 3154 sites demonstrated exceedances; this equated to 0.8 % of the sites assessed. This demonstrates that the number of exceedances is broadly similar when EQS are developed for nationally relevant conditions; and that the inclusion of more sensitive waters in the assessment, through the use of the extended pH range BLM, does not significantly increase the number of exceedances observed while simultaneously significantly increasing the number of sites that are in the applicability domain for assessment.

3.20P.6

Validation of Bio-met for the site-specific bioavailability normalisation of the EQS for zinc

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The importance of considering the bioavailability of metals in understanding and assessing their toxicity in freshwaters has been recognised for many years. Currently, Biotic Ligand Models (BLM) are being applied for the derivation of Environmental Quality Standards for zinc under the Water Framework Directive by several countries in Europe. Bio-met is a user-friendly tool which was developed for implementing bioavailability-based EQS for metals in European freshwaters. A total of 72 validation tests are available for zinc, which have been conducted on 14 different species and in 23 different natural waters. This study validates the relationship between the full BLM predictions and the thresholds (HC5 values) predicted by bio-met in two stages. First, ecotoxicity data for specific species from laboratory tests in natural waters are compared against predictions by the full BLMs for the same species. Second, the site-specific HC5 values predicted by bio-met for the natural waters used for ecotoxicity testing are compared against those provided by the full BLMs. Both relationships are validated for zinc in this study in order to demonstrate the link between the validation tests performed on individual species and the HC5 values calculated for protection of the whole aquatic community. Calculations using a larger set of European natural waters additionally demonstrate the validity of bio-met for predicting zinc sensitivity over a broad range of water chemistry conditions. Bio-met is therefore an appropriate tool for performing compliance assessments against bioavailability based EQS for zinc in Europe, due to the demonstrated consistency with the toxicity test data.

3.20P.7

Validation of Bio-met for the site-specific bioavailability normalisation of the EQS for nickel

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The importance of considering the bioavailability of metals in understanding and assessing their toxicity in freshwaters has been recognised for many years. Currently, the nickel Biotic Ligand Model (BLM) is being applied for the implementation of the Environmental Quality Standard for nickel under the Water Framework Directive throughout Europe. Bio-met is a user-friendly tool which was developed for implementing bioavailability-based EQS for metals in European freshwaters. A total of 54 validation tests are available for nickel, which have been conducted on 9 different species and in 16 different natural waters. This study validates the relationship between the full BLM predictions and the thresholds (HC5 values) predicted by bio-met in two stages. First, ecotoxicity data for specific species from laboratory tests in natural waters are compared against predictions by the full nickel BLM for the same species. Second, the site-specific HC5 values predicted by bio-met for the natural waters used for ecotoxicity testing are compared against those provided by the full nickel BLM. Both relationships are validated for nickel in this study in order to demonstrate the link between the validation tests performed on individual species and the HC5 values calculated for protection of the whole aquatic community. Calculations using a larger set of European natural waters additionally demonstrate the validity of bio-met for predicting nickel sensitivity over a broad range of water chemistry conditions. Bio-met is therefore an appropriate tool for performing compliance assessments against bioavailability based EQS for nickel in Europe, due to the demonstrated consistency with the toxicity test data.

3.20P.8

Validation of Bio-met for the site-specific bioavailability normalisation of the EQS for copper

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The importance of considering the bioavailability of metals in understanding and assessing their toxicity in freshwaters has been recognised for many years. Currently, Biotic Ligand Models (BLM) are being applied for the derivation of Environmental Quality Standards for copper under the Water Framework Directive by several countries in Europe. Bio-met is a user-friendly tool which was developed for implementing bioavailability-based EQS for metals in European freshwaters. A total of 53 validation tests are available for copper, which have been conducted on 11 different species and in 17 different natural waters. This study validates the relationship between the full BLM predictions and the thresholds (HC5 values) predicted by bio-met in two stages. First, ecotoxicity data for specific species from laboratory tests in natural waters are compared against predictions by the full BLMs for the same species. Second, the site-specific HC5 values predicted by bio-met for the natural waters used for ecotoxicity testing are compared against those provided by the full BLMs. Both relationships are validated for copper in this study in order to demonstrate the link between the validation tests performed on individual species and the HC5 values calculated for protection of the whole aquatic community. Calculations using a larger set of European natural waters additionally demonstrate the validity of bio-met for predicting copper sensitivity over a broad range of water chemistry conditions. Bio-met is therefore an appropriate tool for performing compliance assessments against bioavailability based EQS for copper in Europe, due to the demonstrated consistency with the toxicity test data.

3.20P.9

Sources and time trends of copper emissions to water as reported in the E-PRTR

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The European Pollutant Release and Transfer Register (E-PRTR) contains the reported emissions from facilities that are covered by at least one of the 65 E-PRTR economic activities, meet the capacity threshold and emit more than 50 kg of copper (Cu), or copper compounds, per year to the aquatic environment. This study has assessed the Cu emissions to water reported in the E-PRTR for the years 2007 to 2016, in order to better understand the sources of Cu to the aquatic environment. The waste and wastewater management sector were identified as the highest contributor for all years assessed, except one, with an average contribution of 42% of all Cu emitted over the period assessed. Other sectors contributing to the reported emissions are the mineral industry (average 18%), intensive livestock production and aquaculture (15%), the energy sector (12%), and production & processing of metals (5%). Although the exact numbers need to be treated with caution, due to different measuring and reporting practices in different countries, some conclusions can be drawn. The emissions from waste & wastewater treatment mainly consist of the effluents of large municipal wastewater treatment plants; however, it is possible that emissions from industrial sites may also be included in this sector if wastewaters are treated by an external company, or if after treatment on site they proceed to municipal wastewater treatment plants; this may potentially also lead to “double-counting” of emissions. For the mineral industry, around 90% of the reported emissions originate from only 3 locations in Eastern Europe, and these emissions vary drastically from year to year. The emissions from intensive livestock production & aquaculture are mainly related to fish farms, likely due to the use of copper compounds as antifouling agents. Within the emissions from the production & processing of metals sector, on average around 20% (corresponding to around 1% of all copper emissions to water) originate from copper smelting and refining activities. These emissions have decreased by about a factor 2 over the studied period of 10 years. Given that the majority of copper emissions to water are reported under waste and wastewater management, and that background concentrations could potentially contribute to reported emissions for large discharges, understanding the potential contribution of background sources to the emissions under this category would provide a useful context to the emissions from industrial sources.

3.20P.10

Influence of Spiking on Spatial Distribution and Fractionation of Lead (Pb) in Crude Oil Spill Soil

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One of the most critical environmental problems is a result of soil contamination by heavy metals because of its impact on the health of the ecosystem. This study was conducted to investigate the spatial distribution and fractionation of lead (Pb) in crude oil spill soil. Crude oil spill soil and soil with no history of crude oil spill (control) were spiked with Pb as $Pb(NO_3)$ at varying concentrations and time intervals. The chemical fractionation of the soil samples by sequential extraction method define the relative mobility of the metal and this correlates with Pb potential bioavailability. Results of the spatial distribution shows that about 22-56% Pb was mainly in the soluble exchangeable phase in the control soil, 12-32% in carbonate phase and 0-22% was associated with Fe-Mn oxide fractions when compared with the crude oil spill soil which was mainly associated with the Fe-Mn oxide fraction (12–67%) on day one of spiking. After 21 days of Pb spiking, there was significant changes ($P< 0.05$) in the associated fractions in the control soil when compared with the crude oil spill soil which did not show significant change in Pb associated with Fe-Mn oxide fraction. Generally, the proportions of Pb associated with soluble-exchangeable and carbonate fractions seem to decrease with time, with concomitant increases in Fe-Mn oxide and organic fractions. The study reveals that the differences in soil physicochemical properties may affect the retention of Pb in soils and this may influence the chemical fractionation, mobility and bioavailability of Pb. The results also reveals that time can significantly reduce the bioavailability of Pb. The results obtained suggest that the dangers from Pb contaminated soil is influenced by the physicochemical properties, fractionation and mobility of the Pb species in the soil.

3.20P.11

In situ and ex situ approaches to assess bioavailability of metal(loid)s to land snails

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The assessment of organism exposure is a key factor to describe environmental risk which results of the combination of the exposure and the toxicity of chemical in soils. Exposure can be evaluated using bioavailability assays using bioindicators of the soil fauna, such as the terrestrial land snail Cantareus aspersus. Although methologies exist to assess in situ (by snail caging) and ex situ (laboratory bioassay) bioavailability of metal(loid)s to C. aspersus, the answers they provide have never been compared on common soils. Yet such comparison could offer relevant knowledges on the influence of environmental parameters on the bioavailability of metal(loid)s to snails exposed in the field to structured soils or in the laboratory to excavated soils. Expected knowledges could also be useful to stackeholders for the management of contaminated soils. The aims of this study is to compare responses of in situ and ex situ approaches for environmental risk assessment (ERA) of contaminated soils. A site contaminated by metal(loid)s in Vieux-Charmont (France) was used for in situ and ex situ exposure of snails and determinate ERA indices of metal(loid)s transfer and risk to snails. Results showed various patterns depending on the plots (five plots investigated on 21.6 km²), the concentrations in soils, the approaches (in situ vs ex situ), the ability to snail to excrete and to accumulate metal(loid)s, the influence of plant, humus, the metal(loid) behaviors in soils and pedoclimatic conditions. For Co, Cr, Cu, Sb and Sr no obvious risk was detected according to the values of risk coefficient (RC: excess of transfer x toxicity point) (< 100). For As, Hg, Ni, Pb, Sn and Zn, RC were higher ex situ than in situ, while for Cd and Mo, RC the inverse occured. Overall, for the 13 metal(loid)s studied, complementarities and limits of the in situ and the ex situ approaches in ERA of contaminated soils were highlighted. In situ and ex situ approaches are not interchangeable. Indeed, e.g., for Cd and Mo on this site, we found that the ex situ laboratory assay could underestimate the risk whereas it was the opposite for Pb. In situ exposure could be recommended for biomonitoring in a posteriori ERA and ex situ approach either in a priori ERA of chemicals or to assess and compare the risk of excavated soils and to orientate the final destination of contaminated soils (e.g. as embankments, incorportation into road surfacing).

3.20P.12

Photosynthetic activity as a proxy for algal growth inhibition: relevance for the ecotoxicity assessment of the poorly soluble Cr(III)

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Chromium is an environmental contaminant that commonly occurs in two redox forms, Cr(III) and Cr(VI), showing contrasting geochemical and ecotoxicological behaviour. Despite the consensus that Cr(VI) is more (eco)toxic than Cr(III), an increasing number of studies suggests that Cr(III) also has the potential to elicit adverse effects in biota. Understanding Cr(III) ecotoxicity using standardized laboratory tests is complicated by its peculiar chemistry that can cause a rapid decrease in exposure concentrations due to the formation of insoluble, nanoparticulate chemical species. The availability of ecotoxicological endpoints allowing a rapid evaluation of Cr(III) ecotoxicity will therefore facilitate the interpretation of experimental results. In the present study, we used the model green alga *Raphidocelis subcapitata* to compare the effects of Cr(III) and Cr(VI) on the activity of photosystem II (PSII) after 24 hours of exposure and those on algal growth at 72h ; i.e. the endpoint specified in the standard ISO and OECD norms. Algae (initial cell density 200,000 cell/mL) were exposed to trivalent Cr (0–0.625 mg/L) and hexavalent Cr (0–0.859 mg/L) in Erlenmeyer flasks under controlled conditions. Exposure concentrations (< 0.22 μm) decreased by 50–95% (24 hours) and by 70 – 99 % (72 hours) in the case of Cr(III), but remained stable for solutions amended with Cr(VI). The inhibitory effects of Cr(VI) reached 90% for both metrics, while Cr(III) effects did not exceed 60% for algal growth and 20% for PSII inhibition. The inhibitory effect of Cr(III) on PSII after 24h was however higher than that of Cr(VI). Significant correlations between PSII inhibition at 24h and growth inhibition at 72h were observed for both Cr forms. The corresponding linear regressions (duplicate experiences) showed that a 50% inhibition in algal growth at 72h corresponded to an 11% — Cr(III) — and a 3.5–5.0% — Cr(VI) — inhibition of PSII at 24h. However, the slope of the linear regressions differed among duplicate experiences and the regressions' intercepts were not always equal to zero (implying possible effects on growth even in the absence of effects on PSII). Additional experiences should investigate (I) the actual suitability of early PSII measurements as a proxy for growth inhibition at longer exposure times, (II) the existence of PSII vs. growth relationships for other contaminants and (III) the possibility of detecting PSII effects at shorter exposure times.

3.20P.13

Ecotoxicity and speciation of Ni and Cr in a small ultramafic catchment

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Ultramafic rocks have naturally high levels of Ni and Cr, which can lead to potentially ecotoxic concentrations of these elements in the associated soils, sediments and waters. Nickel is a priority pollutant under EU legislation and is regulated via an Environmental Quality Standard based on the filterable (0.45µm) Ni bioavailable concentration (EQSbioav). Estimation of the Ni bioavailable concentration can be obtained with user-friendly Biotic Ligand Model (BLM) tools. Chromium occurs in two redox forms of which Cr(VI) is a recognized human carcinogen and Cr(III) is being reconsidered for its possible ecotoxicological importance as opposed to its putative low ecotoxicity. The present study examined Ni and Cr levels in the Pluhuv Bor (PLB) catchment, a small stream draining an ultramafic substrate in the Slavkov Forest, Czech Republic. The PLB catchment is part of several Czech and international observation networks with average filterable aqueous concentrations (n=29) of 130±48 µg/L for Ni (range 44.3–230.2) and 22±9.9 µg/L for total Cr (range 3.2–38.2); total meaning the sum of Cr(III) and Cr(VI). Estimated bioavailable Ni concentrations constantly exceeded the EQSbioav of 4 µg/L by 2 to 7-fold, despite the high dissolved organic carbon content (24.6±10.8 mg/L; range 6.5–50.4) of PLB waters. Average and maximum Cr levels were comparable to EC20 values determined for Cr(III) and to EC10 values determined for Cr(VI), respectively. Both EC values were determined in experiments performed at LIEC following standardized ecotoxicological procedures in synthetic algal ISO medium. To better understand the bioavailability and ecotoxic potential of Ni and Cr in ultramafic environments, diffusive gradients in thin film (DGT) devices were deployed at the closing section of PLB during baseflow and retrieved after 24, 48 and 72h of exposure. Devices loaded with Chelex resin were used to obtain information on labile Ni and Cr(III) concentrations, while DGT loaded with N-methyl-D-glucamine were used to selectively accumulate Cr(VI). Analyses of DGT eluates are pending. Samples for ecotoxicity testing were also collected at times of DGT retrieval and are being tested for the joint ecotoxic effects of Ni and Cr towards the green alga Raphidocelis subcapitata. The overall results should provide information on the potential risks posed by naturally high Ni and Cr levels in ultramafic regions.

3.20P.14

Establishment of Potamopyrgus antipodarum in outdoor stream mesocosms and validation with tributyltin (TBT)

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In 2019 22.775 new substances were approved via ECHA (European chemicals agency) in Europe. With 48 % of these new approvals Germany had the largest share. Different methods are used to evaluate the potential risks of these chemicals on the environment. One of these methods are mesocosm experiments. Other then pond mesocosm systems, stream mesocosms provide different biocoenosis and abiotic properties. The lotic character of stream mesocosms makes it possible to utilize different test species and focus on other objectives, in outdoor experiments. To establish the New-Zealand mudsnail in an outdoor stream mesocosm system the water was adjusted according to OECD guideline 242. Potamopyrgus antipodarum was introduced in the water body and also in bioassays. In the first experiment five to ten snails and one bioassay per stream were removed weekly, for eight weeks. To count the number of embryos the organisms were narcotised, measured and cracked. The second experiment included TBT, which is known to have endocrine disrupting effects. Each one stream was spiked with nominal concentrations of 20 ng·l-1, 200 ng·l-1 and 2000 ng·l-1 (TBT-Sn). These concentrations were chosen to determine if there is a similar effect concentration in this outdoor system like in laboratory tests (EC50 35-190 ng·l-1). Snails from two different collecting dates were used to compare potential influences of the laboratory condition of the breeding. Each five bioassays with five organisms were introduced. After four weeks all bioassays and every collectable organism from the water body were removed and the number of embryos were counted. P. antipodarum seems to be a suited test organism in stream mesocosms. The first experiment showed that over eight weeks there were, except occasionally exceptions, no significant differences in the number of embryos compared to the amount before the experiment. TBT had no significant influences on the number of embryos of the mudsnails from both breedings at the tested concentrations. The number of embryos showed a decreasing trend however. Number of embryos of P. antipodarum from the water body showed significant differences to the control group at a concentrations of 20 ng·l-1. Because of the high abundances and facile evaluable endpoints, P. antipodarum seems to be a suited test organism in outdoor stream mesocosms, whereas its preferable to have, if possible, high abundances in the waterbody additional to the bioassays.

3.20P.15

Investigation of the molecular mechanism of toxicity of REE on the model organism Daphnia magna

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Rare earth elements (REE) have an essential role and growing importance in world's economy. They are attracting interest from society, policy makers and scientists. The rapidly growing global demand of REE in several strategic industrial and agricultural sectors led many countries to consider the (re)-opening of mining activities for REE extraction. Hence, their increasing use led to the disruption of their biogeochemical cycles with anthropic abnormalities already observed in aquatic ecosystems. 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 the molecular mechanisms of toxicity of REE on the model organism *Daphnia magna* through molecular approaches. Effects of REE were investigated using non-targeted approaches at transcriptome (RNA sequencing) and proteome (shotgun analysis) level.In this study, transcriptome sequencing analysis was performed to identify the effects induced by three REE, namely Gadolinium (Gd), Ytterbium (Yb) and Neodymium (Nd), used as single elements or as a mixture, on *D. magna*juveniles. *D. magna* neonates were exposed for 48h to the EC5 of Gd, Yb and Nd (3.07, 2.5, and 3.5 mg.L^{-1}, respectively) and the EC5 of their mixture (3.03 mg.L^{-1}). A RNA-seq analysis was performed on treated organisms and control group. Comparative transcriptome analysis between exposed and non-exposed organisms showed 1966, 987, and 2456 differentially expressed genes in *D. magna* exposed to Gd, Yb and Nd respectively. REE mixture led to 1588 differentially expressed genes including 585 up-regulated and 1003 down-regulated genes. Within the three REE tested, Yb seems to present different molecular mechanisms as 393, 442 and 134 differentially expressed genes were observed when compared to Gd, Nd and Mixture, respectively. Further analyses are still ongoing to better identify the REE-impacted genes and pathways. Additionally, proteomic analyses are on progress to identify differently expressed proteins. The present study allows obtaining first insights to understand the molecular mechanisms underlying REE effects on *D. magna*.

3.20P.16

Background values of selected metals in the sediments of the lagoon of Venice

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The concentration of trace elements naturally occurring in the sediment as a combination of waterborne (freshwater and seawater) and atmospheric inputs should be carefully estimated for a robust and consistent assessment of the environmental quality of Venice lagoon. Such concentrations, also called background values, can be used to support data evaluation and time trend analysis with the goal of evaluating the extent of anthropogenic influence on the chemical state. The assessment of sediment quality is indeed essential to pursue the achievement of the good ecological status for transitional water bodies as required by the EU Water Framework Directive. In addition, the assessment of sediment quality is crucial to allow the reuse of dredged sediments while preserving the quality and the overall biodiversity of the lagoon ecosystems, from the perspective of sustainable lagoon management. Our research work aimed to i) collect and analyse the background values available in the literature proposed for selected metals (i.e. As, Cd, Cr, Cu, Hg, Ni, Pb, Zn) in the Venice lagoon; ii) review the approaches and methods used to derive such background values; iii) process data available in the literature (e.g. undisturbed radio dated cores from the Venice lagoon) to propose background values for the entire lagoon as well as its four sub-basins; and finally iv) compare those values with literature background values as well as relevant environmental quality standards. Results show a quite large variability in the existing background values, which reflects both the complexity of the lagoon system and the uneven spatial distribution of the available data, and the need to combine both vertical approaches (i.e. sediment core profile) and horizontal approach (i.e. surface distribution of contaminated vs. uncontaminated sediment) in order to obtain spatially resolved background values. The analysis of the distribution of metal concentrations reveals that, for some elements, geochemical enrichment is possible; for some metals, the dataset show exceedance of the sediment national benchmarks that do not reflect the local distribution. Although this study presents a certain level of uncertainty, the results suggest that Hg and Zn are diffuse trace elements also in the pre-industrial period, probably due to the influence of the drainage basin and as a consequence of the historical anthropic presence in the lagoon.

3.20P.17

Soil-plant transfer of critical high-tech metals: Extraction potential by plants

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The diversity of metals used for industrial purposes (electrical engineering, solar panels, batteries...) has increased substantially over the last twenty years. This boom doesn't only concern traditional metals but also elements that have hitherto hardly been used. These high-tech metals (HTM's) have become crucial for most of the industrial sectors and as the growing demand these elements strains the production conditions, environmental protection measures are tending to be neglected. During the extraction process large amounts of highly corrosive residues are produced, containing a veritable cocktail of potentially toxic metals and metalloids. Due to the fact that the biogeochemical cycles of many of these elements are not yet completely understood their environmental behaviour and ecological impact are unpredictable and countermeasures in case of a release are difficult to initiate. This work aims to understand the rhizospheric processes that are involved in the transfer from soil to plant of HTM's. For this purpose, the geological site of Jas Roux, situated in the French alps has been chosen as a study-area. Due to a sulfosalt forming hydrothermal mineralisation this site is naturally rich in several scarce metals and metalloids with high sulphur affinity and offers a unique possibility to study the effect of a metal cocktail containing poorly studied HTM's like Tl, Sb and Ba, rare earth elements (Y, Ce) and others. Unlike recent manmade pollutions generated by mining activities, the Jas Roux deposit allows to study a plant community that had centuries to develop an individual functionality and local adaptions. First in situ experiments show promising results concerning the two alpine species *Hippocrepis comosa* and *Saxifraga paniculata* that are accumulating each different HTM's in their aerial parts. Further ex situ experiments are in progress to analyse speciation change of the elements during the soil-plant transfer.

3.20P.18

Impact of historical land use and gardening practices on metal concentrations and bioaccessibility in garden soils in Kelowna and West Kelowna, BC

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To assess the potential risk associated with exposure to metal contaminants in garden soils, 128 samples were taken from 28 home and 16 community garden plots in Kelowna and West Kelowna. The two municipalities are in the Okanagan, BC, Canada, a region known for its fruit orchards and wineries. Additional information gathered through a questionnaire during the field program included year of the buildings near gardens, historical land use, garden type (in-ground or raised bed), and the use of pesticides and soil amendments (compost, mulch, fertilizer, etc.). Total metal concentrations in the samples were determine by X-ray fluorescence (XRF). For confirmatory analysis, inductively coupled plasma-mass spectrometry, (ICPMS) was used to analyze a subset of the samples. There was good correlation between the XRF and ICPMS data for most of the metals. Apart from Cu (92 mg/kg), the mean concentrations of potentially toxic metals such as As (3.1 mg/kg), Ba (119 mg/kg), Co (5.6 mg/kg), Cr (25 mg/kg), Ni (15 mg/kg), Pb (17 mg/kg), and Zn (147 mg/kg) were all below the Canadian Council of Ministers of the Environment (CCME) soil quality guidelines for residential land use. Soil conditioning products were used in 75% of the gardens studied. Historical land use of the gardens varied from agricultural, residential, flood plains, a parking lot and a metal fabrication company. A comparative review of the elevated metals and land use, age of house, use of soil amendments and currently registered pesticides suggested historical agricultural land use had the most impact on metal concentrations in the gardens surveyed. Metal concentrations in samples collected from raised beds were generally lower than those obtained from in ground gardens. An in vitro bioaccessibility assay (IVBA) was conducted on a subset of samples. The mean metal bioaccessibility were As (37%), Cr (8%), Cu (73%), Pb (63%), Ni (21%), and Zn (54%) implying incorporation of bioavalabilty data in the estimation of risk will provide a more environmentally realistic scenario. Using the mean concentrations, the calculated estimated daily intake (CDI) incorporating bioavailability adjustments were below the respective tolerable daily intake (TDI) suggesting that the risk associated with ingestion of metal contaminants by gardeners were low for most of the gardens studied.

3.20P.19

Assessing toxicity of metal contaminated soil from shooting range

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The contamination of shooting range soils resulting from shots and bullets has received increased attention due to contamination of these sites and adverse health effects. Shooting ranges are a place for training in the use of ammunitions. Lead (Pb) is the primary contaminant at shooting ranges because bullets and pellets are mainly composed of Pb. Evaluation of concentration of heavy metals was found in shooting range soil is important in assessing the pollution risk posed to biota. Seed germination and root elongation tests using lettuce (*Lactuca sativa*) showed decreases in germination percentage and root length in soil from shooting range. Higher concentrations were found in the roots compared with those in the leaves. The study showed that the oxidation of lead pellets over many years had produced metal concentrations in soils and plants exceeding the limit levels for environmental and human health. The remarkable amount of lead was recorded which had not yet weathered. This indicated the need of remediation since the risk of contamination would remain for long time.

3.20P.20

Biological Response of Eisenia fetida Exposed to Lead Contaminated Field Soils of the Shooting Range

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Soil contamination with heavy metals is a major problem around the world. Various shooting activities play an important role in increasing soil pollution with trace elements. The outdoor shooting range contamination typically depends on shooting activity and soil characteristics. The main issue is soil contamination with lead and other heavy metals, mainly depending on use of these elements in ammunition. Although, outdoor shooting ranges are typically isolated and area dependent locations, their accessibility to soil organism creates an environmental concern. The aim of the study was to assess the toxicity of main contaminant of examined shooting range soils to earthworm *Eisenia fetida*. The object of the study were soils taken from recreational shooting range in Alytus, Lithuania. Study shows that soils were heavily contaminated with lead, total Pb concentrations exceeded limit concentration (100 mg/kg). Higher concentrations of other heavy metals, like Cu and Zn, were also observed. The earthworms were exposed to field soils and the biological response of *E.fetida* was determined. The content of malondialdehyde (MDA) and antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT)) were measured as endpoints. Concentrations of MDA increased with increasing concentration of lead as well as activities of SOD and CAT. The soil of shooting range clearly made and adverse effects to earthworms.

3.20P.21

Ecological risk assessment of heavy metals in marine sediments of the brownfield site of Ilva of Bagnoli-Coroglio (Campania-Italy)

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The contamination status by heavy metals (HM) in the surface marine sediments of the brownfield site of Ilva of Bagnoli-Coroglio, and the consequent potential ecological adverse effects are a question of great concern. In fact, the studied area is a National Relevance Contaminated Site (Italy) and several studies highlighted a critical HM contamination of sediment. More, since the disused industrial site of Bagnoli is located in a geologically complex volcano-tectonic system (Campi-Flegrei), the HM stored in marine sediments could result by anthropogenic source (industrial pollution) in addition to a geogenic contribution (natural volcanic activity). Moreover, the local background concentration for metals could be significantly higher than their average concentration in the upper surface crust and this local peculiarity should be considered for properly evaluating the contamination. Therefore, the ecological risk assessment posed by As, Cd, Cr, Cu, Hg, Ni, V, Zn in surface marine sediments of the coastal areas of Bagnoli-Coroglio was carried out using the Potential Ecological Risk Index (RI). RI is based on the HM analyses of 126 superficial sediment samples and on the estimated local natural background values needful to correctly assess the pollution degree. In addition, HM concentrations in surface sediments have been compared with consensus-based Sediment Quality Guidelines (SQG) usually adopted: TEL (Threshold Effects Levels) and PEL (Probability Effects Levels), ERL (Effects Range-Low) and ERM (Effects Range-Median). Furthermore, to evaluate the potential toxicity of investigated HM complex mixtures in sediment samples, the mean ERM quotient method (ERMq) has been applied. Preliminary findings show that the HM concentrations detected in surface sediments are significantly higher than those of the estimated local background values, and the rank of metal contamination factor is: Zn>Cd>Pb>Hg>Cr>As>Cu>V>Ni. Instead, the order for risk factors, taking into account the potential hazard of each metals the following: Cd>Hg>As>Pb>Zn>Cu>Ni>Cr>V. Thus, the RI coefficient suggests a *considerable ecological risk* in the Bagnoli-bay surface sediments (300< RI< 600). Finally, the ERMq values show that 50% of sediment samples are *slightly toxic*, 36% are *medium toxic* and 14% are *highly toxic*. This work highlights the needs of a deeper evaluation of the actual risks posed by HM in a complex volcanic area and of the potential risks linked to a contamination diffusion.

3.20P.22

History of lead contamination downstream of Sunny Corner mine (NSW): a meta-analysis approach

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Abandoned mines can be an ongoing source of heavy metals discharged to the surface water and groundwater, which in many cases, continue unabated since the cessation of mining. In this study, we assessed the spatial and temporal patterns of lead (Pb) contamination in water in Daylight Creek, downstream of the abandoned Sunny Corner silver-lead-zinc mine (base metal mine), in New South Wales, Australia. We collated data for water quality downstream of this mine from different studies conducted from 1979 to 2018 and analysed these data using meta-analysis. The meta-analysis results illustrated that the concentration of Pb in water downstream of the mine had not changed significantly over time. Patterns of lead concentrations downstream of the mine showed some dramatic increases or decreases in some years, which could be related to the precipitation rate in different years. The results illustrated that there is a positive correlation between rainfall and concentration of Pb in the water. An increase in precipitation rate could increase the volume of discharge from the mine, which results in larger contaminant concentrations. Also, remediation activities that took place in the mine could affect Pb concentration as well. The results illustrated that concentration of Pb in 2018 were lower than 2014, which could be the result of clay capping of the mine and sealing the adits in these years.

3.20P.23

Occurrence of mineral elements and heavy metals in bee pollen from Greece

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Occurrence of mineral elements and heavy metals in bee pollen from Greece **Effrosyni Zafeiraki, Konstantinos M. Kasiotis, Kyriaki Machera** Benaki Phytopathological Institute, Laboratory of Pesticides' Toxicology, 8 St. Delta Street, Athens, Kifissia 14561, Greece *E-mail: E.Zafeiraki@bpi.gr, K.Kasiotis@bpi.gr and K.Machera@bpi.gr* Bee pollen is an apicultural product, well known for its nutritional value, as well as for its anti-inflammatory and antioxidant properties. It consists the primary source of nutrition for bees and an important source of mineral elements to humans. Alongside the essential nutrients, bee pollen may also contain various contaminants, including heavy metals and pesticides. In particular, due to heavy metals occurrence in air, water, soil and plants, they can contaminate bee pollen, and raise concerns about its safety for human consumption as well as for bee health. To this end, 20 samples of bee pollen were collected from Greece and analysed for the determination of 30 trace elements, which renders it the most comprehensive study in this region. After being wet digested with Nitric acid (HNO_3) and Hydrogen peroxide (H_2O_2), all samples were analysed by Inductive Coupled Plasma-Mass Spectrometry (ICP-MS). For the confirmation of the elements, internal standards were added at a constant rate and concentration to all calibration standards and unknown samples, while the accuracy of the measurements was assured by the analysis of the available certified reference material. The repeatability of the method and the background contamination were controlled by the analysis of quality-control (QC) standards and blank samples in every sequence, respectively. The recoveries of internal standards were monitored and found to be within acceptable ranges. According to the results, essential elements for human life and nutrition, such as Nickel (Ni) (average: 7511ppb), Zinc (Zn) (average: 230449ppb) and Magnesium (Mg) (average: 3907ppm) were detected in high concentrations. On the contrary, heavy metals were detected in low levels in all the analysed pollen samples. Preliminary human health risk assessment (considering also the daily intake reference dose (RfD) for each metal) demonstrated hazard quotient (HQ) values below the threshold value of 1. The above information verifies that bee pollen can be a prominent source of mineral elements. Considering also the detected levels of heavy metals, it can be supported that pollen samples studied herein is a commodity that does not raise any concerns related to safety for human or bee consumption. Finally, since pollen accumulates a plethora of concentrations of mineral elements and heavy metals, it can be used as bio indicator of the environmental pollution by heavy metals. **Keywords:** pollen, heavy metals, mineral elements, ICP-MS

3.20P.24

Derivation of metal(loid) Effective Residues in freshwater macroinvertebrates from a mining river basin in North Spain

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Monitoring the levels of metal(loid)s in benthic macroinvertebrate tissue allows to evaluate the risk posed to the aquatic communities due to metal(loid) toxicity. The measurement of the alterations in the field macroinvertebrate community composition and structure is an essential component of the integrated assessment of water quality, as established by the Water Framework Directive. The aim of present study is to estimate Effective Residues (ER25 & ER50) for As, Cu, Hg and Se in biota, derived from Generalised non-Linear Models, showing the relationships between tissue residue data from ten field macroinvertebrates (representing 5 feeding styles: scrapers, filterers, predators, generalists and desposit-feeders) and metrics on the ecological status (METI, NORTI, EPT richness and abundance). The ERs are intended for application in ecological risk assessments in mining basins of North Spain and offer specific level of protection against adverse effects associated with bioaccumulation of selected metal(loid)s in macroinvertebrates.Overall, 65 dose-response models were calculated: 28 for As, 24 for Cu, 6 for Hg and 7 for Se. A total of 48 ER25 and 57 ER50 were estimated and are potentially usefull for future ERA. Mercury ERs showed the lowest range in the ratios of ER25 to ER50, suggesting a higher risk for macroinvertebrate communities with smaller increments in Hg bioaccumulation. Species sensitivity distribution (SSD) models on ER50 values could only be performed for As and Cu due to limitation of data. The HC5 parameters for METI-NORTI ER50 and for Nº families and abundance of EPT ER50 were within the range of ETTC values estimated for each metal(loid) using a reference condition approach, which supports their suitability as EQSs for bioaccumulation risk assessment in mining areas of North Spain. We have identified the taxa most suitable as bioindicators to assess the environmental risk due to As, Cu, Hg and Se bioaccumulation in North Spain mining basins. The use of macroinvertebrate community metrics provides a relevant information to identify the environmental risk associated to the tissue residue concentrations and to develop biota EQSs.

3.20P.25

Ecotoxicological study of metallic pollution along the Tunisian coast : Focus on two molluscan models (Solen marginatus, Patella caerulea)

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Marine molluscs are successfully used in ecotoxicology as bioindicators of water pollution. Our study focuses on the analyses of genetic diversity, antioxidant responses and lipid peroxidation of two marine mollusc species in order to detect the impact of pollution on the marine environment and establish a comparative pattern of ecotoxicological responses. Two species of marine invertebrates, Patella caerulea and Solen marginatus, have been collected from six coastal stations all along the Tunisian coast, from north to south. We assessed trace metal contents in seawater, sediments and mollusc soft tissues to have more accuracy for the antioxidant responses studied. Results showed the same metal accumulation pattern for both species. Moreover, the highest concentrations of trace metals and of antioxidant responses were found in the central area of the Gulf of Gabes. This fact is probably due to the chemical pollution generated from the "Tunisian Chemical Group" in Gabes City. The median-joining population networks based on COI (mtDNa) and ITS1/ITS2 (rDNA) showed for the two species a high genetic population interconnection with high number of migrants per generation estimates (Nm). This high population connectivity could be related to ocean currents transporting larvae and juveniles between distant patches of native habitat. This genetic continuum along the Tunisian coast prevents from linking the eventual impact of pollution to disturbance of genetic diversity.Key words: Trace metals, Ecotoxicology, Bioindicators, Genetic diversity, Antioxidant response

3.20P.26

Distribution of S, Ca, K and Fe in the lichen Punctelia hypoleucites transplanted to Bajo de la Alumbrera mine, Catamarca (Argentina)

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Open-pit mining activity impacts air quality, mainly through the emission of particulate matter. At present, the lichen *Punctelia hypoleucites* (Nyl.) Krog is being evaluated as bioindicator of air pollution in Bajo de la Alumbrera, an important open pit mine in Catamarca, Argentina. To interpret the bioindicator response it is important to know how the elements are distributed in different lichen structures. Knowing the origin of the accumulated elements is also important for associating this response to a specific source of pollution. Therefore, the aim of this work is to analyze the elemental distribution of Ca, K, S, Fe within the vegetative and reproductive lichen structures, in order to estimate element accumulation in *P. hypoleucites* exposed in sites with different degree of mine influence. Lichens were transplanted in bags to three monitoring sites: one site inside the mine, an off-mine site 15 km away, and a site far away of the mine where the lichens were collected (control). After three months, the samples were analyzed by particle induced x-ray emission (PIXE). 2D maps of elemental concentration were obtained by micro-PIXE by scanning a 50-MeV $^{16}O^{5+}$ beam over cryosectioned tissue samples at Buenos Aires Tandar accelerator. In all transplant sites, the K/Ca ratio in *P. hypoleucites* was higher in apothecia (0.9 - 1.7) than in thalli (0.3 - 0.5), while Fe was preferably distributed in the upper and lower cortex of both structures. At the site inside the mine, Fe content for both structures was the highest. Sulphur was distributed homogeneously in all the layers of lichen thalli and apothecia at the off-mine and control sites. At the site inside the mine it was observed an overlap of S and Fe maximun content in the apothecium cortex, suggesting a mineralogical association of these elements. Consequently, Mössbauer spectroscopy was applied in order to determine iron species. It was identified pyrite particles together with others minerals of iron in different degrees of oxidation. These results allow inferring the mining origin of the atmospheric particulate material trapped by the lichen in the mining area. On the contrary, the distribution of Ca and K would indicate a different origin for these elements. With the analytical techniques applied in this study the traceability of the accumulated Fe in *P. hypoleucites* was achieved. This information is valuable for air biomonitoring in the Bajo la Alumbrera mine area.

3.20P.28

Phytoeffects of chemical soil contamination in acute and chronic experiments

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This study was aimed to compare the effects of heavy metal polluted soils on higher plants in acute and chronic experiments. We investigated the impact of polymetallic pollution (lead, copper and zinc) on plant growth parameters. White mustard (*Sinapis alba* L.) was used for the bioassay as it is the recommended plant species for soil quality assessments according to the Russian standard (Russian Federal Register FR 1.31.2012.11560). Samples of agro soddy-podzolic soil (Eutric Albic Retisol (Loamic, Aric, Cutanic, Ochric)) were collected on two experimental fields located in the Moscow region (56°02'01.9°N 37°10'04.9°E and 56°01'41.7°N 37°11'04.3°E) (IUSS Working Group, 2014). Samples were collected from the upper soil layer (0–20 cm), differing in the amount of organic carbon (s1 C_{org} - 3.86% and s2 C_{org} - 1.30%) and were prepared in accordance with ISO 11464:2006 "Soil quality – Pretreatment of samples for physico-chemical analysis" (ISO 11464, 2006). Control and metal-spiked (650 mg/kg Pb + 1100 mg/kg Zn + 660 mg/kg Cu) soil samples were analyzed.. Seed germination, root and shoot length of *Sinapis alba* seedlings were estimated in acute and chronic phytotests using eluate and applicative experimental design. According to acute method, specially designed boxes were used. Each box has two compartments; the volume of each compartment is 92 cm^3. The lower compartment is for root growth. In applicative design of experiment it is filled in with soil, then filter paper was placed in the lower box compartment and moistened with deionized water. In eluate design of experiment the lower compartment is filled in with filter paper soaked in aqueous extracts of soil samples. For the eluate design of experiment aqueous extracts were prepared immediately before the bioassay using soil/water ratio of ¼.The upper compartment is for shoot growth. Then, ten seeds of *Sinapis alba* were placed on the filter paper in each box and incubated for 4 days at 22-24°C and 60%. For chronic phytotest, the soil was placed in pots. Ten seeds of white mustard were planted in each pot (400 g of soil) The pots with soil samples were incubated for 30 days in an open greenhouse with natural temperature fluctuations (in June at about 20-24 °C and 60% relative soil humidity. Each experimental had 3 replicates. According to the data obtained, test responses of mustard seedlings affected by polymetallic contamination were identical (strong growth inhibition) in the chronic experiment and in the acute experiment using applicative method. Similar effects were observed both in humus-rich (s1) and humus-poor (s2) soils. In contrast, in acute experiment with soil water extracts (eluate design) significant differences in test responces were observed, both for the bulk soil, and between soils that differ in humus status. Water extracts of humus-rich (s1) soil markedly stimulated plant growth. Thus, the eluate method which is recommended for laboratory express phytotesting of soil toxicity using soil aqueous extract has limitations. It cannot adequately indicate the level of toxicity caused by metall contamination in soils rich in organic carbon

The Polar Regions: Pollutants & Environmental Change - Multiple Stressors, Ecosystem Response and Environmental Policy (P)

3.21P.1

Assessing the bioaccumulation of organic contaminants along an Arctic marine food web using an ecosystem modeling tool

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The Arctic is progressively recognized as the "canary in the coalmine" for global multiple stressors. Organic contaminants are associated with global stressors such as ozone layer depletion, climate change, ocean acidification, changes in nitrogen-phosphorous cycles, biodiversity, and ecosystem function. Modeling efforts to assess the impact of chemicals on organisms have been focusing on predicting contaminant toxity and exposure potential. Exposure potential is typically assessed by estimating chemical persistence, long-range transport potential, and bioaccumulation. Tools to assess bioaccumulation include quantitative structure-activity relationships, multimedia models, pharmacokinetics, and ecosystem models. Ecopath with Ecosim (EwE) is a well-established ecosystem modeling suite consisting of three components; Ecopath, Ecosim, and Ecospace. Ecopath is a static, mass balanced representation of the trophic interactions of the ecosystem. Ecosim and Ecospace are, respectively, a time dynamic and a spatiotemporal dynamic simulation for the ecosystem. Ecotracer is an EwE tool that describes the accumulation of contaminants and tracers in the ecosystem, based on the initial Ecopath model and for either of the simulation modules. Given the relative success of EwE among the policy-making community we seek to assess Ecotracer as an alternative modeling tool to estimate the bioaccumulation of organic contaminants. As a case study we employ an established Ecopath model for the Barents Sea ecosystem and simulate a contamination of this region with 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153). PCB-153 is a well-known persistent organic pollutant that has been consistently detected in virtually all biotic and abiotic matrices in the Arctic over the past decades. We compare reported and estimated concentrations for the modeled Barents Sea and its species, make suggestions for optimal Ecotracer configuration for organic contaminants, and outline Ecotracer limits and limitations.

3.21P.2

Modelling polar bear subpopulation decline due to persistent organic pollutants

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Arctic ecosystems are subjected to many threats including pollution with toxic substances, such as persistent organic pollutants (POPs). Due to the lipophilic nature of these compounds, POPs accumulate easily in food chains, affecting especially organisms at higher trophic levels (e.g. polar bears). There, these compounds are known to cause altered reproduction and immunological effects. Due to a combination of chemical properties of POPs in cold conditions (e.g. high thermal stability, high volatility and slow degradation) and marine currents and air flows, POPs are transporting northwards, resulting in POP high levels in polar bear individuals,. Consequently, many studies focused on individual effects of POPs on individual health effects, disregarding possible population-level effects. In the present study we estimated the impact of POPs on the 19 polar bear populations, by deriving exposure-response curves for survival and reproduction. As data on toxicological endpoints for polar bears is grossly lacking, endpoints in the present study were extrapolated from endpoints for typical laboratory species (*Rattus norvegicus* and *Mus musculus*). Finally, temporal and spatial differences in population trends were analyzed, by the construction of maps and statistical analysis.

3.21P.3

Temporal trends of legacy POPs and perfluoroalkyl substances in landlocked char from High Arctic Lakes

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Temporal trends and climate related parameters affecting the fate of legacy persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), organochlorine pesticides (HCHs, DDTs, HCB) and perfluoroalkyl substances (PFASs) were examined in landlocked Arctic char (Salvelinus alpinus) in two paired High Arctic lakes in Melville Island (West and East lakes). Research over the past 10 years in the study area has revealed ongoing permafrost disturbances, which are of significant magnitude and importance on the West Lake watershed and lake. Adult char were collected in late July from almost every year from 2008 to 2018, by gill netting; numbering 7 to 25 adult fish per lake and year. All fish were dissected in situ and subsamples of muscle+skin, liver, otoliths and GI tract were kept frozen for transport (-30ºC). In total, more than 100 samples from muscle+skin arctic char were collected and analyzed for legacy and emerging POPs in this study with the aim of i) examining temporal trends of legacy and emerging POPs ii) study which parameters are affecting the occurrence of POPs in Arctic char and iii) investigate whether or not climatic disturbances affecting West catchment and lake may affect the temporal trends of legacy and emerging POPs. Σ71PCB showed significant declining trends of concentrations in Arctic char from East Lake (-1.4 % per year) as expected due to the past national and regional bans/ restrictions on use and emissions of these chemicals in circumpolar and neighbouring countries. However, the concentrations of PCBs and ΣDDTs were found to increase significantly (+2.1 % per year) in char collected in West Lake. The increases of PCBs and DDTs in char from West Lake in comparison to East Lake may be in response to greater inputs of PCBs bound to terrestrial carbon, due to permafrost disturbances (e.g. huge input of DOC and POC) greatly affecting West Lake and its catchment. On the other hand, temporal trends of total perfluorocarboxylates (C4-C14) PFCA in char from the two Melville Island lakes showed decreasing concentrations at a rate of 31 %/y in West Lake and 22 %/y in East Lake over the period 2008 to 2016. Concentrations of PFASs in West and East lakes were similar in both lake water and char muscle, despite major differences in water chemistry. These results suggest that climate disturbances due to warming are affecting, in different magnitude, temporal trends of legacy and emerging pollutants of Arctic freshwater biota.

3.21P.4

Comparison of trends of perfluoroalkyl substances (PFASs) in ringed seals and in Ocean waters across the Canadian Arctic

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The ringed seal (*Phoca hispida)* is a key biomonitoring species for the evaluation of spatial and temporal trends of persistent organic pollutants in the Arctic. Early temporal trend studies on perfluoroalkyl substances (PFASs) in liver of ringed seals in the Canadian Arctic, showed that perfluorooctane sulfonate (PFOS) achieved maximum concentrations in 1998-2000 and declined from 2000 to 2005, while C9-C15 perfluorocarboxylates (PFCAs) had increasing levels until 2005. Ocean transport of PFOS was proposed as the main route of delivery to the arctic, however, the rapid response to phase out and bans of PFOS suggested the importance of an atmospheric pathway as well. The objective of this study was to compare temporal trend data for PFASs in ringed seals in several locations in the Canadian arctic, as well as newly available time trends in ocean waters from the central Canadian archipelago. Ringed seal samples were obtained from subsistence harvesting in four regions: Beaufort Sea (Sachs Harbour), Lancaster Sound (Resolute), Hudson Bay (Arviat) and coastal Labrador (Nain). Hunters recorded information on the date and location of collection, sex, girth, length, and blubber thickness. Seawater (1L) was collected near Resolute Bay, in 2005 as part of an Arctic transect and subsequently in 11 other years between 2007 and 2019 at the same location at depths of 1 to100 m under ice and in open water. Seal liver and seawater were analyzed for a suite of PFASs by LC-MS/MS. Highest total PFASs (PFOS + C7–C15 PFCAs (ΣPFCAs)) in ringed seals over the period 2011-2018 were observed in the seals from Hudson Bay (Arviat; 58 ng/g ww) compared to other locations (Sachs Harbour; 39 ng/g; Resolute 30 ng/g and Nain 33 ng/g ww). Since 2011 PFOS and ΣPFCAs concentrations in liver have increased at Resolute and Arviat by about 2-fold. PFOS has declined significantly in seawater at Resolute with an overall disappearance half-life of 4.4 yrs, while PFOA, the major C8-C15 PFCA in seawater, showed no trend between 2005-2019. An increase in PFOS was also observed in atmospheric measurements including high volume air and ice core samples in the Canadian Arctic where PFOS had a doubling time of 2.9 yrs between 2006 and 2014. Thus trends of PFOS in ringed seals appear to reflect atmospheric rather than oceanic trends in concentrations in the Canadian arctic possibly due to annual delivery of PFASs into the productive near surface ocean waters during spring snow melt.

3.21P.5

Long-term effects of thin layer capping in the Grenland fjords, Norway: Reduced uptake of dioxins in passive samplers and sediment-dwelling organisms

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In order to evaluate potential remediation strategies using thin layer capping for fjord sediments, a field experiment was set up in 2009 in an area contaminated from previous emissions of dioxins from a closed down magnesium smelter. Test field plots at 30 and 100m depth were covered with thin layers (< 5cm) of 1) crushed limestone, 2) dredged marine clay or 3) dredged clay mixed with powdered activated carbon (AC). After capping, several investigations have been carried out to determine cap thickness, dioxin fluxes and potential impacts of the capping treatments on macrobenthic communities. The objective of this investigation was to assess the effects of various thin layer capping treatments on bioavailability of dioxins. This was done by collecting intact boxcore sediment samples from the capped field plots and uncapped reference locations and maintaining them as mesocosms circulated with natural seawater at in situ temperature. Bioavailability was measured by using passive samplers (SPMDs) exposed in the overlying water and by exposing polychaetes and gastropods collected from the wild to the boxcore sediments. For documentation of cap status and possible recontamination, the study was supplemented with SPI-images and chemical analyses of samples from sectioned sediment cores. Our results show that thin caps of clay or limestone without AC had little or no effect on uptake of dioxins for more than one year after capping. Thin caps with AC, however, reduced uptake by up to 88% during the first year, slightly declining to 64-67% nine years after capping. This occurred despite that natural sedimentation after the capping in 2009, has buried the cap layers in 2-5 cm new material contaminated with dioxins. The results indicated that capping with a thin layer of clay mixed with powdered AC (~2 kg m^{-2}) significantly reduces the uptake and bioavailability of dioxins and other dioxin-like compounds by ca 65 % still 9 years after treatment. The results are in line with sediment-to-water fluxes measured *in situ* with flux chambers. However, before this technique can be recommended for sediment remediation, the positive effects on contaminant release and bioavailability need to be carefully evaluated and weighed against negative effects on benthic macrofauna revealed by our companion studies.

3.21P.6

Long-term effects of thin-layer capping in the Grenland fjords, Norway: Effects on benthic communities

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The Grenland fjord in Norway is contaminated by large historic emissions of dioxins and mercury from a magnesium plant operating 1951-2002. As a possible *in situ* remediation option, thin-layer sediment surface capping with powdered activated carbon (AC) mixed with clay was applied at two test sites at 30 m and 95 m depth in 2009. This study describes the long-term biological effects of the AC treatment on marine benthic communities. Our results show that the capping with powdered AC strongly reduced the benthic species diversity, abundance, and biomass and that the effects were still evident 9 years after capping. At 30 m depth, the brittle star *Amphiura fiiformis* was eradicated, a key species for important ecosystem functioning e.g. mixing of the sediment (bioturbation). At 95 m depth, the biodiversity was higher and the community was more resilient to the AC treatment. Nevertheless, there was also a significant general reduction in species abundance and biomass. Such a long-lasting effect of powdered AC on marine benthic communities is alarming and has so far not been reported. Along the positive effects of AC capping for reducing contaminant release and bioavailability, negative effects on benthic macrofauna and long-term disruption of the benthic community should be considered. Our results show that before AC can be applied for *in situ* treatment of contaminated sediments on a large-scale basis, a careful assessment of the vulnerability of the benthic community needs to be done on a case by case basis. Further, more research is needed to obtain and use an AC type or other sorbent that is less detrimental to the benthic organisms.

3.21P.7

Studies of distribution of marine microplastics in the Russian Arctic Seas during the RV "Academic Mstislav Keldysh" cruises in 2018 and 2019

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Marine plastic litter has been reported globally and recognized as an emerging threat to biota and habitats. Microplastics have been found ubiquitously in the Global Ocean, however, the Arctic Ocean, which has a focal role in the global climate change scenario, remains largely unexplored with only scarce data available on microplastics. The present study was aimed to quantitatively assess the distribution of microplastics in the Russian Arctic shelf seas. A complex approach was used to sample water surface, subsurface and bottom sediments, supported by hydrophysical and biogeochemical data. Sampling was carried out along the Northern Sea Route streaming from Arkhangelsk (White Sea) eastwards to the Laptev Sea in 2018 and to the East Siberian Sea in 2019. Floating plastic particles were collected with a neuston net (a total of 49 tows in 2019); water samples were taken with a continuous flow system from 3m depth (22 samples in 2018 and 61 samples in 2019); sediment samples were collected with a box-corer sampler with 50x50 cm capture volume and washed over a 0.5mm metal mesh (16 stations in 2018 and 23 stations in 2019). Sampling protocol and particle characterization followed recently recommended guidelines for MP samples allowing this data to be comparable to other global seas. Chemical identification of plastic type was performed using spectroscopy methods (Cary 630 ATR-FTIR or PerkinElmer Spotlight ATR-FTIR). Linear and surface size of each particle were measured and the abundance of microplastics was calculated (items/m^3). The present study revealed that Siberian Arctic (east from Novaya Zemlya archipelago) was not plastic-free: microplastics were registered in all five examined shelf seas. The average concentration of floating microplastics was 0.01 items/m^3, which is significantly lower than in the other parts of the Global Ocean. Areas of the highest concentration of microplastics in the Russian Arctic corresponded to the areas of river plumes. Large microplastic particles were concentrated in the outer parts of the plumes, which is presumably caused by a specific discharge regime of the large Arctic rivers. We suggest that river discharge and distribution of buoyant riverine plumes need to be considered as well as global thermohaline circulation to better understand the influx and distribution of microplastics in the Arctic Ocean.

3.21P.8
Chemical identification of microplastics ingested by Red Phalaropes using Fourier Transform Infrared (FTIR) spectrometry and evaluation of exposure to associated contaminants
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Red Phalaropes (Phalaropus fulicarius) are vulnerable birds who breed in the Arctic. Typically, their diet consists of zooplankton found offshore in upwelling areas. However, it is hypothesised that phalaropes feed closer to shore during warmer ocean conditions, where they are exposed to greater amount of micro -and macro- plastic debris. Given the historical record of studies reporting that Red Phalaropes mistake floating plastic pieces for prey, it is crucial to evaluate this as a potential route of exposure to chemical contaminants associated with plastics. The individuals used in this study were collected during southward migration in the fall of 2016, on the northern coast of British Columbia, Canada. Microplastic debris were found upon examination of the guts in all carcasses (n=9), and the veterinary report indicated that the large amount of ingested plastics contributed to the cause of death. The chemical identification of microplastic debris allows us to shed light on this particular exposure route to plastic-associated chemical contaminants, as different polymer types can be linked to a variety of additives and sorbed environmental contaminants. Thus, knowing which types of polymers are being ingested will allow us to determine what contaminant types the Red Phalaropes are exposed to via ingestion of plastic pollution, and what constitutes a threat to this understudied species.

Tire Wear and Microrubber Particles - From Problems to Solutions (P)

3.22P.1
Modelling of the distribution and fate of tyre and road wear particles by the combination of a novel GIS and probabilistic model approach
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Tire abrasion has become an environmental topic that is increasingly coming into the focus of society and science. It is estimated that in the European Union 1,327,000 t/a tyre wear particles (TWP) are released into the environment through road traffic [1]. Whereas several emission factors and predicting models for the fate of airborne TWP are published, the fate of TWP via surface runoffs is hardly investigated. The authors developed and combined a probabilistic and GIS (Geo Information System) model in order to calculate the TWP distribution for the whole of Germany. Basing on this, the atmospheric transport of TWP will be modeled for Germany whereas due to the large complexity of the water and wastewater networks the propagation in water will be demonstrated only for two river basins: Wupper and Panke. The GIS modelling was done by the use of ArcGIS (ESRI) and bases on the digital landscape model (DLM) of the Federal Office of Cartography and Geodesy supplemented by the road data set of OpenStreetMap. The probabilistic model aims to estimate the average driving forces for each street segment, which is required for the implementation into the GIS model. Therefore, the street related parameters like maximum allowed speed, curvature, slope, acceleration / braking were integrated in the model, while individual parameters of the car (air pressure, axis alignment) or driving style were not considered. The determination of the traffic strengths by existing data sets, supplementary sources and by the estimation in residential, commercial and mixed areas resulted in a cross-city traffic strength map representing the initial situation for the dispersion modelling. As expected, the highest traffic volumes are recorded on the motorways accounting up to 85,000 vehicles per day which corresponds to maximum of 8 kg/m/a TWP. The next step is to determine the emission quantities of tyre abrasion and the emission factors for each vehicle category, which together define the source strength of the emissions. To evaluate the distribution model a comprehensive mapping should be carried out by the use of novel screening measuring methods such as SEM/EDX to analyze TWP in soil samples. Since the probabilistic model is based on many assumptions, finally a sensitivity analysis will be performed. [1] Wagner S; Hüffer T; Klöckner P et al. 2018. Tire wear particles in the aquatic environment - A review on generation, analysis, occurrence, fate and effects. Water research 139:83–100

3.22P.3
Chemical and bioanalytical characterization of polycyclic aromatic compounds (PACs) in tire granulates
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Ulrika Eriksson[1], Anna Larsson[1], Ivan A. Titaley[1], Maria Larsson[1] [1]Man-Technology-Environment (MTM) Research Centre, School of Science and Technology, Örebro University, Örebro 701 82, Sweden Artificial turf contains granulates made of recycled car tires, which contain polycyclic aromatic hydrocarbons (PAHs). Current characterization of PAHs in car tires are limited to a list of 8 PAHs as defined by REACH (PAH_8), even though other PAHs have been previously measured in car tire particles. Furthermore, there is limited knowledge on the bioactivity of PAHs found in tire particles. In this study, several PAC classes were analyzed in tire granulates of three different size fraction; PAHs, alkylated PAHs, oxy-PACs, N-PACs, and S-PACs, in total 85 compounds. The bioactivity of PACs in the tire particles was assessed using the H4llE-luc bioassay for aryl hydrocarbon receptor (AhR). Chemically derived TCDD equivalents (chem-TEQs) were calculated from the relative response potency factors (REPs) specific to the H4IIE-luc assay and compared to the derived bio-TEQ values. Extraction of PACs in tire granulates was performed using sonication of samples in toluene, with further clean-up with basic silica. The results showed that only a minor portion (0,2-0,8%) of the bio-TEQ could be explained by the chem-TEQ values. The total PAC concentrations were 39 – 59 µg/g tire granulate. A number of 49 PAC homologues were detected in the samples. PAH_8 accounted for 4.7 – 8.1% of the total PAC concentrations .This indicates that current characterization of PAHs using PAH_8 is insufficient for risk assessment of car tire granulates.

3.22P.4
Processes for Transport of Tyre and Road Wear Microplastic Particles, Metals and Polycyclic Aromatic Hydrocarbons in Road Runoff - Characterisations and a Sorption and Desorption Laboratory Study
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Microplastics are emerging environmental pollutants with potential severe negative impacts to living organisms. Tyre and road wear particles (TRWP) in urban runoff account for a high proportion of the microplastics load into European rivers and to oceans globally. During tyre and road wear microplastics are released from the tyre tread, road markings and polymer modified bitumen. Other particles and substances are released from the road surface (asphalt or concrete), or the vehicle (e.g. brake wear, exhaust emissions, motor oils). These emissions contain a cocktail of microplastics, organic pollutants and metals. Microplastic research is still in its infancy and there are knowledge gaps that need to be filled, as for example research on emissions, transport processes and treatment options for TRWP in road runoff. In this study we investigate the transport processes of TRWP, polycyclic aromatic hydrocarbons (PAHs) and metals from roads via road runoff to different stormwater treatment systems. Road runoff, washwater and road sand originating from street sweeping, stormwater and stormwater sediment, treatment filter materials and soil-bed filter materials from rain gardens and road infiltration ditches are characterized for the content of TRWP, PAHs and metals. Preliminary results show that road runoff, washwater and road sand as well as urban stormwater contain high concentrations of tyre wear particles, nanoparticles, metals, PAHs and aliphatic petroleum hydrocarbons. The sorption – desorption tests are performed in a laboratory study including investigations such as processes for filtration/sorption and desorption/leaching of TRWP, metals and PAHs to and from (1) sand and dust from street sweeping; (2) sediments from ponds and sedimentation chambers; (3) soil from rain gardens and road infiltration ditches. The studies are performed in column tests on both clean and polluted materials, with simulated road runoff solutions and both TRWP spiked and unspiked samples. Microplastics (TRWP) and other pollutants as PAHs and metals will be analysed. The results from the study may also be useful and used as input data in soil and water modelling studies for TRWP transport and treatment.

3.22P.5
Analyses of tire and road wear particles (TRWP) in environmental road dust samples with an automated SEM/EDX and single particle classification system
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Wear and tear from tires and asphalt (polymer modified bitumen) is known as one of the major sources to microplastics in the environment. The amount of tire wear produced is approximately 0,1g/vkm resulting in 13 000 tons of tire wear produced in Sweden every year. However, the complexity of this problem is huge since the calculations are doubtful and hard to confirm due to difficulties when analyzing environmental samples. Tire wear are black particles with a high content of SBR (styrene-butadiene-rubber), carbon black, silica and trapped

particles. The size range varies from several 100µm to nm where the maximum in terms of mass concentration has been found to be between 20-100µm. Because of the blackness and heterogeneous nature, tire wear is a challenge to analyze. In most analyses a time-consuming sample preparation is required, also the analyses itself tend to be very time consuming. Particle Vision (Fribourg, Switzerland) has developed a method to identify and quantify tire wear basedon automated SEM/EDX single particle analysis and a morpho-chemical classification system. By using an extensive particle library and a machine learning algorithm, the system can distinguish between several particle types e.g. tire wear, minerals, organics, metals and bitumen. In addition to the less time-consuming analyzing step, the sample preparation applying this method, is less time-consuming compared to analysis methods demanding density separation of TRWP prior analysis. In a Swedish project funded by the government and in the frame of a PhD-project funded by FORMAS, VTI has sampled road dust collected with a Wet Dust Sampler (WDSII) on urban streets and on a rural highway in Sweden. Samples have been taken with de-ionized high-pressure water adjacent to the kerb and in-between wheel tracks. Before the sample preparation a subsample was taken out to determine the size distribution. The samples were then sieved into two fractions, ≤20µm and ≥20-≤125µm and the material was collected on polycarbonate filters. The method delivered promising results for TRWP particles in the analyzed WDS samples. First results indicate that approximately 5-10% of the particles in the size fraction 20-125µm of the WDS sample consist of TRWP. The performed analyses also evidence that tire wear particles can have different shapes, sizes and appearances and not only the form of elongated "sausages" which is the most common description in the literature.

3.22P.6
Acute and chronic effects of tire particles and microfibers on Daphnia magna
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Tire debris and synthetic fibers are emitted in large quantities into the environment. The effects of these microplastic particles on aquatic organisms are still largely unknown. Therefore, this study aimed to determine the acute and chronic effects of car tire particles and plastic fibers on the freshwater invertebrate species *Daphnia magna*. Fibers of a length between 100 to 2200 µm and a width of 29 µm were generated through washing polyester (PET) fleece blankets. Tire debris was milled from end-of-life passenger tires (Genan, Denmark) and sieved to obtain a final particle size between 25 and 75 µm. Acute (48 hours) and chronic (21 days) toxicity studies were carried out based on respective standard protocols (OECD 202/211). The test organisms were exposed to a range of up to eight concentrations, including environmentally relevant concentrations (0.00005 - 0.15 g/L). For acute exposure, the endpoints ingestion and immobility were measured, while for chronic exposure mortality and reproduction were assessed. No acute effects were observed for both particle types. While tire particles were ingested by juvenile and adult *Daphnia* and ingestion increased proportionally to exposure concentration, fibers were not. Although *D. magna* did not ingest fibers, reproduction and survival were negatively influenced during chronic exposure at high concentrations. This was probably due to physical effects caused by an impeded movement due to entanglement of the organisms in agglomerates formed by fibers and algae provided as food. Tire particle decreased *Daphnias*' reproduction at high concentrations, which might be also in relation with associated contaminants in the tire particles that are released to the body after ingestion or due to leaching. The study indicates that the tested microplastic types do not negatively affect *D. manga* at environmentally relevant concentrations. However, in future scenarios for which higher concentrations are predicted, microplastics may affect freshwater invertebrates. Furthermore, this study highlights that microplastics with different characteristics (e.g. shape, composition, possible associated substances) may cause different kinds of effects. This confirms the need to assess the effects of different microplastic types.

3.22P.7
Effects of tire rubber and polystyrene microplastics in the gene expression of Chironomus riparius
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The contamination of worldwide waters with plastics in general and microplastics in particular is one of the most important **water risks** and **chemical pollution** episodes that has ever existed during the history of mankind. Although microplastics are nowadays a massive field of study in environmental sciences, there is still inconclusive and scarce information about the toxicity of different plastic polymers, particularly at the molecular level. We studied the gene expression profile of the aquatic insect *Chironomus riparius* in response to microplastics. The model organism, *Chironomus riparius* is a standardized species in toxicity tests of chemicals and an essential member of aquatic and terrestrial trophic chains. The microplastic materials tested were tire rubber and polystyrene (PS) at concentrations of 1 and 10mg L^{-1} in artificial freshwater. The particle sizes were 89.8 ± 44.7 µm for tire rubber and 38.9 ± 28.6 µm for PS. In addition, a control treatment without microplastics was set at the same conditions (20 ±1ºC, 16:8h light:dark, no feeding and constant aeration; n=3, 10 larvae per replicate). Chironomids were added as fourth instar larvae and they were exposed for 36h. Larvae were sampled, counted and frozen at -80ºC for later extraction of RNA and retrotranscription to cDNA. The genes monitored are related to the endocrine system, detoxification mechanisms, stress- and immune- responses and DNA repair. The results obtained showed that when organisms were exposed to microplastics, the transcriptional activity of several heat shock protein genes (*hsc70*, *hsp90*, *Gp93*, *hsp40*, *hsp60* and *hsp17*) was upregulated compared to controls. This occurred mostly at 10mg L^{-1} of tire rubber. In addition, the expression of the genes *SOD Mn* and *FKBP 39*, related to the mitigation of oxidative stress and to the endocrine system, respectively, was altered. Overall, the present study is the first that describes changes in the gene expression of insects in response to microplastics of two commonly used plastic materials. Furthermore, the study opens questions about the effects of long-term exposure to plastics in *Chironomus riparius* and aquatic insects.This work has been funded by the Ministerio de Ciencia, Competitividad y Universidades (SPAIN), CTM RTI2018-094598-B-I00, the Raija ja Ossi Tuuliainen foundation and The Kuopio Naturalists' Society. A.B.M.G is the receiver of a pre-doctoral contract from the National University of Distance Education (UNED).

3.22P.8
Crumb rubber toxicity in coastal marine systems
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Crumb rubber granulate (CRG) produced from end of life tires (ELTs) is commonly applied on indoor and outdoor synthetic turf pitches (STPs), playgrounds, safety surfaces and walkways. In addition to fillers, stabilizers, cross-linking agents and secondary components (e.g. pigments, oils, resins, fibers), ELTs contain high levels of organic additive compounds and heavy metals. While previous environmental studies have focused on terrestrial soil and freshwater ecosystems, in Norway many sites applying CRG are coastal. In the current study, the organic chemical and metal content of 'fresh' and 'weathered' CRG and their seawater leachates was investigated, and the uptake of crumb rubber by the brown crab (*Cancer pagurus*) was studied as an example of an exposure route for CRG to coastal marine organisms. A combination of pyrolysis gas chromatography mass spectrometry (py-GC-MS) and chemical extraction followed by GC-MS analysis revealed similar organic chemical profiles for pristine and weathered CRG, including additives such as benzothiazole, N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine and a range of polycyclic aromatic hydrocarbons (PAHs) and phenolic compounds (e.g. bisphenols). ICP-MS analysis indicated g/kg quantities of Zn and mg/kg quantities of Fe, Mn, Cu, Co, Cr, Pb and Ni in the CRG. A mixture of organic additives, metals and other inorganic compounds readily leached from the CRG into seawater. Benzothiazole was the organic compound with highest concentration (average of 136 mg/L), while PAHs (ranging from < LOD to 0.58 mg/L) and phenolic compounds (e.g. 2,4-bisphenol F and 4,4'-bisphenol F at 0.012 and 0.006 mg/L, respectively) were present in low abundance. Zn was the most abundant metal in the leachates (23.8 mg/L) followed by Fe (0.08 mg/L) and Co (0.06 mg/L). While organic chemical concentrations in the leachates stabilized within days, metals continued to leach out over the 30 day period. Brown crabs were exposed to two concentrations of CRG (0.5 and 0.05 g/L) in two size fractions (5 mm and 250 µm diameter) for 24 hours. Ingestion of the rubber and subsequent gut evacuation were studied over 5 days. Image analysis of filtered stomach contents confirmed uptake of rubber particles in different sizes, but also efficient gut evacuation upon transfer to clean water. We discuss the implications of CRG and leachate toxicity in acute and long-term exposure scenarios for marine coastal ecosystems.

Trace Metal Biogeochemistry and Fate in Ecosystems (P)

3.23P.1
Bioaccumulation of Arsenic and Heavy Metals in the White Muscle of Fish from Pools in the Upper uMzingwane Catchment Area Associated with Artisanal Small-scale Gold Mining in Zimbabwe
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There is evidence of extensive alluvial gold panning activity masked under the guise of Artisanal Small-scale Gold Mining (ASGM) along major river channels in the uMzingwane District in the Southern parts of Zimbabwe. This often illicit, unregulated practice is associated with a geological feature (greenstone) rich in, easily accessible, native gold. The present study was conducted to obtain an in depth assessment of the levels and distribution of selected metals associated with greenstone gold deposits (Arsenic, Cadmium, Copper, Chromium, Lead, Lithium, Manganese, Nickel and Zinc) and ore processing (Mercury) in the white muscle of 15 fish species endemic to the study site; bottom sediment and water samples collected at pools in the Upper uMzingwane catchment area. Bioaccumulation

factors were computed based on the habitat occupied by each fish species studied. The total Arsenic and heavy metal concentration in the respective fish white muscle tissue, bottom sediment and water samples were measured using an ICP-MS. The concentration of the various metals under study in the respective environmental samples were, to a greater extent, above international legislation limits and this suggests that ASGM may be involved in the introduction of toxic metals into aquatic ecosystems in the study area.

3.23P.2

Modeling Nickel Leaching from Abandoned Mine Tailing Deposits in Jossingfjord

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Underwater disposal of tailing and mineral products from mines in lakes and seas has been considered favourable due to the geochemical stability obtained during long-term storage in anoxic sediments. Some ores contain sulfide minerals. Metal sulfides are stable in the ore, but bioavailability and risk of toxic effects may increase in the water environment due to oxidation and transformation into more soluble forms. The goal of this work was to elaborate a model for simulations of Ni cycle in the water column, the Bottom Boundary Layer (BBL), and upper sediments and apply it to the mine tailing sea deposit in the Jossingsfjord. 1D benthic-pelagic coupled biogeochemical model, BROM, supplemented by a Ni module specifically developed for the study was used. The model was optimized using the field data collected in the fjord. We first simulated the "baseline solution" for the system - for conditions of the natural fjord seasonal variability with relatively small content of iron and nickel species entering the system and low burying rate (model years 1955-1960). Then there was simulated a 25 years period of intensive tailing contained particulate Si, Fe_2O_3 and NiS (from 1960 to 1984, SS=2.3 10^6 tons/yr) and since 1985 the period of restauration with modern day small scale tailing (SS= 455 tons/yr). Intensive tailing period resulted in changes of biogeochemical composition both in water column and upper sediment layer. Concentration of oxygen slightly decreased in the water column while high burying rate allowed it to penetrate in low concentration deeper into the sediment. Heterotrophs disappeared in the sediment under intense tailing deposition. Concentration of many dissolved parameters and content of all solid parameters decreased in the sediment (Mn(II), DOM, MnO2, POM). Concentrations of dissolved Fe(II) and total dissolved Ni increased a thousand times caused by reduction of incoming Fe(III) and oxidation and dissolution of incoming NiS. Numerical experiment with total closure of mining activities showed that Ni leaching from the sediments can be a source of Ni input to the water for about one decade. Ni leaching from the sea deposits may be prolonged due to sediment reworking by bioturbation at the sediment-water interface. Modelling results showed that doubling of bioturbation activity results in dissolved Ni concentration doubles in the water column and increases 10 times in pore water, Ni benthic flux triples.

3.23P.3

Sources of geogenic arsenic in surface water, a case study from the Spessart ranges, Germany

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The sources of arsenic in natural surface water is soften not known in detail. First investigations of As in surface waters in the Spessart ranges (mainly basic rocks diorite, gneis und amphibolite, carbonatic Zechstein sediments, Bunter sandstone), Germany, started in 1996 when the water concentrations exceeded the new limits of the European Union (10 mg/l). Some wells which were used as drinking water resources had to be closed because the As concentrations exceeded the limit. From hydrogeological catchment investigations, carbonatic Zechstein sediments combined with Baryt dikes were identified in some areas as natural As sources with highest As output. Bunter sandstone springs showed always As values below 10 µg/L. Since drinking water resources are limited in the Spessart ranges, new potential drinking water catchments are needed. In this study, two potential drinking water catchments with different geological settings were investigated, but high As concentrations were found, as well. The origin of potential As sources in this catchments were investigated. From literature studies, the potential As containing minerals and geological formations were identified: Zechstein copper shales and Permian rhyolite. Water samples were taken from creeks under differing hydrogeological conditions (dry and wet season). Total As concentration were analyzed using an ICP-MS (Zeiss Jena). Results show that total As concentrations are higher if copper shales are present in the catchment. The total As concentrations were lower during the wet season, when shallow and deeper groundwater is feeding the creeks. As is also present when rhyolite is the main geological formation in a catchment, but at lower concentrations. This is probably due to sparingly soluble As minerals in rhyolite. Preliminary calculations showed a dominance of the species As V. The aim of future research is the investigation of the distribution pattern of As species in water of the investigated catchments.

3.23P.4

A comparison of uranium exposure route toxicokinetics and toxicodynamics in the freshwater crustacean Daphnia magna

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Uranium (U) is a naturally occurring radionuclide with important economic value due to energy production and weapons development. Sources of U in the freshwater environment are vast and it is important to understand the potential impacts placed upon biota. Information lacks regarding dietary transfer of U along the aquatic food chain even though literature suggests that the primary pathway for U is through waterborne accumulation. There are few studies that compare U exposure route (dietary vs waterborne) kinetics, distribution and subsequent effects exerted upon organisms. The work presented here compares both aqueous and dietary uranium exposure routes in *Daphnia magna*. Aqueous exposures consisted of daphnia exposed for 48 hrs across a range of depleted U (DU) concentrations (10-250 µg/L) followed by 72 hrs of depuration. Dietary exposures consisted of algae (*Raphidocelis subcapitata*) exposed for 4 hrs to DU (50-500 µg/L) and subsequently fed to daphnia for 96 hrs. We show aqueous U accumulation is higher compared to dietary exposures, although a strong relationship exists between the transfer of uranium in the feed (algae) to the consumer (daphnia). Even though dietary transfer is comparably lower, results demonstrate that daphnia are affected differently based on exposure route. Mitochondrial ROS (reactive oxygen species) production increased due to dietary exposure, but not over a wider range of exposure to aqueous U concentrations. Furthermore, results demonstrate that major ion (Ca, Mg, Na, K) homeostasis becomes imbalanced when daphnia are exposed to uranium in the water but not by diet. The work highlighted here stresses the importance of understanding both uptake pathways as it pertains to potential risk placed upon aquatic organisms.

3.23P.5

Boron and Strontium isotope ratio analysis in freshwater samples of the German Rhine River as a tracer of anthropogenic contamination

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Boron (B) is applied in many industrial and everyday applications, e.g. as perborates in bleaching agents or as doping additive in the semiconductor industry. Due to its versatile and rising use, an increasing amount of B is discharged into the aquatic systems through wastewater. This is reflected not only in the B concentration, but also in the B isotopic composition of a sample. Therefore, isotopic analysis of stable B is a potential anthropogenic tracer. Due to many different inputs, such as urban waste water, diffuse emissions, or tributaries, rivers are considered rather complex aquatic systems. Therefore, it is challenging to identify possible contaminant sources and differentiate them from each other. The aim of this study is to identify anthropogenic sources and to distinguish them from geological sources, such as tributaries, using B and Strontium (Sr) isotopic compositions in freshwater. Thus, 76 freshwater samples from one of the heavily polluted rivers in Germany, the Rhine, were analyzed for their B and Sr isotopic composition. The B isotopic composition of the Rhine varies from $\delta^{11}B/^{10}B_{NIST951a}$ (-0.5 ‰ ± 1.1 ‰) to (+32.8 ‰ ± 0.9 ‰) with concentrations of 11.6 µg L^{-1} ± 1.3 µg L^{-1} to 65 µg L^{-1} ± 6 µg L^{-1}. The tributaries are distinguishable from the main river by higher B loads (33 µg L^{-1} ± 2 µg L^{-1} to 253 µg L^{-1} ± 17 µg L^{-1}). The Sr isotopic composition of the Rhine is rather heterogeneous with $n(^{87}Sr)/n(^{86}Sr)$ ratios ranging from 0.70858 ± 0.00015 to 0.70925 ± 0.00014 with a mean Sr concentration of 388 µg L^{-1} ± 68 µg L^{-1}. The tributaries are characterized by different Sr concentrations and Sr isotopic compositions (0.70871 ± 0.00014 to 0.71195 ± 0.00015). Although the B and Sr isotopic compositions of the tributaries differ from the main river, they were found not to significantly change the respective isotopic signature of the main river. Overall, the B isotopic composition of the Rhine shows a trend, with $\delta^{11}B/^{10}B_{NIST951a}$ values depleted in light isotopes and the B concentration increasing downstream. Looking at the industrial and population density along the Rhine, it is noticeable that both are increasing downstream of the Rhine. Thus, it is very likely that the B isotopic composition is mainly influenced by anthropogenic impact rather than due to the geogenic properties of the river basin. This study shows that the combination of Sr and B isotope ratio analysis can be used for a better understanding of pollution sources in river systems.

3.23P.6

Rapid Loss of Copper from the Water Column: A Comparison with PCBs

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Under the UN Globally Harmonized System of Classification Labelling and EU Classification, Labelling and Packaging regulation (CLP), the aquatic classification of a substance for chronic hazards is based on chronic toxicity and assessment of its degradability. Inclusion of degradability recognizes that, in the event of a release, effects from rapidly degraded substances are localized and of short duration due to reduced environmental exposure. This has been termed "rapid loss from the environment." For organic chemicals, rapid loss is determined by a standard biodegradability study demonstrating 70% conversion of the substance to CO_2 in 28 days. A similar approach has not been accepted for inorganics substances (i.e., metals). However, several publications have demonstrated rapid loss of metals from the water column with limited remobilization. One of the arguments against adopting the concept of rapid removal for metals in a regulatory context has been the fact that the principal mechanism of removal has been sorption to solids. This same removal process also applies to persistent, bioaccumulative and toxic organic substances, which do not biodegrade 70% in 28 days. This presentation compares and contrasts copper with PCBs. Data are presented to show that the total PCB production from 1930-1975 was 1.4 billion lbs whereas, copper production was 304 billion lbs with an additional 720 billion lbs coming from natural sources. PCBs have become a global contaminant of concern, whereas, copper has not. The key properties of bioaccumulation, biomagnification, lipid solubility, homeostatic control, and permanence in the sediments provide an explanation of why copper and PCBs in aquatic environment behave differently and have different concerns in spite of the fact that copper production/release far exceeds that of PCBs. These data argue that copper is rapidly lost from the water column.

3.23P.7

Physiological performances of common carp (Cyprinus carpio) are more impacted by tertiary Cu/Cd/Zn metal mixture at high temperature

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Metal contamination of the aquatic environment is problematic due to the bioaccumulative, non-biodegradable and toxic proprieties of these elements. Several studies have demonstrated the impact of single metal exposures on the physiological performance of fish but their results are sometimes contradictory, with metals showing different effects according to the concentration used and/or the duration of exposure. Moreover, these experiments were done on single metal exposures and rarely looked at the impact of combined factors. In our study, we investigated the combined impact of tertiary metal mixture (Cu/Cd/Zn) and temperature on the physiological performances of a fish model species, the common carp *Cyprinus carpio*. At 10°C and 20°C, fish were subjected to Cu/Zn/Cd mixture exposure at concentrations representing 10% of their 96 h LC_{50} (Cu = 4.8 µg.L^{-1}; Cd = 2.9 µg.L^{-1} and Zn = 206.8 µg.L^{-1}) for 12 hours, 1 day, 3 days and one week. Their standard (SMR) and maximum metabolic rate (MMR) were measured, aerobic scope (AS) was calculated and hematocrit, metal accumulation (Cu, Cd and Zn) and tissue damage in the gills were analyzed. Our results prove that temperature has a more profound effect than the metal mixture on the fish performance, especially at this sublethal concentration. Aerobic scope, MMR and SMR are elevated at 20 °C. Standard metabolic rate and hematocrit content were the only parameters affected by the metal mixture treatment and increased after 3 days of exposure at 20°C, demonstrating that the fish are more impacted by the metal mixture at the higher temperature. Finally, the accumulation of metal (especially Cu and Cd) in the gills during the exposure to the metal mixture reflect the variation of the SMR observed at 20 °C. To conclude, these results, and the absence of lactate accumulation, indicated that common carp can cope with these levels of metal pollution during short-term exposure.

Water Quality under Pressure - Understanding Fate and Effects of Organic Pollutants in Rivers by Integrating Field, Laboratory and Modeling Approach (P)

3.24P.1

Agricultural practices and fishpond management, a comprehensive inventory of phytopharmaceuticals fluxes from the watershed to downstream rivers.

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In France the use of phytopharmaceutical products (PPP) is significant, leading during the last decade to a seventh place worldwide in term of consumption, with more than 70 000 tons of active substances sold in 2015. Our laboratory works on processes of contamination of the aquatic matrix by the agricultural lands subjected to phytosanitary treatments, and then on the dynamics of these pollutants inside peculiar ecosystems that are fish ponds. In France, these small water bodies represent more than 3500 km² and are often found to be at the very beginning of hydrographic networks (first and second Strahler's order) thus impacting downstream river quality. However, because of their relatively small size, their qualitative monitoring does not fall within the limits defined by the Water Framework Directive (WFD), making them understudied regardless of their high remediation potential. Indeed, our previous study carried out on three dam ponds have demonstrated a reduction of pesticide concentration peaks as well as a reduction in potentially deleterious effects for downstream ecosystems. However, this buffer effect regarding PPP, was lacking several elements to understand the functioning, as well as the effects ponds have on pesticides pollution. In the present study, we wanted to clarify and deepen our knowledge on the functioning of ponds, and processes involved in potential remediation between upstream and downstream rivers. During a complete fish production cycle, we monitored the concentrations of several pesticides, between the agricultural catchment area and streams located upstream and downstream of a Lorraine pond (54). Dissolved and particular fluxes were monitored for 31 pesticides and 60 associated degradation products during the sampling period. In parallel with the upstream / downstream monitoring, a monthly sampling was also carried out on the aquatic and sedimentary matrix, in order to highlight the processes inducing the buffer effect of the ponds. Our work allowed to highlight the importance of the conjugated monitoring of parent molecules (found upstream of the pond) but also degradation products (found downstream). In addition, we have also highlighted the importance of the sedimentary compartment in the storage of PPPs, as well as its supply through indigenous and allochthonous suspended matter. The latter may be non-negligible when one considers the molecules having a high Koc, and thus a high affinity with the particular compartment.

3.24P.2

Spatial and Temporal Variability of Metformin Transformation in a Flume Study

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The antidiabetic drug metformin is among the most commonly prescribed pharmaceuticals globally. Extremely high concentrations of metformin (up to 47 µg/L) and its major transformation product (TP) guanylurea (up to 222 µg/L) have been reported in effluents and surface water. Here we present the results of a large-scale flume study investigating spatial and temporal variability of metformin degradation in relation to seasonality, TP formation, hyporheic exchange flow, bacterial abundance and diversity, oxygen and nutrient gradients. Mesocosm experiments were conducted using 100 m long recirculating flumes at the artificial stream facility and pond system of the German Environment Agency (UBA, Berlin, Germany) that were spiked with 10 µg/L metformin. The experiment was run from October-November 2016, and then repeated from May-August 2018. Surface and pore water were sampled and analyzed for non-reactive tracers and inorganic solutes (BO33−, LiBr, NH4, SO4, Fe2+) while metformin and TPs were quantified and identified using (UHP)LC-MS/MS and LC-HRMS/MS methods. O2 profiles, and pore water flow velocity were also measured during the experiment. Sediment microbial communities were characterized using quantitative real-time PCR and time-resolved amplicon Illumina MiSeq sequencing. Pore water concentration time series and tracer data were subsequently used to calculate first-order removal rates and retardation coefficients via one-dimensional reactive transport modeling. Flume data were further compared to our previous field and mesocosm studies. The DT50 of metformin in flume surface water was lower in summer (42 days) compared to fall (136 days), consistent with field observations. Metformin as well as its major TP guanylurea were removed in hyporheic sediments and we observed unusually high retardation of metformin. Both higher bacterial abundance and diversity improved metformin degradation, consistent with prior flume studies. Shorter flow paths and sediment dune structures appeared to encourage degradation which might be due to higher O2 availability. Our data suggest that restoration measures designed to increase hyporheic exchange flow will increase the reactivity of metformin and guanylurea on the reach-scale and the efficiency of rivers towards contaminant removal. We further conclude that promoting bacterial abundance and diversity will stimulate the in-stream removal capacity of rivers.

3.24P.4

Benthic organisms affect the degradation of trace organic compounds during bank filtration: Effects on redox zonation of littoral sediments

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Trace organic compounds (TrOCs) such as pharmaceuticals or personal day care products are measured in surface waters in the range of ng/l to µg/l. During bank filtration surface water flows through littoral and aquifer sediments towards drinking water abstraction wells. In the littoral sediments transformation and/or degradation of TrOCs can occur depending on the redox conditions. Existing studies on these processes focus on simplified sediments while in reality, benthic organisms such as submerged plants and mussels cover littoral sediments. We hypothesize that these organisms considerably impact the redox zonation and consequently the degradation of TrOCs during bank filtration. To test this hypothesis, we conducted a field investigation in a temperate lake used for bank filtration (Lake Müggelsee, Berlin, Germany) in June 2019. We installed dialysis membrane samplers at three different sites (covered by submerged plants, dreissenid mussels or none of these) within the sandy littoral zone for two weeks to sample pore water. Depth profiles of redox parameters, e.g. concentrations of sulfate and manganese, and oxygen were measured via IC, ICP-OES and oxygen microsensors. Oxygen loggers were installed above the sediment and sediment cores were taken. First results show that the mussel coverage has an impact on oxygen concentrations and its daily dynamics in the water column: Maximum oxygen concentrations in the surface water were lower and delayed at the site covered by mussels compared to the plant and control site. Mussels altered the sediment's hydraulic conductivity and sediment composition which affects water residence time and flow paths. Despite lower oxygen concentrations in the overlying water, higher sulfate pore water concentrations were found at the site covered by mussels compared to the other two sites indicating less anoxic conditions. Our field results, thus, show that benthic organisms, especially mussels, considerably modify oxygen concentrations and redox zonation of littoral sediments. The effect of these changes on the degradation of selected TrOCs (mainly pharmaceuticals like Valsartan and Gabapentin) will be further investigated in a follow up laboratory column study.

3.24P.5

Evaluation of a global multiscale multimedia fate model framework applied to home and personal care products in Europe and North America

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Regulatory environmental exposure scenarios are typically conducted using coarse grained approaches and are often criticized for their lack of spatial and temporal resolution which can create large variations in predicted exposure concentration for many ingredients. This is a typical scenario particularly for home care and beauty and personal care (HC & BPC) products which have a wide dispersive use and are generally discharged into fresh or marine surface water via sewer systems. Here we present an evaluation of the updated previously published Pangea/EcoHope multi-scale multimedia model, incorporating the hydrological dataset HydroBASINs. The focus is on the geographies of North America (NA) and Europe to compare freshwater and sediment predicted environmental concentrations (PECs) to measured environmental concentrations, building on a previous evaluation in Asia. An emissions inventory for five HC & BPC case study ingredients was created including: Linear Alkylbenzene Sulphonate (LAS), Triclosan, D5, Octocrylene and Octyl Methoxycinnamate. ScenAT, an environmental exposure model, and SimpleTreat were used to derive predicted, spatially explicit emission inventories for Europe and NA, which incorporates population, water use, connectivity to sewage treatment and removal mechanisms in sewage treatment plants. The Pangea/EcoHope framework was then run to simulate the fate and transport of these ingredients across Europe and NA with higher spatial resolution in regions of high emissions and populations. Georeferenced monitoring data across Europe and NA were collected from the scientific literature. In total 951 monitoring samples (freshwater: 689, sediment: 262) across 16 countries (NA: United States and Canada; Europe: Denmark, Germany, Greece, Hungary, Italy, Norway, Poland, Portugal, Romania, Slovenia, Spain, Sweden, Switzerland and United Kingdom) were collated in order to perform a comparison between modelled and monitoring values. Triclosan was the most data rich ingredient with 262 and 115 monitoring points identified for Europe and NA respectively. Here we present initial outputs of the comparison.

3.24P.6

Fate and effects of agricultural and urban organic pollutants in a small river catchment

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Water quality in rivers is affected by pollutant emissions from agricultural and urban areas under different weather and, thus, discharge conditions. Transformation processes in the field are not well understood with results from lab experiments being hardly transferable to environmental conditions. The aim of the study, in the frame of the CRC CAMPOS – Catchments as Reactors, was to identify dominant input sources of organic pollutants into the Ammer River and its tributaries, in the vicinity of Tübingen, Germany, and dominant attenuation processes and their driving factors along the main river by analysing chemical and toxicological profiles. We integrated field sampling of the main river Ammer, its tributaries and headwaters with chemical target and non-target analysis as well as with toxicity analysis with cell-based bioassays. First screenings provided a chemical pollutant inventory of over one hundred target chemicals in the low ng L^{-1} concentration range, among them pesticides (herbicides, fungicides and insecticides) and their metabolites, pharmaceuticals, industrial and household chemicals. Chemicals associated with agricultural activities such as pest control were found both in the main stem and in the tributaries. Under base flow conditions, the chemical and toxicological pollutant profile in the Ammer main stem was dominated by inputs via wastewater treatment plants (WWTPs). Lagrangian sampling along a selected river stretch downstream of the WWTP allowed quantification of attenuation processes of target compounds selected as representatives for different transformation pathways like photo- and biodegradation. Sampling campaigns following moderate to strong rain events indicated different pollutant mass flux dynamics related to the pollutant source (urban vs. agriculture) and particle-associated pollutant mobilization. Next steps will focus on urban in comparison to agricultural areas as pollutant source and further analyse the pollutant emissions and dynamics following rain events of different strength. **Acknowledgements**: This work was supported by the Collaborative Research Centre 1253 CAMPOS (Project P1: Rivers), funded by the German Research Foundation (DFG, Grant Agreement SFB 1253/1 20147).

3.24P.8

Dynamics of mixture effects and causative chemicals during rain events in rivers in agricultural and urban areas

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During dry weather conditions, the main inputs of organic pollutants into rivers stem from wastewater treatment plant effluents and diffuse pollution sources. During rain events, fast run-off components from agriculture and sealed surfaces of urban areas are additional input sources. An integrated analytical and bioanalytical approach was applied to track changes in the water quality and to characterize potential input sources of organic micropollutants into the Ammer river, Tübingen (Germany). The case study comprised three predominantly rural and two predominantly urban sites. Grab samples were taken on four consecutive days following a moderate rain event, with a reference sample drawn on each site after a longer dry spell. All samples and blanks were enriched by solid phase extraction and analysed with liquid chromatography and tandem mass spectrometry. In total, 51 pesticides were quantified. Concentrations and calculated mass fluxes indicate that the rain event site-specifically mobilized additional pesticides that were only present at low concentrations during dry weather while other chemicals were diluted by the additional discharge. The water extracts were measured by four in-vitro bioassays covering the environmentally relevant mode of actions of activation of the aryl hydrocarbon receptor (AhR), estrogenicity (ER), oxidative stress response (AREc32) and photosynthesis inhibition (combined alga assay). Iceberg modelling using the bioanalytical equivalent concentration approach was applied to estimate the contribution of the detected target analytes (pesticides) to the observed effects and to identify, which of the known chemicals are forcing agents for the observed mixture toxicity. This study shows that even moderate rain events can lead to a substantial increase in concentrations of individual pesticides almost without changing the non-specific toxicity and modes of action that are not necessarily associated to pesticides (AhR, ER) but had a substantial influence on algal toxicity in cases where there was an additional input of herbicides. Acknowledgements: This work was supported by the Collaborative Research Centre 1253 CAMPOS (Project P1: Rivers), funded by the German Research Foundation (DFG, Grant Agreement SFB 1253/1 20147).

3.24P.9

Landscape level exposure assessment of pesticide concentration at drinking water abstraction locations - the road towards an EU-wide tiered approach

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The exposure assessment of plant protection products (PPP) at drinking water abstraction points is of growing interest for authorities, water suppliers, industry, and other stake holders and is hence particularly addressed in the EU regulatory framework (regulation 1107/2009). However, there is no generic guidance available on the derivation of drinking water abstraction concentrations in the EU.

An exception is the national approach of the Netherlands, a simplistic but very solid first Tier approach, which considers edge-of-field PEC_{sw}, use intensity including cropping area within a drinking water catchment, application practice and dissipation in the water system amongst others. The Dutch approach underlies worst-case assumptions e.g. all agricultural land is connected and releases water to a water body. Our work explores the feasibility of a general tiered EU-wide approach to derive realistic PPP concentrations at drinking water abstraction points. Specifically, our goals are: (i) the characterization of EU-wise drinking water catchments, (ii) the identification of vulnerable catchments based on agricultural area or specific crops, (iii) to enable substance specific modelling for agricultural area/crop using a landscape-level assessment model. On this account, we analyzed the European catchments for specific crops on the basis of the Water Framework Directive (WFD). The focus was on catchment characteristics (e.g. crop area, soil hydraulic properties) which have a strong impact on runoff as well as drainage generation and therefore on the mixing of PPPs in surface water. In a first step, the spatial variation of the mixing factor by crop area was investigated taking into account the stream course from headwater catchment to a larger main river. In the second step, we identified typical abstraction areas for surface water and groundwater using proxy data (e.g. protection zones and other proxy data) with the aim to explore the most vulnerable combinations in the EU. These data can then be used for the definition of specific (vulnerable) scenarios regarding the mixing of PPPs in surface water for a specific crop on EU level. It is expected that these data in combination with landscape-level modelling using the Soil and Water Assessment tool (SWAT) can be used as starting point for a tiered exposure assessment to derive generic mixing factors and drinking water concentrations at abstraction locations.

Non-Extractable Residue (NER) in Regulatory Testing of Chemicals (P)

3.25P.1

Implications of application solution on NER formation and substance degradation in soil

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In this work, we will present the story of the "persistence" investigation of a development candidate of a fungicide. In former experiments, this compound showed no degradation during a 120 day OECD 307 soil degradation study (DT_{50} >1000 days). Because persistence would be a cut off criteria, we started a new OECD 307 guideline study with ^{14}C-radiolabeled test item with six sampling points (0, 10, 20, 40, 61, 122 days) with two concentrations (1 mg/kg, 10 mg/kg) in two soils (clay loam, sandy loam) and two replicates. Full material balances were established (CO_2, NER, soil extracts) as well as extract analysis by radio-HPLC. In contrast to the original data, in this study good degradation could be observed. To explain the different findings, we investigated in a new approach the experimental differences between the two studies, *the solvent of the application solution*. In this investigation, ^{14}C-radiolabeled test item was applied on the same two soils, with three sampling points (0, 62, 120 days) with a concentration of 1 mg/kg - once with 200 µL acetonitrile, once with 400 µL MeOH:H_2O (1:1, *v:v*).As a main result, we can clearly show, that the solvent of the application solution has significant impact on the microbial degradation and mineralization (CO_2) of the test item as well as on the NER formation. The MeOH:H_2O application showed in both soils higher mineralization, NER formation and test item degradation as the acetonitrile. Acetonitrile, which is considered toxic for the microbial community, together with the low mobility of the substance (e.g. low water solubility) and the fungicidal activity, could have let to the initial determined "persistence". To sum up, with this experimental comparision, we could elucidate one reason of the difference between the original "persistence showing" study, which was performed with acetonitrile as application solvent, and the recently done OECD 307 study with our "standard" MeOH:H_2O application solution. In addition, in this case we can state, that by reviewing the overall distribution of the residues, the formed NERs could be considered as fully metabolized biogenic bound residues and not as entrapped and bound parent substance.

3.25P.2

Remobilization of non-extractable residues of 14C-labelled ionic compounds in soil

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Many organic compounds form non-extractable residues (NER) in degradation simulation tests with soil or sediment. Due to the lack of analytic methods, substances non-extractably bound to the soil matrix are so far not included in the persistence assessment. However, it remains unclear whether NER acts as an irreversible sink, or if the formation of NER can be seen as a temporary stabilizing process, thereby making remobilization of the NER possible. NER can be divided into three types: Sequestered (type I) NER are physically trapped or strongly sorbed to the soil matrix and are considered potentially remobilisable. Covalently bound (type II) NER are considered to be tightly bound to the soil matrix, therefore it is unlikely that they will be released. Biogenic (type III) NER consists of e.g. amino acid, fatty acids etc. and may also be part of type I and II NER. Since they are indistinguishable from compounds formed from degradation of natural organic matter, type III NER are of no environmental concern.In addition, it is currently unclear how the environmental fate of organic compounds is affected by the presence of an ionic functional group. However, many organic compounds such as biocides, pesticides pharmaceuticals and industrial chemicals are ionic at environmental pH.In simulation studies according to OECD 307, the remobilization of NER of the three model substances, 4-n-dodecylphenol [phenyl ring-14C (U)] (14C-DP), 4-n-dodecylbenzenesulfonic acid sodium salt [phenyl ring-14C (U)] (14C-DS) and 4-n-dodecylbenzyltrimethylammonium chloride [phenyl ring-14C (U)] (14C-DA) with high structural similarity were investigated. At neutral pH DP is present as non-charged molecule, DS as anion and DA as cation. For the investigation of NER-remobilization, extracted soil containing 14C-NER of DP, DS and DA after 7 and 84 days of incubation were respectively mixed with fresh soil (ratio 1: 2) and incubated for 54 days. The samples were sequentially extracted to quantify the formation of volatile, mineralized and extractable residues (ER) and portions still remaining non-extractable in soil. To characterize the present NER, type I and II NER are quantified by silylation procedure followed by thin-layer-chromatography (TLC) analysis to clarify if type I NER contain the parent substance. Type III NER were quantified by acid hydrolysis followed by two-dimensional TLC. NER characterization will be compared for the neutral, the anionic and cationic chemicals.

3.25P.3

Characterization of NER and comparison with bioavailable fraction in aerobic soil degradation of 14C-Phenanthrene

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Regulation of chemicals in Europe is subject to the REACh (EC 1907/2006), Biocidal Products Regulation (EC 528/2012) or plant protection products regulation (EC 1107/2009). In these regulations, the degradation of chemicals is tested by application of simulation tests such as soil degradation according to OECD test guideline 307. During degradation in the soil most chemicals will form non-extractable residues (NER) which can be determined only if the test has been performed with isotopic labelled chemicals. NER may contain possible remobilizable and potentially hazardous (so-called type I NER), non-remobilizable covalently bound (i.e. type II NER) as well as biogenic (type III NER) fractions. Up to now, a standardized method for characterizing and quantifying these different fractions is still lacking. The aim of this project is to evaluate methods for differentiation between different types of NER as well as to compare the results with complementary methods for determination of potential bioavailable fractions. Therefore, we tested the degradation of ^{14}C-labelled Phenanthrene in two different soils according to OECD 307. The soil samples were exhaustively extracted by aqueous and solvent extraction followed by harsh extraction using accelerated solvent extraction (ASE). The remaining NERs were quantified by combustion analysis. These NERs were subjected to different further extraction procedures in order to quantify the different types. For release of type I NER (entrapped parent compound and/or transformation products) and differentiation to type II (covalently bound residues) the soil samples were subjected to a silylation procedure, while for determination of the biogenic fraction the soil residues were extracted by 6N HCl for hydrolysis of peptides and other biological macromolecules. Simultaneously an extraction of another soil aliquot with 0.1M EDTA will be performed to compare with silylation. In additionto this work, soil samples were incubated in parallel under identical conditions and extracted using a 3-phase extraction with Tenax according to ISO/TS 16751:2018 in order to determine the potentially bioavailable fraction. These results were compared with those for the dissolved phase (aqueous extraction) and total extractable concentration (solvent extraction with ASE) to understand temporal trends in test substance bioavailability.

3.25P.4

Comparison of EPA and ECHA guidance on characterization of non-extractable residues (NER) in degradation assessment

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Following the publication of a guidance for addressing unextracted residues in laboratory studies involving soils and sediments in September of 2014 by the Environmental Fate and Effects Division (EFED) of the United States Environmental Protection Agency (US EPA), the European Chemicals Agency (ECHA) has recently published a discussion paper on improving the interpretation of Non-Extractable Residues (NER) in degradation assessment (June 2018). The

EPA promulgated an approach aimed at removing weakly sorbed residues using solvents of different polarities. The ECHA recommendation on the other hand offers an elaborate extraction procedure and a way of discriminating between the different NER fractions and identifying the relevant fraction for persistence assessment. This presentation will discuss results of several soil and sediment degradation studies performed based on each guidance and the challenges associated with their execution. These studies were mainly performed with plant protection products (PPP); but results of a few pharmaceutical API's and household products will also be presented. It was generally observed that the EPA recommended additional extractions with less polar and non-polar solvents collectively removed ≤5% of applied radioactivity following polar solvent extractions. More work is needed on the ECHA approach in order to better understand the NER and how to implement procedures in the routine work in most laboratories. Non-extracted residues were further characterized via kinetics modeling. The kinetics modeling approach provides an additional tool that was used to explain the formation of radioactive residues and CO2 by fitting the data to NER and CO2 as separate compartments. The data fit that will be presented as an illustration and explained here as a PPP parent compound that was demonstrated to be incorporated into NER. The optimized parameters for this fitting indicate that approximately 50% parent is becoming bound while the other 50% is degrading to metabolites (kParent_NER and kParent_Met = 0.13), with very little mineralization (kMet_CO2 = 0.0014).

Semi- and Non-volatile Organic Compounds in Indoor Dust and Air: Early Warning Signals for the Outdoor Environment and Indicators of Indoor Environmental Quality (P)

3.26P.1

Influence of insulation materials and position on human indoor exposure to SVOCs

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Insulation is a key element for every new building or during renovation process to achieve high energy-efficiency performance under Western Europe climate. However, insulation materials may release additional pollutants, including semi-volatile compounds (SVOCs), with adverse health effects in the indoor and outdoor environment. Therefore, the choice of insulation is also crucial for the indoor air quality issue, and we propose in this study to compare indoor exposure to insulation materials according to their relative position in the wall. Insulation can be situated either on the inner side of the wall (i.e. before the concrete layer) or alternatively on the outer side of this concrete layer. The present study focuses on organic substances contained in three organic rigid foamy insulation materials (polyurethane (PU), polystyrene extruded (XPS) and polystyrene expanded (EPS)) and in additives used in concrete masonry units. Four exposure pathways are considered: inhalation, dust ingestion, gaseous skin uptake, direct dermal contact. The concrete layer acts as a buffer zone for SVOCs (e.g., intake fraction of TCPP contained in PU is almost null with outer insulation), while on the contrary, VOCs diffuse quickly enough through concrete to reach indoor air compartment over the material's lifetime (e.g., indoor air concentration of formaldehyde contained in PU is dampened with outer insulation but the mean concentration over 50 years is the same). Triethanolamine contained in concrete additive is entirely emitted indoors when insulation is outer, and its non-carcinogenic effect is high. As a result, for both polystyrene insulations with outer insulation, indoor damage increases with outer insulation because it is dominated by additives emissions-related impact. On the contrary, human toxicity from exposure to PU's emissions is dominated by TCPP and formaldehyde, and damage is halved with outer insulation. To conclude, materials' relative position highly influence contaminant of SVOCs indoors. Outer insulation is a good mean to reduce indoor exposure to insulation materials offgasing emissions. However, exposure to chemicals contained in concrete's additives increases and care should be taken to avoid nocive additives within concrete.

3.26P.2

Greenhouse gases and volatile organic compounds from degrading plastics

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The global production of plastics has grown exponentially in recent decades due to their low cost, malleability and many other advantages. However, as plastic materials continue to accumulate in the natural environment, there is still a lack of understanding regarding mechanisms of degradation and the eventual fate of these materials. The identification of greenhouse gases (GHGs) and volatile organic compounds (VOCs) released from the degradation of plastics is crucial in understanding the fate of plastics and what secondary threats are posed. Many studies have investigated the release of compounds from marine environments, on landfill sites and through recycling processes but few have quantified the release of gaseous compounds through abiotic methods, such as thermal degradation or photo degradation with UV light. This study aims to characterise and quantify the amount of GHGs and VOCs released through these abiotic chemical reactions as well as biodegradation, using selected strains of bacteria. The plastic polymers used in the study will be common types of plastics found in everyday life, typically within indoor environments, including Polyvinyl chloride (PVC), Polypropylene (PP) and Low density polyethylene (LDPE). Different chamber methods for degrading these plastics will be evaluated to assess their potential for capturing and measuring GHGs and VOCs released. Once a suitable method is devised, air samples will be analysed through Gas Chromatography Mass Spectrometry (GC-MS) and Proton Transfer Reaction Mass Spectrometry (PTR-MS) to determine concentrations of gaseous products as the plastics break down. The results will be discussed and also, further insight from indoor air quality models will be presented.

3.26P.3

In vitro assessment of cytotoxicity and receptor activity of flame retardants and phthalates

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According to recent research and studies, there are concerns of pollution of indoor environments with semi-volatile organic compounds as a result of increase in atmospheric temperature which consequently affects indoor temperature. Particularly, flame retardants and phthalates from many household and industrial products have been hypothesized to become the main pollutants in indoor environments. These pollutants are known to persist in the indoor air and have the potential to bioaccumulate in exposed individuals. With limited toxicological knowledge about the mechanisms underlying their activities, there is the need to further investigate the biological responses associated with these compounds as an effort to establish their potential cellular effects for better risk assessment. We analyzed biological responses using in vitro assays for toxicity endpoints of high relevance for human health for three flame retardants triphenyl phosphate (TPP), Tris(2-chloroethyl) phosphate (TCEP) and, Tributoxyethyl Phosphate (TBEP) and five phthalates (Diethyl phthalate (DEP), Dibutyl phthalate (DBP), Butylbenzyl phthalate (BBP), di-2-ethylhexyl phthalate (DEHP), di-(2-ethylhexyl) terephthalate (DEHTP), a replacement for di-2-ethylhexyl phthalate, and 2-ethyl-hexanol (2EH), precursor for the synthesis of DEHP. The non-cytotoxic concentrations of the chemicals used ranged from 1.96 µM to 125 µM for the flame retardants and phthalates and 62.5 µM and 1 mM for 2EH. Reporter gene assays were used to analyze receptor activity of the aryl hydrocarbon receptor (AhR), estrogen receptor (ER), androgen receptor (AR) and induction of oxidative stress by the nuclear factor erythroid 2-related factor 2 (Nrf2) in Hepg2, VM7Luc4E2, CHO and MCF7c32ARE cells respectively. All the cells used were stably transfected with the firefly reporter construct except for the HepG2 cells which we transfected with pGud-luc and Renilla plasmid DNA. ER and AR antagonist activities were detected in all the flame retardants and phthalates although at high concentrations. However, a slight estrogenic activity was observed in BBP. Further, an induction of AhR was observed in TBEP at high concentration (31 µM – 125 µM). No Nrf2 activity was observed in any of the chemicals tested.

Chemicals in Short- and Medium-lifespan Products: Opportunities and Challenges to a Circular Economy (P)

3.27P.1

Food packaging in the circular economy: Identification of hazardous chemicals and human exposure

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Food packaging is a type of food contact article (FCA) that protects food, enables storage, transport, transfer of information, marketing, and can facilitate food consumption. It is a short- and medium lifespan product since many packaged foods are consumed within days after purchase and some have a shelf life of up to three years. Since many foodstuffs are products of mass consumption, their packaging is, too. But food packaging is also a main component in European household waste, with ca. 100kg per person annually. To remediate environmental impacts associated with food packaging production, use, and end-of-life, the European Commission has set packaging recycling targets that support the transition to a circular economy. However, a critical rebound effect of food packaging in a circular economy is often not discussed, namely chemical contamination and related impacts on human health. To investigate this, we compiled the publicly available information on what chemicals are used in the manufacture of FCAs, including food packaging. The Food Contact Chemicals Database (FCCdb) contains more than 11'500 unique food contact chemicals (FCCs) that may be used in the manufacture or that may be present in finished FCAs. We compiled the FCCdb from global regulatory lists and industry inventories. We then explored FCCs' hazards by consulting harmonized classifications for human health and the environment, plus endocrine disruption and persistence-related properties. This analysis shows that ca. 600 hazardous FCCs are candidates for substitution. Moreover, structure-based predictions revealed several hundred more FCCs as potentially having similar levels of concern but lacking official classifications. Importantly, a large proportion of FCCs have no toxicity data, indicating a major knowledge gap. In a next step, we developed and applied a systematic mapping protocol to integrate the scientific evidence on FCCs that migrated or were extracted from FCAs. The resulting evidence map is based on ca. 1500 data sources and can be used to recognize trends, enable systematic reviews and address knowledge gaps. In the course of the project, FCCs with migration data will be compared to human biomonitoring studies to obtain evidence of human exposure to FCCs. This information will be useful for establishing the chemical safety of food packaging in the circular economy, because it highlights known hazardous chemicals as well as pertinent knowledge gaps.

3.27P.2

Establishing a comprehensive database of chemical additives used in plastics

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Plastics pervade every aspect of our modern life due to their cheap price and versatility associated with different chemical additives therein. These plastic additives may provide not only desired functionalities such as flame retardancy and plasticity, but also features such as color and smell that make the products more attractive to consumers. However, many additives are not chemically bound to plastics, and may be released during the production, use, disposal and recycling of plastics, leading to human and environmental exposure to these chemicals and causing adverse effects in some cases. In contrast to the broad range of plastic additives that are currently used, the current research and risk assessment have focused on a limited number of chemicals such as heavy metals, phthalates and brominated flame retardants. One major cause of such phenomenon is that the identities of many plastic additives are yet unknown/unfamiliar to the wide scientific and regulatory community. To combat this ongoing shortcoming, a comprehensive database of plastic additives is compiled in this study. The database builds on publicly accessible sources including existing regulatory databases, industry databases, industry lists, industrial handbooks, and peer-reviewed literature. The uses (in terms of industrial sectors or plastic products), performed functionalities (e.g. color, flame-retardancy), regulatory status, as well as toxicological and physicochemical properties are compiled for all plastic additives. More than 7000 compounds are potentially present in plastic products. In addition, there are substantive information and transparency gaps: for a majority of plastic additives it is not possible to identify the products/polymers in which they are used, the concentrations at which they are used, or their intended functionalities. Only a limited number of plastic additives have been (rather) well-studied, and comprehensive hazard profiles can be found. The common use of many metal element-containing substances further complicates the hazard and risk assessments of all plastic additives, particularly in terms of a lack of applicable QSARs to fill in data gaps.

Assessment and Management of Wastewater Effluents (P)

4.01P.1

Are micropollutant concentrations driving biodegradation in activated sludge treatment?

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A large number of micropollutants (MP) is entering our wastewater treatment plants (WWTP) and ultimately ends up in the environment. It is impossible to quantify all effluent concentrations, while emission estimates are important for assessing environmental risks. Therefore, environmental fate models are often used to predict MP removal and effluent concentrations, however, prediction estimates of biological removal remain poor. Here, we aim at assessing the influence of concentration on biodegradation of a large set of MPs, which we expect to be related to microbial activity such as enhanced transcription rates of specific enzymes or changes in microbial community composition. The influence of MP concentration on biodegradation was assessed with a meta-analysis, including activated sludge treatment plants around the globe, and in addition a laboratory experiment to underpin our findings. Our global dataset included data on 93 pharmaceuticals and personal care products from 41 studies. Preliminary data analysis shows that we find a significant effect of concentration on the biodegradation rate in 34 % of all MPs included in our global literature dataset. It is very likely that in 34% of the MPs included in our analyses, the microbial community composition changed in different WWTPs as a resultant of exposure history. An additional laboratory experiment should provide further insight on whether microbial community composition or enhanced enzyme transcription influence biodegradation. The efficiency of biological removal of MPs is dependent of concentration in some cases, which can contribute to modelling practices by further explaining variation in biodegradation rates of individual MPs.

4.01P.2

Photocatalytic atrazine degradation with the nanocomposite aerogel sPS/N-TiO2 under UV and visible light irradiation and toxicity evaluation of the process.

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The continuous release into the environment of recalcitrant and persistent micro-pollutants such as pesticides threatens both environmental ecosystem and human health. Wastewater treatment plants (WWTPs) based on activated sludge (AS) processes were not originally designed to deal with pesticides, which can therefore pass through them partially or completely unaltered. Removal of such molecules needs improved WWTs such as advanced oxidation processes (AOPs). Titanium dioxide photocatalysis has been extensively investigated in laboratory settings, but its scaling up is still limited by the cost associated to the energy consumption of UV lamps required for the photocatalysis and by the use of powdered catalysts. Intensive research has focused on the development of semiconductor photocatalytic nanoparticles (NPs) for the degradation and mineralisation of recalcitrant compounds. However, the efficiency of these processes is reduced by particle aggregation in water and the most common photocatalysts, such as TiO2 and ZnO, are toxic to both humans and aquatic life. AOPs must therefore be evaluated both for their degradation efficiency and, even when the parent compound is completely degraded, for the absence of ecotoxicity in the treated effluent. In this work, a visible light active photocatalyst (N-doped TiO2) was dispersed in a high porous polymeric matrix based on syndiotactic polystyrene aerogels (sPS) in order to obtain a nanocomposite sPS/N-doped TiO2 with high porosity and homogeneous catalyst dispersion. Photocatalytic tests were carried out in a pyrex cylindrical photoreactor operating in batch mode and irradiated with visible or UV light emitted by LEDs. Atrazine was chosen as a model pesticide at an initial concentration of 100 ppb. Although its use in the European Union was banned in 2003, atrazine is still present in the environment and studies on its degradation remain necessary. After 180 minutes of irradiation, tests showed an atrazine removal of 29 and 47% for visible and UV light, respectively. Toxicity assays with the model alga Raphidocelis subcapitata showed that treated effluents were still toxic after 180 minutes and inhibited cell growth by 82 and 93% for visible and UV light treatments, respectively. These results suggest that toxic by-products can be formed following treatment of atrazine-containing aqueous solution using AOPs. However, increasing the time of the process up to 3 days no toxicity is observed to model algae.

4.01P.3

PHARMACEUTICALS: STUDIES ON REMOVAL EFFICIENCY IN WASTE TREATMENT STATIONS AND TOXICITY TESTS CONSIDERING DIFFERENT FORMULATIONS

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Considering the increase of knowledge in the area of environmental chemistry and the performance of the scientific committee of toxicology, ecotoxicology and environment the need to obtain data on the effects of drugs on the environment was identified. The scientific literature comments that the presence of drugs in the environment is generally small compared to other chemicals however, the high persistence of several of these compounds and their continuous replacement increase the risk of chronic exposure to aquatic organisms, as well as to the humans. One of these compounds, Fluoxetine Hydrochloride, has been reported to cause disturbances in aquatic organisms. The present work presents the results obtained in the evaluation of drug removal efficiency in wastewater treatment plants, based on bench test, and toxicity tests for said compound in different formulations. The results obtained demonstrated that the study drug was partially degraded by the organisms in the test system with an approximate removal of 27%. The data obtained experimentally, are in agreement with what was predicted by the model of quantitative relation between structure and activity (Q'SARS), around 32%. The toxicity assessment of samples generated by the respirometry system showed a reduction in toxicity as biodegradation proceeds. The tests with *Ceriodaphinia dubia* showed reduction around 25% (EC50-24 hours = 0.87ppm -

1.16ppm) and with *Vibrio fischeri* organism reduction around 12% (EC50 = 28.50% and EC50 40.92%). The acute toxicity test (*Vibrio fischeri*) showed not only the toxicity of fluoxetine hydrochloride and its decrease in the biodegradation process (respirometry test), but also the problem inherent in the type of excipient used in the various dosage forms. The compounds analyzed presented different toxicities, being the generic drug more toxic than the commercial form and the pure drug (content < 90%) that presented the lowest toxicity. Tests indicate possible environmental accumulation with consequences harmful to aquatic organisms. Pharmacodynamic activities linked to drug ecotoxicology are sciences that can present more conclusive results of their risk and their formulations to environmental problems.

4.01P.4
Whole effluent toxicity testing: Are there alternative approaches?
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The US EPA has freshwater whole effluent testing (WET) methods that include 3 cladoceran species (Ceriodaphnia dubia, Daphnia magna, D. pulex), 4 fish species (fathead minnow (Pimephales promelas), bannerfish shiner (Cyprinella leedsi), rainbow trout (Oncorhynchus mykiss) and brown trout (Salvelinus fontinalis)), and 1 alga species (Raphidocelis subcapitata). These species are used in 11 codified methods with 3 cladoceran species and 4 fish species acute tests while the short-term chronic test methods use 1 cladoceran (C. dubia), 1 fish (P. promelas), and an alga (R. subcapitata). This is a small set of species for toxicity assessments and additional methods for sensitive species from different taxonomic groups are being investigated. . One such species is the fatmucket mussel (Lampsilis siliquoidea), as it has been demonstrated to be among the most sensitive of all aquatic species to some contaminants. The tests are initiated with juveniles (1- to 3-wk-old) and after 7-d, growth rates are determined. We are also developing a 4-7-d sublethal growth test for D. magna to be performed at 25°C. We will be testing several single chemicals and effluents for both species along with the standard species at two of EPA's research labs (i.e., Duluth and Cincinnati). Longer-term plans are to develop protocols for other taxonomic groups, e.g., mayflies, amphipods, midges, and a plant. With these additional species, we expect to enhance our understanding of the range of species sensitivities, aid in the detection of both known and unknown chemical and biological contaminants in wastewater and ambient waters and provide procedures to aid in prioritizing problem contaminants and mixtures. In this presentation, we will outline the general study plans for each species and the progress to date. We will discuss options for joining a listserv (or a similar system) to communicate the methods and incorporate the input from the scientific community during this development stage. Disclaimer: This presenta¬tion does not necessarily reflect the views or the policies of the USEPA.

4.01P.5
On-line biosurveillance of wastewater discharges by analysing the behaviour fo three invertebrates species : feedback in an urban context and on its relevance in different major industrial sectors
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Context Water resources, bio-diversity and human health are major societal concerns. One of the main prerogatives for preserving them is to limit the entry of chemical contaminants into the environment, but very few tools are available to assess these inputs online. To meet this need, a collaboration between the INRAE-Lyon ecotoxicology laboratory and ViewPoint has made it possible to develop a station that measures the toxic quality of treated water on site, in real time and with a 30-day autonomy. **Methodology used** Some of the effluent to be analyzed is diverted and enters a conditioning module to control its temperature and oxygen content. A constant flow (650 ml.min-1) comes into contact with the biological probes and their individual locomotor behaviour is measured in real time by video-tracking methods. The species used (Gammarus fossarum, Radix auricularia, Erpobdella testacea) for this online toxicity assessment method belong to different large phylogenetic groups and are representative of the receiving environments. Finally, the station's on-board computer measures the toxicity index from the individual distances travelled and is sent every two minutes to the water manager. F**eedback on urban and industrial step** The project with Saur industrial group consists in evaluating the purifying performance of a new chemical micropollutant treatment at the Saint-Fons urban wastewater treatment plant (1 million inhabitants equivalent). The site is equipped with online bio-monitoring tools upstream and downstream of this process. A year of continuous monitoring showed 1- different episodes of chemical contamination of the effluent, and 2- improvement of toxic quality through this organic chemical contaminant removal process. A project financed by the RMC water agency consists in demonstrating the relevance of the tool to the industrial context. Four sites with different types of waste, classified by sector of activity, were equipped (chemistry, surface treatment, dyeing and agro-alimentary). The first follow-ups show the need to adapt the station (filtration, dilution) or the acclimatization of biological probes according to the sectors of activity. the first months of follow-up show that this technology would be relevant to the industrial sector. The sensitivity of the tool has made it possible to provide useful knowledge on the variability of their effluent and thus to provide them with a real decision-making tool.

4.01P.6
Mitochondrial toxicity of chemical mixtures: a tool for whole effluent testing
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Mitochondria are cellular powerhouses as well as metabolic and signalling hubs, regulating diverse cellular functions, varying from basic physiology to phenotypic fate determination. Chemical contaminants may have an adverse effect on mitochondrial functioning, possibly targeting not only specific molecules within the mitochondria but also having broader effects such as alteration of electron transport chain activity, formation of mitochondrial DNA mutations, mitochondrial uncoupling, amongst others. In fact, chemicals introduced into the aquatic environment via wastewater are known to trigger disruptions of mitochondrial respiration. Understanding the role of anthropogenic contaminants in mitochondrial dysfunction, and its consequences in organismal fitness and survival, are important as these are key cellular organelles involved in a number of biological processes. In this study, mitochondrial toxicity of the effluent from a wastewater treatment plant (WWTP) was quantified in two different cell lines, human liver cells, HepG2, and gill cells of rainbow trout (*Oncorhynchus mykiss*), RTgill-W1, and its sensitivity compared. To that end, after collection, part of the effluent samples were submitted to a solid-phase extraction (SPE) and the resulting extracts tested for their toxicity with both HepG2 and RTgill-W1 cell lines. In parallel, for the remaining aliquots of the same samples, osmolality was corrected by dissolving solid amounts of salts, galactose and pyruvate up to the concentrations found in the Leibovitz's L-15 culture media. Such procedure allows the application of whole-water samples directly to the RTgill-W1 cells, being the response of the referred cell line when exposed to extracts and to whole-water samples compared. Subsequently, mitochondrial toxicity was quantified by measuring the change of the oxygen consumption rate (OCR) with the Agilent Seahorse XFᵉ24 Analyzer. As part of the 3Rs policy, the proposed methodology aims at improving the understanding of the effects of chemical mixtures on mitochondria functioning of an ecologically relevant fish species while presenting a new tool for the rapid assessment of the mitochondrial toxicity of effluents and chemicals.

4.01P.7
Endocrine effects of an advanced wastewater treatment plant using effect-based methods in situ and in vitro
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Currently, the pollution of surface water with micropollutants (MP) is one of the most crucial environmental problems. The inability of wastewater treatment plants (WWTP) to remove these MPs has become a growing concern. Effluent discharges still contain hormone active substances, which have the potential to elicit adverse effects on aquatic organisms at very low concentrations. These so-called endocrine disruptors can impact population structures longterm. Due to the inefficiency of conventional WWTPs to eliminate MPs, multiple studies have been performed to determine if a quaternary cleaning step can improve the quality of wastewater effluent being released back into the environment. One possible method is the ozonation of precleared water. This technique has been established on the WWTP Aachen-Soers within framework of the DemO3AC-project. The main goal of this project is to evaluate if ozonation can reduce the vulnerability of the biocenosis and significantly enhance the water quality of the wastewater effluent. By using a broad biotest battery and analytical chemistry, the status quo

of the Wurm River, which represents the receiving stream of the WWTP Aachen-Soers, has been investigated before and after implementation of the large scale ozonation plant. Within this work specifically, three different effect-based methods were used to detect potential endocrine disruption within the Wurm River, up- and downstream of the effluent discharge. Two in situ experiments, a reproduction test with the New Zealand mud snail and a vitellogenesis observation in rainbow trout were conducted. Furthermore, an in vitro bioassay panel was performed to investigate the estrogenic, anti-estrogenic and anti-androgenic potential in water samples, respectively. A significantly increased reproductive output in mud snails and an increased vitellogenin concentration in rainbow trout was found downstream the effluent discharge. On the other hand, a decreased estrogenic and an eliminated anti-androgenic potential was shown within the cell-based assays after ozone treatment. Anti-estrogenic potential has not been detected. All in all, the in vitro and in situ experiments showed different results, but the effects found within the in situ tests can potentially affect on population level, due to the reproductive endpoint. The project is funded by the Ministry for Climate Protection, Environment, Agriculture, Conservation and Consumer Protection of the State of North Rhine-Westphalia, Germany.

4.01P.9

Determination of 18 opioid compounds in urban wastewater (Valencia, Spain) by HPLC-MS/MS

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Recently, the human consumption of opioids has increased in an important way. This class of pharmaceuticals is used for treatment of moderate or severe pain, generally, associated with palliative therapies against the effects of cancer. The relatively low cost and the limited therapeutic alternatives for chronic pain have combined to create an overreliance of opioid medications with associated overdose and addiction phenomena [1-2]. The liberalization of laws, the no controlled prescriptions and illegal use have turned the abuse of these drugs into a major social, environmental and public health problem. The work focused on the development and validation of an analytical method and the determination of 11 opioids and 7 metabolites for their high consumption in influent and effluent wastewater samples from three Wastewater Treatment Plants (WWTPs) in Valencia (Spain) where the population reach about 1.5 million inhabitants. The sampling was carried out for 1 week to simulate a real representative of opioid day consume, considering the different release of contaminants in wastewater during 24h. Different analytical columns and elution gradients were tested to determine the best condition for the separation of opioids. The best performance was obtained with the Luna® Omega Polar C18. The mobile phase consisted of (I) deionized water and (II) methanol, both with 0.1% formic acid. The extraction was based on solid-phase extraction (SPE) using polymeric reversed phase (Strata-X) and polymeric ion-exchange phase (Strata-X-CW). The determination was carried out by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a triple-quad using two precursor → product ion transitions for each compound in the multiple selected reaction monitoring mode (MRM). The current work has presented a sensitive method for the quantitative determination of 18 substances. The recovery results obtained are ranging from 60% to 120%. The most abundant opioids are heroin, hydromorphone, hydrocodone, tramadol and its metabolite. The results pinpointed the need of further studies on the short and long term to supervise the real consumption of these compounds and to assess both, the removal efficiencies of the WWTPs and the eco-toxicological impact in animal and vegetal species. References [1]. Volkow ND, McLellan AT. Opioid Abuse in Chronic Pain — Misconceptions and Mitigation Strategies. New England Journal of Medicine. 2016;374(13):1253-63. [2]. Kolodny A, Courtwright DT, Hwang CS, Kreiner P, Eadie JL, Clark TW, et al. The Prescription Opioid and Heroin Crisis: A Public Health Approach to an Epidemic of Addiction. 2015;36(1):559-74. Acknowlegment: This work has been supported by the Spanish Ministry of Economy and Competitiveness and the ERDF (European Regional Development Fund) through the project CICLIC -subproject WETANPACK (RTI2018-097158-B-C31) and by the Generalitat Valenciana through the project ANTROPOCEN@ (PROMETEO/2018/155); D. Sadutto also acknowledges the Generalitat Valenciana for his "Santiago Grisolía" grant "GRISOLIAP/2018/102, Ref CPI-18-118".

4.01P.10

Low-Cost and Rapid Sensors for Profiling Sewage for Public Health Assessment

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Wastewater analysis has recently been shown to be an innovative and promising tool for the estimation of community-wide drug use and public health. Sensors has emerging as a rapid analytical tool for the biomedical diagnosis and environmental monitoring, and we recently proposed an unique community sewage sensors as rapid and inexpensive alternatives to classical analytical methods for the detection of sewage biomarkers for public health assessment[1]. We demonstrated a range of sensors, such as electrochemical, colorimetric and optical (e.g. Rama spectrometry) sensors for the analysis of cocaine and methylamphetamine. Here, we will present our most recent work on the development of a range of sensors, combining with nanomaterial and DNA aptamer for the evaluation of illicit drugs for public health assessment. As an example, we will show a new and low-cost paper-based substrate for surface-enhanced Raman Scattering (SERS), Au@Ag-GNFP, for sensitive and selective detection of methamphetamine (METH) and ketamine (KET), with negligible interference, promising reproducibility and excellent stability. This assay has a clear potential to be implemented for field test using a portable Raman spectrometer. We will also present an emerging lateral flow-based paper device; together with an isothermal amplification strategy to improve the sensitivity and selectivity or our assay, which ultimately aims to analysis wastewater in the field with a rapid and low-cost way.

4.01P.11

Characterisation of dental wastewaters associated with the use of mercury-free materials

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Abstract Amalgam (mercury-based) materials have been in use in dentistry for nearly 150 years. However, Ireland's recent ratification of the Minamata Convention, which incorporates measures for the phase down and phase out of mercury, has seen a rise in use of *mercury-free* materials in dentistry. Amalgam is now largely prohibited for dental treatment in certain patient categories unless deemed strictly necessary by the dental practitioner and the discharge of mercury waste and wastewater is strictly regulated. However, while the long-term effects of mercury release into the environment are widely understood, little is known about any potential effects of accidental discharge of untreated dental wastewater into the environment, or indeed the properties and constituents of wastewater generated from use of replacement mercury-free materials that have been introduced. These mercury-free materials often contain nano- and micro-particle fillers and a range of other, sometimes unspecified, chemical constituents. There are also questions as to whether the wastewater separation techniques already *in situ* in dental practices, which were largely designed with amalgam separation in mind and particles >100 μm^2, are also appropriate for new dental materials. In this study, we examine the dental wastewater (DWW) resulting from the application and removal of mercury-free dental materials. We report the results of DWW analyses, including total suspended solids, and other physicochemical data, as well as particle size distributions that could be potentially be discharged from DWW, despite filtering technologies in place. We conclude that particles from a range of materials such as glass ionomers or ceramic fillers are detectable in dental wastewater. Analyses indicated that each mL of dental wastewater sample analysed contains high concentrations of micro- and nanoparticles, including Al and Zn nano-fillers. We report the results of preliminary toxicity testing of these waste streams using OECD 202 *Daphnia magna* acute immobilisation tests. This study addresses a knowledge gap, which has the potential to aid further guidelines and policymaking and make recommendations for dental wastewater treatment before release into the environment. *Keywords* – Dental, Wastewater, Materials, toxicology

4.01P.12

Toxicological biomarkers on Eisenia andrei exposed to effluent-contaminated soil

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Disposal of sanitary effluents in the soil has numerous benefits, including reduced use of chemical fertilizers. However, its effects on human health and biota, including edaphic fauna, result in the need for investigation, monitoring and remediation of these areas. Considering the ecological importance and the potential indicator of earthworms, the objective of this work was to evaluate the toxicity of sewage discharge in the soil of an area, in *Eisenia andrei* oligochaetes. Behavior (ISO 17512-1 / 2011), Reproduction (ISO 11268-2) and OECD 222, Acetylcholinesterase (AChE) enzyme activity (Ellman et al., 1961), Catalase (CAT) (Nelson and Kiesow, 1972), superoxide dismutase (SOD) (Misra and Fridovich, 1972) and lipid peroxidation (TBARS) (Buege and Aust, 1978) were evaluated to determine the toxicological effects on organisms caused by soil effluent disposal. The results indicated that the disposal of untreated sanitary effluent in the soil was toxic to the reproduction and behavior of the organisms. An increase in the activity of SOD and CAT enzymes as antioxidant defense mechanisms was observed. inhibition of AChE activity, indicate the occurrence of lipid peroxidation of the cell membrane and changes in neurotransmission, respectively due to soil contaminants The toxic effect could not be specifically attributed to a contaminant as sanitary effluents consisted of complex mixtures of substances. However, the application of ecotoxicological tests was efficient to analyze the toxic potential of soil contaminated by sanitary effluent.

4.01P.13

Assessing a polluted river environment by the study of the biological and physiological responses in the gastropod Lymnaea stagnalis

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El Malah river is one of the most important stream in the north-western region of Algeria and it is subjected to anthropogenic pressure i.e., agricultural activities and wastewaters. In this study, we assessed the water quality of this river by studying the physiological and biochemical state of the snail Lymnaea stagnalis, used as freshwater sentinel. We also considered the ecological distribution of the population. Sampling was done for two seasons, winter and spring, in four locations along the El Malah river in 2019. Three sites are located in an agricultural region (S1 to S3, up to downstream) and the fourth is located downstream from urban wastewater discharges (S4). Four parameters were selected for this study: the abundance of the populations, the morpho-biometric indices, the condition indices and biomarker responses. The results show a collapse of 54.4 % of the total density of the population in both seasons from the site S1 to the site S3, and no specimen was recorded in the site subjected to a flow of wastewaters (S4). Morphometrics data describe bigger snails in winter than in spring for all the sites. Moreover, condition indices based on the wet weight (CI), and based on the shell volume (VCI), demonstrated no significant differences between sites in winter. However, in spring, they indicate significant differences for CI, with lower values in the site S1 and S3, due to the breeding season. Measured biomarkers, Ethoxyresorufin-O-deethylase (EROD) activity and Glutathion S-transferase (GST) gave significant higher values in the site S2 in spring. The Lipid peroxidation (LPO) shows detectable values in all the study sites with high values in the site S1 in spring and in site S3 in both seasons. However, Acetylcholinesterase (AChE) activity shows low values in all the sampling sites. This study allowed establishing a link between the absence of Lymnaea stagnalis and urban wastewater discharges and highlights the toxic effects of pollutants in the sites situated within the agricultural region. As a conclusion, the land use in the catchment of the El Malah river has a great influence on the biology in the stream and does not allow the development of healthy populations. Measures must be implemented to maintain the integrity and sustainability of the fluvial ecosystem, such as urban wastewater treatment and the promotion of extensive agriculture. Keyswords : river, anthropogenic pressure, snails, biomarkers.

4.01P.14

Pharmaceutical and personal care product (PPCP) profiling in a South African urban surface water setting: Using a multi-faceted approach towards investigating emerging health challenges

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Rapid urbanisation and population growth in low- and middle-income Countries (LMICs) are associated with various socio-economic challenges that limits sustainable development, along with poor infrastructure, water and sanitation service delivery that leads to the slumming of peri-urban communities. Such challenges not only impact the well-being of the natural environment, but also public health challenges such as heightened spread and development of (non)communicable disease. The presentation will discuss spaciotemporal changes of various chemical biomarkers over a two-year sampling period in a South African urban environment, including: 1) change in chemical biomarker levels along surface waters in the urban setting, 2) evaluating wastewater treatment performance, 3) per capita pharmaceutical- and drug use profiles, and 4) identifying chemical burdened ‘hotspots’ in a LMIC urban setting. Aqueous samples from seven river locations and wastewater treatment works (WWTW) influent and effluent located near the town of Stellenbosch (South Africa) were sampled every second month for seven days between May 2018 and May 2019. Sample collection and processing was done as described by previous work in our groups (Petrie et al., 2016; Archer et al., 2017). Target analyte acquisition using ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was achieved using a previously developed method for the quantification of 90 pharmaceutical and personal care products (PPCPs; Petrie et al., 2016). Flow-proportional mass loading (g/day) of the PPCPs were determined by obtaining daily flow rates (ML/day) from the WWTW operator and using a URMOD rainfall-runoff model for mass load estimations of the environmental sampling locations. Although the WWTW showed adequate removal of most of the target analytes, some recalcitrant pharmaceuticals were still shown to be discharged. Moreover, various chemical biomarkers such as lifestyle chemicals, illicit drugs, antiretrovirals and antibiotics were regularly detected in the low µg/L level at a heavily contaminated river system in the area, confirming that human waste from the adjacent communities are being discharged directly into the natural environment and not directed towards the urban sewage infrastructure. This provides more evidence for the need of applying the UWP approach to provide additional per capita loadings of urban communities that are not necessarily connected to the larger urban infrastructure.

4.01P.15

Chemicals of emerging concern in the urban environment - a catchment perspective

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Micropollutants are known to be present in the environment. One of the primary sources of entry are through wastewater treatments works (WwTW). Although there is legislation to protect the environment from priority micropollutants, this list is minimal compared to the thousands in use. This work looks at 142 micropollutants in wastewater in influent, effluent and receiving waters at 5 locations across the catchment, proving a more extensive list of chemicals of emerging concerning (CECs), covering 75% of the population of the studied area. Robust sampling and analytical methods were employed, including solid phase extraction of samples followed by ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry for the accurate analysis of these CECs throughout multiple matrices across the catchment. The results showed clear trends between population and urbanisation as well as increasing CEC loads downstream despite high removal efficiencies at the WwTWs. This study confirms the necessity of considering the overall contributions of WwTWs within a catchment as a whole rather than the individual performance of a WwTW and the risk it may pose.

Assessment of Chemical Mixtures and Multiple Stressors: From Additivity and Synergy to Policy Options (P)

4.02P.1

Quantifying synergistic interactions between pathogens and chemicals on mortality in invertebrates

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One stressor may render the organism more sensitive to a second stressor. Hence, environmental pollutants may interact with naturally occurring pathogens resulting in effects not readily predictable from neither the ecology of the organism nor the inherent toxicity of the pollutant. When two stressors potentiate the effect of each other, the interaction is termed synergistic. During the last decade, the exploitation of combining traditional pesticides with pathogenic infections has gained increasing interest, mainly to improve methods for controlling pest in agricultural settings and for controlling insect vectors of human diseases. Additionally, however, concern of synergistic interactions between sub-lethal doses of chemical pollutants making beneficial (and other non-target) insects more susceptible to diseases has also received increasing attention. Resultantly, an increasing number of studies claim synergistic effects of combined exposure to pesticides and pathogens. However, the reference model used to define synergy (whether a reference model is even used), vary significantly between studies. In this study, we survey the literature for well described studies of combinations of pathogen and chemical exposure and quantify their joint effect relative to the reference model of independent action (IA). Contrary to studies quantifying mixture effects relative to the model of concentration addition (CA), where model deviation ratios (or synergy ratios) of observed versus predicted effect concentrations are used, quantifying deviations relative to IA needs a different approach. As IA predicts *effects*, which can only vary between zero and 100% mortality, rather than *concentrations* eliciting a specific effect, which can vary indefinitely relative to each other, deviations from the reference model is quantified as the difference in mortality between observed and predicted at the timepoint where this difference is the largest. A total of 222 publications were extracted from Web-of-Science and 70 studies (881 combinations) lived up to the criteria for IA predictions. The frequency of synergistic interactions was significantly higher than antagonistic interactions, and highest for combinations including microsporidium and virus infections in combinations with chemicals. Combinations with fungal infections did, however, yield the most severe deviations from the reference model. Observed patterns of interactions will be discussed in a mechanistic perspective.

4.02P.2

Exploring synergistic biocide mixtures: Concentration dependent synergy for high efficacy at target sites with low environmental impacts

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The benefits we get from the use of chemicals is, and should, always be weighed against the risk towards human health and the environment. The "easy to grasp" benefits versus the inherent complexity of chemical risk assessment leaves us in a tricky situation where chemicals are widely used and the risk assessment is lagging and fraught with uncertainty. Today, a multitude of antimicrobial and antifouling chemicals are used to protect surfaces and materials in a wide variety of sectors. However, an unnecessary excess of chemicals beyond what is needed for the desired effects is often used to prolong the protection. We aim at minimizing this problem via the directions: Synergism between chemicals and slow, controlled release facilitated by encapsulation. An important aspect of chemical synergism for this purpose is that it should only demonstrate synergy at the target site/organism, but not towards non-target species at environmentally relevant concentrations. The aim is to use a carrier compound together with a lipophilic biocide and our proposed mechanism of synergy depends on micelle formation of the carrier compound. We hypothesize that when the concentrations of the chemicals used decreases further away from the target site, the concentrations will be too low to form micelles (i.e. below the critical micelle concentration (CMC) of the compounds), and thus the synergistic interactions will no longer occur. By using this concept we hope to find highly concentration-dependent synergistic interactions that can drastically lower the doses required to achieve desirable antimicrobial effect and lower environmental impacts at non-target sites. In addition to synergistic interactions, we hope to further reduce the leakage from antimicrobial materials by developing bio-based microcapsules to encapsulate the biocides for a controlled and prolonged low-dose release, further lowering the environmental impacts. The presented approach has the potential to be implemented in a wide variety of areas, and is an important step to reduce the numbers of harmful toxins in the environment. In this presentation we will present the overall outline of the approach and some details on the first case study, exploring the combination effects of binary mixtures of biocides with potential to demonstrate the proposed synergistic interactions.

4.02P.3
Can organophosphates and carbamates cause synergisms by inhibiting esterases responsible for biotransformation of other chemicals?
Y. Cao, University of Copenhagen / Department of Plant and Environmental Sciences; N. Cedergreen, University of Copenhagen / Plant and Environmental Sciences
The main detoxification routes of xenobiotics are oxidation and hydrolysis. The hydrolysis by general esterases (GE) being the most efficient route of hydrolyzation. Organophosphates (OP's) and carbamates (CB's) inhibit the activity of multiple esterases, with their insecticidal target being acetylcholine esterase (AChE). It implies that synergism could be caused by OPs and CBs if they decrease biotransformation of xenobiotics depending on hydrolysis for detoxification. An important detoxification route of insecticide alpha-cypermethrin (α-cyp) is detoxified by hydrolysis. To test if the toxicity of α-cyp towards Daphnia magna was enhanced by inhibiting GE, increasing constant concentrations of the GE inhibitor iso-OMPA was applied with α-cyp and mortality was monitored. The model deviation ratios at LC50 were positively correlated with the inhibitions of GE measured in vivo, confirming that synergy is obtained with co-exposure of α-cyp and a GE-inhibitor. The next step was to test realistic insecticides mixture in a ray-design. Three thion-OP's (diazinon, chlorpyriphos and parathion), one oxon-OP (chlorfenvinphos) and one CB (aldicarb), respectively, were mixed with α-cyp at 50:50 effect ratios and tested together with the individual compounds. No synergy was found for any of the mixtures during 96h tests (adding food after 48h). The three thion-OP's, which need to be bio-activated by cytochrome P450 before exerting toxicity, showed antagonistic effect, while the combination of the oxon-OP and α-cyp followed a concentration addition model. The result of the mixture test between aldicarb and α-cyp was also antagonistic. The results were contrary to hypothesized knowing OP's and CB's inhibit esterases in general. A fundamental difference between iso-OMPA and OP's or CB's, however, is that iso-OMPA has a low affinity for AChE and primarily targets GE, while OP's and CB's are selected for their high affinity for AChE to obtain their insecticidal properties having a proportionally lower affinity for GE. The insecticidal activity of the OP's and CB's therefore seemed to dominate their action when tested on a sensitive organism as D. magna.

4.02P.4
Using the concept of chemical activity to assess mixture toxicity in Daphnia magna
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Organic chemical substances can occur in various mixture compositions in the environment. Yet, knowledge of the impact of chemical mixtures on organisms and environmental systems is scarce. Two factors that contribute to this gap in knowledge are 1) the challenges to measure environmental concentrations of all chemicals, and 2) that concentrations of chemicals cannot be added up to inform about the mixture toxicity, as toxicity-exerting concentrations are compound-specific. Thus, currently, risk and exposure assessments of toxic chemicals in the environment are exceedingly demanding. The *chemical activity approach* provides a concept to address hazard potential of organic chemical mixtures in which every single chemical is present below its specific toxic concentration by converting the concentration of all chemicals into a common, unitless currency. The chemical activity of different chemicals is additive. Further, it is often observed that toxicity adds up for neutral chemicals at low concentrations. This conforms even when the substances are not related chemically or have different modes of action at higher concentrations. This is known as baseline toxicity, or narcosis and is related to chemical activity. Hence, chemical activity offers an integrative tool to quantify the potential effect of low levels in chemical mixtures on biota expressed as baseline toxicity. To identify the effects of chemical mixtures and explore the applicability of the chemical activity approach, we will expose the model organism *Daphnia magna* to various organic, hydrophobic chemical mixtures that differ in chemical composition and chemical activity levels. The concentration of each chemical will be kept below the threshold level of its specific toxicity. The latter will ensure that the observed effects are not related to the specific regulatory mechanism of the chemicals present in the mixture. Immobilisation of *D. magna* will be used as endpoints. The results from these experiments may help to test hypotheses on how the concept of chemical activity can be used to assess chemical mixtures and may help provide a new framework to evaluate ecological effects caused by chemical mixtures in aquatic environments with background levels of contaminants.

4.02P.5
Predicting mixture toxicity effects over time with toxicokinetic-toxicodynamic models (GUTS): assumptions, experimental testing & predictive power
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The assessment of mixture toxicity is one of the most challenging tasks in ecotoxicology. To date, the methods to assess the impact of chemical mixtures on organisms ignore the time dimension. As toxicity is a process in time, so is the action of mixtures. Explaining and predicting the effects of mixtures thus requires the use of toxicokinetic-toxicodynamic models (TKTD models) which simulate the time-course of processes leading to toxic effects on organisms. Among TKTD models, the General Unified Threshold model for Survival (GUTS) framework is a promising tool to increase mechanistic understanding of mixture effects on survival over time. Here, we present GUTS-RED mixture models (for the Stochastic Death (SD) and Individual Tolerances (IT) approach) under the assumptions of i) independent action and ii) same mode of action of the tested chemicals (or same form of damage). Depending on the assumption, we present how to use single substance exposure data to calibrate the model, and predict the survival probability over time for mixtures. We used *Enchytraeus crypticus,* a model species in ecotoxicology, and several suitable combinations of chemicals (prochloraz, triflumizole, cypermethrin, chlorothalonil, sulfoxaflor, MGK 264, copper oxychloride) to test the model assumptions and evaluate the ability of the models to assess and predict mixture toxicity effects on survival. For this, individuals were exposed to chemicals in single (for model calibration), and in binary mixtures (to enable comparison between model prediction and the data). All the exposures were performed in water solution at constant exposure in 24 well plates for 96 hours and the survival was monitored over time at 0, 3, 6, 24, 48, 72 and 96h. The GUTS mixture toxicity modelling predicted survival over time of binary mixtures based on single exposure calibrations. When the correct assumption was followed (based on the chemical mode of action) and the model prediction significantly differed from to the data, it indicates that synergism or antagonism occurs in the mixture (below or above the prediction respectively). In such situations, refined modelling can result in a mechanistic explanation of the synergism/antagonism.

4.02P.6
Physiological responses to fluctuating pCO2/pH drive opposing interactions with copper toxicity
A. Wilson McNeal, R.W. Wilson, C. Lewis, University of Exeter / Department of Biosciences
Global CO_2 increase is altering ocean carbonate chemistry and driving down pH, and coastal organisms must contend with this alongside existing chronic pollution. Many pollutants, such as trace metals, are pH-sensitive, their speciation and hence bioavailability changing with ocean pH. As coastal areas are naturally dynamic, diel variation regularly drives pH far below and above typical open ocean values. Little is known about how this affects the toxicity of pollutants. We addressed this using a combined field and laboratory approach with two marine invertebrates, the mussel *Mytilus edulis* and ragworm *Alitta virens*. We exposed both species to

environmentally relevant concentrations of the pH-sensitive trace metal copper (0.1 µM for mussels and 0.25 µM for ragworms in order to induce comparable toxicity responses), and either a repeating 12-hour $p CO_2$/pH cycle (pH 8.14-7.53) or a static pH 8.14 treatment, as is commonly used as a control in ocean acidification and ecotoxicology experiments, to determine how these two stressors interact to drive toxicological outcomes. After two weeks, physiological and toxicological endpoints were measured at the high pH/low-pCO2 point of the cycle. Differences in the species' internal acid-base physiology drove opposing responses to copper toxicity. Mussels underwent a haemolymph acidosis, which drove two-fold increases in the antioxidant activity and DNA damage induced by copper. In contrast, ragworms experienced an alkalosis, driven by a two-fold increase in coelomic fluid bicarbonate in response to fluctuating pH/pCO2. This moderated the copper-induced oxidative stress to slightly reduce both antioxidant activity and DNA damage. Copper becomes more bioavailable as seawater pH declines, but differences in physiology were as important in determining its toxicity to these two common coastal species.

4.02P.7

Multiple-stressor effects of climate change and copper to temperature acclimatised daphnia

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Climate change will affect the properties of freshwater systems in multiple ways including alteration of flow, drought and flooding events, and prominently temperature. These changes to freshwater ecosystems will act as stressors to the organisms living within them, and will co-occur with anthropogenic stressors such as chemical pollution. Copper represents a common pollutant in the UK, with freshwater exposure primarily occurring from agricultural and industrial sources. Previous studies have investigated the multiple-stressor effect of copper exposure and temperature and the negative effects on daphnia. However, daphnia in such studies were exposed to a sudden change in temperature, either followed by a short period of acclimatisation or small number of generation with combined exposure. In natural ecosystems, the changes to temperature from climate change will occur gradually over multiple generations not necessarily exposed to copper. To improve the realism within laboratory toxicity tests *Daphnia magna* were gradually acclimatised to divergent temperatures of 15, 20 and 25 ºC over multiple generations. The divergent cultures then underwent copper exposure in a reproduction study at the different temperature combinations and the effect on mortality, number of neonates, time to brood and the size of neonates and adults recorded. At increased test temperatures, there was a reduction in neonates and increased mortality and time to first brood at higher temperatures supporting previous findings. Accounting for copper pollution, these data support increasing temperatures marginally exacerbating the effects of copper toxicity in synergistic action.

4.02P.8

Effect of combined exposure to microplastics and organophosphates on the immune system of terrestrial isopod Porcellio scaber

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In recent years, research on the potential environmental effects of microplastics has gained increasing interest. Numerous studies have shown the effects of microplastics on terrestrial organisms. However, the combined effects with other pollutants have rarely been assessed. In this study, we investigated the effects of combined exposures to microplastics (textile microfibers, 220 ± 200 µm) and the organophosphate pesticide chlorpyrifos (CPF) on terrestrial isopods Porcellio scaber. Our prime focus was the immune system of these organisms with total (THC) and differential (DHC) hemocyte count, hemocyte viability and phenoloxidase (PO) activity as immune markers. The activity of acetylcholinesterase (AChE), the enzyme specifically inhibited by CPF, was also assessed in the hemolymph as well as in whole organisms. Animals were exposed to CPF alone (0.3, 0.6, 0.9, 1.2, 3.0 and 6.0 mg/kg dry soil), microplastics alone (0.05, 0.1, 0.5 and 1.5% w/w) and combination of CPF (0.3–6.0 mg/kg dry soil) and microplastics (0.05 and 0.5 % w/w) in Lufa 2.2 soil for 3 weeks. CPF alone induced significant alterations in immune markers and clearly inhibited AChE activity indicating its presence in the hemolymph. Microplastics alone did not cause any notable effects on the isopod immune system. The effects of CPF on survival and feeding activity at the highest two concentrations in combined exposure were clearly decreased, indicating a potential decrease in the bioavailability of CPF by microplastics. In line with this, also the effects on the viability of hemocytes as well as AChE activity in whole organisms were decreased when organisms were exposed to the mixtures of microplastics and CPF in the soil. To conclude, our results indicate that (i) CPF alone induced alterations in immune markers, (ii) microplastics at concentrations of 0.05–1.5 % w/w did not exert any effects, while (iii) in combined exposure the microplastics decreased the bioavailability and immune-related toxicity of CPF.

4.02P.9

Pesticides in the Sado River estuary (Southwest Iberian Peninsula, Portugal) - What levels and who is at risk?

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Estuarine environments are suffering from anthropogenic pressures due to human activities that include beyond others those from intensive agriculture practices. This is the case of the Sado River estuary located close to the Atlantic Ocean and habitat of numerous species from birds to mammals (dolphins). Therefore, with the propose of investigating the presence of 56 pesticides from different categories (fungicides, herbicides and insecticides) in this estuary, and the potential risk for biota, water samples were collected for one year (2017) at several sites located in both margins the Sado Estuary. The analyses involved an extraction step using solid-phase (OASIS HLB cartridges) and pesticide quantification/identification by gas chromatography-mass spectroscopy (GC-MS/MS). Data showed that 88% of fungicides, 91% of herbicides and 98% of insecticides were measured in all samples, some being above the 2013/39/EU Directive levels. In this respect, the total loads of these compounds were respectively ≈ 19 µg/L, ≈ 3 µg/L and ≈ 30 µg/L for fungicides, herbicides and insecticides. Several studies have shown that the concentration-addition model (CA) can predict the observed toxicity of mixtures of pesticides with reasonable accuracy. Therefore, the maximal concentration measured for each of the 56 pesticides in this estuary was analysed using a two-tiered approach based on CA and independent action models using, and three trophic levels. Fish is the trophic level affected the most by these compounds, posing clear risks to their survival as well as those organisms that use these resources as food supplies. In conclusion, legal quality standards are not being followed, what is against both the Portuguese and European legislation. We suggest that specific studies regarding the identification of both the sources and impacts of pesticides must be done together with depollution measures of this critical habitat. Keywords: fungicides, herbicides, insecticides, GC-MS/MS Acknowledgements: Partially supported by FCT and ERDF (UID/Multi/04423/2019).

4.02P.10

Characterisation of cumulative risk of contaminants to organisms exposed to stormwater in Oslo, Norway

A. Ruus, NIVA / Environmental Contaminants; R. Wolf, NIVA Norwegian Institute for Water Research; K. Petersen, K. Tollefsen, NIVA - Norwegian Institute for Water Research / Ecotoxicology and Risk Assessment

Oslo, the capital of Norway, is situated by the Inner Oslofjord. In addition to municipal discharges, the Inner Oslofjord ecosystem is affected by industry, leisure boats and commercial ships. Furthermore, various environmental contaminants enter the fjord by rivers and streams, and through surface water/stormwater. The Oslofjord is a moderately exposed coastal area with an ecologic condition of moderate and a chemical condition of not good according to the water framework directive. 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. [1,2]. 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 toxic effects and identify the main contributing stressors to the predicted environmental risk. “Urban fjord”-data from 2016 [2] was imported to the NIVA Risk Assessment database (NIVA RAdb, www.niva.no/radb) and subjected to quality control, mapped to unique chemical identifiers and aggregated as spatio-temporal data sets of water concentrations of single compounds. These data cover more than 150 individual compounds. Concentrations in stormwater were used to perform a retrospective CRA for different species groups (taxa) including molluscs, algae, invertebrates, worms, crustaceans, and fish by using effect data for relevant compounds. NIVA RAdb was used to calculate the 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, the taxa 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 – This work was funded The Research Council of Norway, project 268294: Cumulative hazard and risk assessment of complex mixtures and multiple stressors (MixRisk).References1. Green, N.W., Schøyen, M., Øxnevad, S., Ruus, A., Allan, I., Hjerman, D., Severinsen, G., 2016. Contaminants in coastal waters of Norway 2015. Niva report 7087-2016, 209p.2. Ruus, A., Bæk, K., Petersen, K., Allan, I., Beylich, B., Schlabach, M., Warner, N., Borgå, K., Helberg, M., 2017. Environmental Contaminants in an Urban Fjord, 2016. Norwegian Environment Agency report M812-2017, 114p.

4.02P.11
Characterisation of cumulative risk of pollutants to marine organisms in Sørfjorden, Norway
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Sørfjorden is located at the south west coast of Norway and is a freshwater influenced, protected fjord with an ecologic condition of moderate and a chemical condition of not good according to the water framework directive. The area has a long industrial history, and has historic as well as active industrial emissions. The main industries in the fjord are metal industries which started in the early 1900s. Peak emissions of approx 1 ton of mercury, 1835 tonnes of zinc, 773 tonnes of lead, and 24 tonnes of cadmium occurred in 1985. In addition to metals, emissions of PAHs also occurred. The condition of Sørfjorden have been monitored by different monitoring programs and concentrations of a wide range of contaminants (metals, PAHs, PCBs, PBDEs and others) have been reported in biota, water and/or sediment samples from different monitoring sites in the fjord [1,2]. The objective of the present study was to use historical monitoring data to perform a retrospective cumulative environmental risk assessment (CRA), identify species groups (taxa) of highest risk for toxicological effects and identify the main contributing stressors to the predicted environmental risk. Monitoring data from 2015 were imported to the NIVA Risk Assessment database (NIVA RAdb, www.niva.no/radb). Water concentrations of pollutants 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. Fish had the highest CRQ for acute effects, whereas molluscs had the highest CRQ for chronic effects. For both species groups, Zn and Cu were in most cases the main contributors to the CRQ. The study show that there is a predicted risk for biological effects in organisms in Sørfjorden due to water-borne exposure to pollutant mixtures. Acknowledgement. This work was funded by The Research Council of Norway, project 268294: Cumulative hazard and risk assessment of complex mixtures and multiple stressors (MixRisk). References 1 Green, NW et al., 2016. Contaminants in coastal waters of Norway 2015. Niva report 7087-2016. 2 Ruus, A. et al., 2016. Operational monitoring of coastal waters in the Hardanger River Basin, 2015. Niva report 6996-2016.

4.02P.12
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 sufide-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 during 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. Analyses of water samples demonstrate still high concentration of trace elements in downstream tributaries and in the following order Al> Mn>Fe>Ce>Ni>La>Nd>U>Ge>Cu, before dilution in the coastal water (Teien et al., 2017). The exposure data from the sites monitored was subjected to a component-based Cumulative Risk Assessment (CRA) using the NIVA Risk Assessment database (www.niva.no/radb) to predict site-specific impacts. The resulting CRA demonstrated that several taxa were at risk (risk quotients > 1), and that the risk profile was clearly depending on the metal composition. Fish had the highest risk (i.e. risk quotient) for acute effects in Kaldvellfjord, followed by molluscs and algae. Molluscs displayed the highest risk for chronic effects, followed by invertebrates and algae. The risk for acute effects of these multi-component mixtures in fish was driven by a selection of metals, typically following the order Fe>Cu>Zn> Ge>Al>La. Analyses of gills of caged Atlantic Salmon smolt exposed to the fjordwater showed significant accumulation of several of the elements identified to cause risk, albeit limit transfer to other organs such as liver was observed and indicated that gills may be the main target organ for these complex mixtures. The observed data suggest that several of the trace metals including rear 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.

4.02P.13
Derogation considerations in the context of guidance for endocrine disruptor (ED) assessment
S. Andrews, Compliance Services International
Under the 'Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009', it is stated "There may be cases in which due to the knowledge on the physico-chemical and (eco)toxicological properties of the substance an ED assessment does not appear scientifically necessary…". There are additional factors which could be taken into consideration when assessing if it is scientifically necessary to perform an ED assessment, e.g. substances ubiquitous in the environment and existing use(s). There are many substances which are ubiquitous in the environment including nitrate, chloride, sulphate and phosphate salts present in between 111,180 and 945,404 km^2 of groundwater bodies in the EU. Thus, humans or non-target organisms can be exposed to these substances as they are exposed to groundwater. The existing use of a substance could be a suitable indicator of the relevance of an ED assessment, for example substances used as food materials and flavourings. This poster will review the relevance of conducting an ED assessment for co-formulants where they are either environmentally ubiquitous or already have a use which causes high environmental or consumer exposure.

Bioaccumulation and Biotransformation: Advances, Challenges, and State of the Science for Chemicals Regulation (P)

4.03P.1
Bioaccumulation of PFOS and GenX in the benthic freshwater amphipod Hyalella azteca
C. Mueller, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Bioaccumulation and Animal Metabolism; S. Kühr, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Department Bioaccumulation and Animal Metabolism; C. Rauert, German Environment Agency UBA / International Chemicals Management; D. Maletzki, German Environment Agency UBA; C. Schlechtriem, Fraunhofer IME / Department Bioaccumulation and Animal Metabolism
The bioconcentration test with the freshwater amphipod *Hyalella azteca* (HYBIT) was recently suggested as alternative test system for bioconcentration studies. About 20 organic compounds with different hydrophobic character (log Kow 2.4-7.8) have been tested. The bioaccumulation potential of compounds is commonly expressed in the form of bioconcentration factors (BCF) determined in flow-through studies with fish according to OECD 305. Bioconcentration studies with *H. azteca* resulted in BCF estimates which show a strong correlation with fish BCF values. So far, only lipid accumulating substances have been tested with *H. azteca*. Further studies are required to elucidate the bioconcentration of non-lipid accumulating substances. PFOS and Gen X were chosen as test items due to their ionic character as well as their environmental and regulatory relevance. PFOS, used since the 1940s, is a global pollutant and a representative of fluorochemicals that can be found in nearly all biota samples from nearly all places around the world, and has been restricted by the Stockholm Convention since 2009. Alternatives, such as the ammonium salt of hexafluoropropylene oxide trimer acid (GenX), were developed to substitute the production and usage of perfluorooctanic acid (PFOA). However, GenX has also been found in surface-, ground-, raining and finished drinking water as well as air emissions in some areas. Semi-static bioconcentration tests with *H. azteca* were carried out. The test design enabled constant exposure conditions during the uptake phase of all tests. Animals were exposed for seven to ten days followed by a depuration phase lasting up to 14 days. Measurement of tissue concentrations in animal samples collected during the studies allowed to calculate BCF_{SS} and BCF_{kin} values. The results are compared with BCF values described in the literature. Gen X seems to be less bioaccumulative than PFOS. Additional tests with PFOS were carried out to investigate the impact of the ionic composition of dilution water on the result of the BCF tests. Results indicate that ionic composition of the exposure medium may alter the bioaccumulation potential of ionic organic compounds.

4.03P.2
Biomagnification of ionic organic compounds (IOCs) in rainbow trout (Oncorhynchus mykiss)
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Regulation of ionic organic compounds (IOCs) is a predominant challenge within the PBT-assessment. However, a commonly acknowledged approach for the estimation of the bioaccumulation potential of IOCs is still missing (Franco *et al.*, 2010, Treu *et al.*, 2015). The group of IOCs comprises a broad range of chemical classes like surfactants, some classes of pesticides, pharmaceuticals and also poly-

and perflourinated acids including chemicals of very diverse structures. The bioaccumulation potential of compounds is commonly expressed as bioconcentration factors (BCF), determined in flow-through studies with fish according to OECD 305. This test was developed and optimized for neutral, water-soluble test substances that are suitable to be administered via a homogenous and stable water concentration and absorbed via the gills of the fish. For certain groups of substances, a dietary exposure test was proposed, where this is considered more suitable than an aqueous exposure test. However, the dietary approach yields a dietary biomagnification factor (BMF) rather than a bioconcentration factor (BCF). In contrast to water-soluble and neutral substances, IOCs are thought to be more effectively absorbed via the gastrointestinal tract (GIT) than via the gills. This might be caused by the lower membrane permeability of the IOCs and by the high trans-epithelial resistance of the gills compared to the trans-epithelial resistance of the GIT and with that the greater permeability of the GIT. A crucial difference between the two uptake pathways is the prolonged retention time in the GIT. Three feeding studies with rainbow trout (*Oncorhynchus mykiss*) were carried out according to OECD 305 to test the biomagnification potential of TBP, TMOA, Benzotriazole, Tecloftalam, Pentachlorophenol and MEE-phosphate. Three experimental diets containing two IOCs each were prepared and tested. Animals were collected during the studies to investigate the uptake and elimination of the different test compounds and to derive kinetic dietary biomagnification factors. Separation of liver, GIT and carcass allowed to further calculate tissue distribution factors for the individual test substances. Differences in the tissue distribution of the ionic substances were observed. However, none of the IOCs tested in this study showed a distinct biomagnification potential.

4.03P.3

Assessing Bioaccumulation of Cyclosiloxanes using the Bioaccumulation Assessment Tool (BAT)

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The Bioaccumulation Assessment Tool (BAT) facilitates the evaluation of multiple kinds of bioaccumulation data with consideration of both endpoint relevance and reliability in a quantitative weight of evidence approach. Bioaccumulation data for cyclosiloxanes octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) were entered into the BAT Ver.1.0 to assess the usefulness of the BAT for calculating, organizing, evaluating and assembling multiple bioaccumulation data and to assess the bioaccumulation potential using a systematic, transparent and weight of the evidence approach. Several observations were made regarding the performance of the tool. BAT Ver.1.0 only allows five entries per bioconcentration factor (BCF), biomagnification factor (BMF), or trophic magnification factor (TMF) metric; yet for cyclosiloxanes often more than five bioaccumulation metrics were available for assessment. Pseudoreplication of bioaccumulation metrics (*e.g.* calculating a BCF using several different approaches from the same underlying data) was also a concern in the final assessment. One recommendation for this aspect is to allow the user to identify and more heavily weigh the most appropriate bioaccumulation metric from each independent piece of data in the weight of evidence assessment. From a B assessment perspective, the BAT indicated that several cyclosiloxanes were 'B' or 'vB' according to BCF results, however the weight of evidence indicated 'not B' based on the BMF and TMF results. The BAT was particularly useful for illustrating potential outliers in the data sets for both D4 and D5. In these cases, the outliers were associated with growth corrected BMFs, a practice which has been criticized in recent scientific discourse. Currently, the relevance weighting of bioaccumulation metrics is user-defined in the BAT, since there is a lack of scientific and regulatory consensus on this matter. Greater consensus in this area would advance the practice of bioaccumulation assessment. In summary, the BAT was useful for organizing bioaccumulation data and performing weight of evidence evaluations for these data rich compounds, and this application provided guidance for improvements that are being incorporated in BAT Ver.2.0.

4.03P.4

Trace metal bioaccumulation in freshwater macroinvertebrates: a comparative approach of lab and field studies

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The isopod *Asellus aquaticus* has been proposed as a potential biomonitor for pollutants in freshwater environments due to its wide range and high tolerance to pollution events. Although often included as part of multi-species biomonitoring programmes, a more widespread adoption of *A. aquaticus* for this purpose is limited by an incomplete understanding of the mechanisms of uptake and accumulation of trace metals in macroinvertebrates, especially in the presence of multiple metal contaminants. Both *Asellus* spp. and *Gammarus* spp. are known to accumulate various trace metals. However, the majority of these studies have been carried out solely *ex-situ*, and rarely consider the impact of environmental factors on the concentrations accumulated. The few studies that do attempt to account for additional stressors, such as temperature variation, do so only in the laboratory environment, which is unlikely to fully represent ambient conditions. We examine the uptake and bioaccumulation of specific trace metals in both *in-situ* and *ex-situ* conditions. In the *ex-situ* tests, trace metals are studied both individually and in metal mixtures. These results are compared to *in-situ* measurements of environmental concentrations and compositions and accumulated concentrations in order to provide a comparison between both conditions. The river system chosen for *ex-situ* study exhibits a diversity of riverside land use, including agricultural, industrial, landfill and urban. *In-situ* experimentation consists of exposing *Asellus* spp. and *Gammarus* spp specimens to single metal analytes and metal mixtures, and examining the uptake and accumulation. *Ex-situ* sediment and macroinvertebrate samples are digested and analysed using Inductively-Coupled Plasma Optical Emission Spectrometry (ICP-OES), while water samples are analysed using ICP-OES and HACH spectrophotometer.

4.03P.5

The application of artificial mussels in conjunction with transplanted bivalves to assess metal exposure in a platinum mining area.

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Platinum (Pt) occur naturally in the environment in very low concentrations. Platinum mining activities in South Africa are known to contribute to Pt and associated element emissions in the environment, but knowledge on the concentrations found in the freshwater environment are scarce. Knowledge of Pt exposure in freshwater systems surrounding the mining areas are of great importance for the both risk assessment for aquatic health and water resource management. Therefore information based on field studies give valuable information on the metals that are associated with these mining activities. Indicator organisms have been used worldwide as biomonitors of environmental contaminant exposure. In addition passive sampling devices such as the artificial mussel (AM) have been successfully applied for a wide range of metals in marine, and more recently, fresh water systems, but never before used for determining Pt concentrations. The AMs were deployed along with a freshwater clam biomonitor species, *Corbicula fluminalis africana,* at three sites along the Hex River, which flows through an intensive Pt mining region. Metals that were analysed in this study are As, Cd, Co, Cr, Ni, Pb, Pt, V and Zn. The results showed differential uptake patterns for Pt, Cr and Ni between the AMs and clams. Clams are able to take up metals from the water in both the dissolved form as well as complexes bound to particulate matter. Thus the physical and chemical conditions at the different sites and during the different surveys influence the form of the metal available for uptake by the clams while the AMs only accumulate the bioavailable fraction. Thus the combination of AMs and bioaccumulation indicator organisms will provide a holistic assessment of metal exposure in aquatic environments associated with mining activities.

4.03P.6

Copper and zinc plant bioaccumulation in Crambe abyssinica fertilised with liquid swine manure and poultry litter

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Animals manure is massively used as an organic fertiliser to increase yields crop and reduce the chemical fertiliser application. However manure often contains high concentrations of Cu and Zn that can be bioaccumulated in soil and plants putting in risk animals, humans and the environment. Fieldwork was performed to verify the effects of different rates of soil fertilisation with liquid swine manure and poultry litter on crop yield of Crambe abyssinica. Their Cu and Zn foliar uptake and bioaccumulation was also evaluated. The investigation was carry out on 24 plots being 6 plots for each 4 treatments in a randomized block design. The treatments consisted in soil fertilisation with (1) poultry litter, (2) liquid swine manure, (3) chemical fertiliser (N, P2O5 e K2O) and the (4) control (no fertilisation). At the end of the crop cycle, grain yields, copper and zinc content in leafs were evaluated. Results showed that grain yields were statically equivalent in relation to both animal's manure and chemical applications, but superior to the control, demonstrating that the fertilisations evaluated was effective in providing nutrients for the crop increase. The leaf content of Cu was similar in all the treatments (~ 5mg kg-1) within a safe value. Application of liquid swine manure resulted in higher leaf content of Zn (~ 45mg kg-1) compared with the others treatments (~ 33mg kg-1), still lower than the range values 100-400mg kg-1 considered harmful for the environment. Crambe abyssinica grain yields was better in the soil enriched with animals manure and did not bioaccumulate high amounts of Cu and Zn, which indicates that animals manure can be a safe biofertiliser if not applied in excess.

4.03P.7

Fish Biotransformation in Bioaccumulation: Output from a HESI Technical Workshop

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Hydrophobic organic chemicals may bioaccumulate in fish and other aquatic organisms, thereby increasing the organism's exposure to the chemical and the risk of potential adverse effects. Chemical registration in most jurisdictions requires an evaluation of a chemical's bioaccumulation potential. The currently available in vivo test (OECD TG305) is resource-intensive and as a result, bioaccumulation assessments are often performed using predictive computational models. These models include QSARs for BCFs and one-compartment (1-Co) and multi-compartment PBTK mass-balance models. For the mass balance models, a key input is the rate of biotransformation, which is a major source of uncertainty in chemical bioaccumulation assessments for fish. To improve modeled predictions of bioaccumulation in fish, reliable biotransformation rate estimates are needed to parameterize the models. Two OECD Test Guidelines (OECD TG 319 A & B) were recently developed to estimate intrinsic clearance using in vitro biotransformation systems derived from rainbow trout liver tissue. The clearance values obtained from the in vitro methods can be used in the models to refine bioaccumulation estimates. The standardization of these methods for fish and recent OECD TG developments will allow additional data to be generated that can inform bioaccumulation assessment specifically and ecological risk assessment more generally. However, additional work is still necessary to enhance uptake, application, and advancement of these approaches to improve the science that underpins chemical evaluations. A HESI technical workshop was held in October 2019 aimed at identifying immediate, short-term, medium-term, and longer-term needs related to research and coordination in this space. Session topics covered in the workshop included: current use of in vitro methods + IVIVE to predict bioconcentration of chemicals that undergo biotransformation in fish; improving current use of IVIVE procedures; "second generation" in vitro methods & extrapolation approaches; and regulatory use & uptake. This poster will provide a summary of the main workshop conclusions and provide an overview of a strategic roadmap for future work.

4.03P.8

Toxicokinetics of silver nanoparticles in a stream mesocosm

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Despite the large volume of (eco)toxicity studies on engineered nanomaterials (ENMs), their environmental risk is still challenging to evaluate. It is crucial to understand the fate, transformations and behaviour of ENMs when released to the environment and their consequent interactions with biota. The present study aimed at assessing the fate and bioaccumulation of a simulating Ag ageing form (Ag2S-NP) and the ionic counterpart as silver nitrate (AgNO3) in a simulated stream scenario. The aims were: 1) to determine toxicokinetics of Ag2S-NPs in freshwater invertebrates; 2) to compare kinetic parameters of Ag uptake with those derived from single-species tests; 3) to evaluate potential trophic transfer. The study was performed in an indoor modular mesocosm system. Each stream was filled with 7 kg of sediment mixed with grounded alder leaves and with 35 L of artificial pond water. The following species were placed in each unit: Physa acuta (snail), Dugesia tigrina (planarian), Chironomus riparius (non-biting midge), Lumbriculus variegatus (oligochaete worm), Daphnia magna (water flea) and Oncorhynchus mykiss (rainbow trout). Biota, water and sediment were sampled destructively at days 2, 7 and 14 (last day). Biota was sampled for Ag analysis and toxicokinetics were evaluated for snails, planarians and chironomids using one-compartment models. Results revealed higher total Ag body concentrations in snails exposed to both forms in the mesocosm. Exposures to Ag2S-NP showed higher uptake rate constants from water (kw) for snails from single-species tests than from the mesocosm test, but similar uptake rate constants from sediment (ks). Elimination rate constant (ke) for snails exposed to Ag2S-NP was also much higher in single-species than in mesocosm exposed snails. For AgNO3 exposures higher kw and ks were found for mesocosm tests. Internal Ag concentrations in chironomids were higher following AgNO3 exposure in mesocosms. Exposures in mesocosms led to almost 20 times higher Ag body burdens in planarians exposed to AgNO3 than to Ag2S-NP. Planarians exposed to Ag2S-NP in single-species tests showed no Ag uptake, and very low Ag uptake from AgNO3 exposure. In the single-species tests planarians were not exposed to contaminated food, therefore mesocosm results may indicate that ingestion of food is an important uptake route for these organisms. Modelling was done to provide a more complete analysis, exploring all possible exposure routes and Ag bioavailability.

4.03P.9

QSAR modelling and prediction of organic chemical half-lives in aquatic and terrestrial organisms

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The bioaccumulation (B) assessment of new and existing chemicals is a legal requirement for many regulatory agencies and a relevant step in exposure and risk assessment. The first-order, whole body, primary biotransformation rate constant (kB) as well as total elimination rate constant (kT) and corresponding half-lives (HLB and HLT) are key parameters determining the overall bioaccumulation potential of a chemical, particularly for hydrophobic chemicals. However few data and predictive tools for these parameters exist. Given the relative importance of kB and kT on the bioaccumulation process, the limited availability of measured data, the extensive costs associated with bioaccumulation testing, and the aim to reduce animal testing, there is a need to develop and evaluate more in silico tools for predicting these toxicokinetic (TK) parameters. This work presents existing and new QSARs for predicting HLB in fish as well as HLT in human and rodents. These HLs have been modelled using a limited number of theoretical molecular descriptors and in compliance with the OECD principles for the validation of (Q)SAR models for regulatory purposes. Performances calculated for the new QSAR models generated using the fish and rodents datasets reflect the good ability to fit the data in the respective training sets. In addition, the models appear to be robust and externally predictive also when applied to generate predictions on different splitting schemes. The analysis of the applicability domain (AD) was performed to identify the chemical space that is not well predicted by the QSARs (chemicals outside the ADs). Finally, the identification of chemicals with slow HLs among those investigated in the studied organisms, provides indication of high B potential in different environments (i.e. aquatic and terrestrial) which may be used to guide future experiments.

4.03P.10

QSAR approach for the analysis and prediction of in vitro intrinsic hepatic clearance in rat and mouse

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The CEFIC LRI ECO44 project "Integrating Bioaccumulation Assessment Tools for Mammals (iBAT-Mam)" aims to integrate experimental data and data generated by *in silico* approaches, to improve mammalian bioaccumulation (B) assessment. Within this context, models based on *in vitro-in vivo* extrapolations (IVIVE) and Quantitative Structure-Activity Relationships (QSAR) are used to maximize the information available from *in vitro* and *in vivo* measurements and to address *in vivo* biotransformation rate data gaps. The present work has the goal to provide an *in silico* QSAR approach for the prediction of *in vitro* intrinsic hepatic clearance ($CL_{int,\ in\ vitro}$) in rodents. $CL_{int,\ in\ vitro}$ reflects the liver's ability to transform the chemical independently of the blood flow and the bioavailability of the

substance *in vivo*. IVIVE models are used to scale $CL_{int,\ in\ vitro}$ to *in vivo* rates such as hepatic clearance, which is a key parameter in bioaccumulation and physiologically-based toxicokinetic (PBTK) models. Experimental data for $CL_{int,\ in\ vitro}$ available in the iBAT-Mam project database were measured for more than 7000 chemicals (mostly drugs) for two rodent species (i.e. *Rattus norvegicus* and *Mus musculus*) using different *in vitro* biotransformation rate assays (i.e. S9 homogenate, hepatocytes, microsomes). Preliminary QSARs were generated following reactivity based patterns, based on sites of metabolism (SoMs) in CYP450 which were identified by using the SMARTCyp method embedded in Toxtree software (JRC EU-Commission). Theoretical molecular descriptors used as input for multivariate analysis and QSAR modelling were generated from canonical SMILES. Particular attention was paid to generate models compliant with OECD guidelines for QSAR models development and validation i.e. effort was made to generate statistically reliable and externally predictive models with defined structural applicability domains (AD). These results demonstrate that QSAR methods can predict biotransformation rate endpoints which can then be used to parameterize PBTK models, to improve bioaccumulation assessment and to refine the evaluation of the hazard and risk that chemicals may pose to humans and the environment.

4.03P.11

Refining bioaccumulation screening of neutral hydrophobic organic chemicals in air-breathing organisms using in vitro liver S9 biotransformation assays

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Using in vitro biotransformation assays combined with in vitro-in vivo extrapolation (IVIVE) for bioaccumulation screening can reduce animal testing and inform a weight of evidence approach to bioaccumulation assessment. Recently, the Organisation for Economic Co-operation and Development (OECD) has developed test guidelines (No. 319B) to determine the the in vitro intrinsic clearance of a test chemical using liver S9 of rainbow trout (RT-S9). As bioaccumulation screening for air-breathing organisms is of increasing concern, there is a need for mammalian bioaccumulation screening protocols involving in vitro approaches. This study reports on the development and testing of a screening approach for bioaccumulation assessment of neutral hydrophobic organic chemicals in air-breathing organisms using rat liver S9 biotransformation assays. It is based on the OECD RT-S9 protocols with modifications to provide all the information needed to conduct IVIVE for the purpose of bioaccumulation assessment. Modifications include the determination of the unbound fraction ($f_{u,inc}$) of the test chemical in the incubation, which is not included in current RT-S9 protocols. We illustrate that $f_{u,inc}$ can be estimated using a composition-based approach by including lipid and protein analysis of rat liver S9, or measured experimentally using a solvent-free passive-dosing approach. Results show good agreement between model estimated and experimental $f_{u,inc}$ values. Results further indicate that an in vitro depletion rate constant in a rat liver S9 greater than 0.3 d^{-1} is sufficient to negate biomagnification in rats.

4.03P.12

Determination of In Vitro Intrinsic Clearance - a Discussion on Evaluation Criteria

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Our research seeks to reduce the uncertainty in the estimation of chemical bioaccumulation in fish when applying non-animal computations and experimental methods. Within the Cefic-LRI ECO.34 project, we followed a tiered strategy that integrates toxicokinetic (TK) models, quantitative structure-activity relationships (QSARs), in vitro experimental data from fish liver, gill, and intestinal tissues, and in vitro-to-in vivo extrapolation methods. We tested eight organic chemicals with varying physico-chemical properties in these different in vitro assays, all derived from rainbow trout (*Oncorhynchus mykiss*): a primary gill cell system, S9 fractions of liver and intestine, and three fish cell lines (RTgill-W1, RTL-W1, RTgutGC). Overall disappearance over time of the test chemicals was quantified in order to determine in vitro intrinsic clearance (CLinvitro) rates for incorporation into the computational models to predict fish bioconcentration factors. To derive CLinvitro, we followed the criteria outlined in the OECD 319 test guideline, including controls, number of time points, abiotic degradation and R^2 values of the log-normalized depletion of the parent compound.Out of 115 experiments performed within the project, 16% required the case-by-case approach mentioned in the OECD guideline because the outcomes were not clear-cut. In addition, out of remaining 97 experiments, 39 failed the R^2>0.85 OECD criterion. That led to the need for the decision whether the CLinvitro determined in these experiments should nevertheless be considered or whether the in vitro intrinsic clearance would have to be assumed to be below the limit of quantification. Finally, in five cases, one biological replicate differed from two others regarding the fulfillment of the OECD criteria.We wish to discuss these issues and suggest more detailed and standardized criteria for data evaluation with the aim to reduce the need for the case-by-case approach and the requirement of detailed expert knowledge. In this way, the OECD 319 guideline should become more widely applicable both with regards to the in vitro systems applied and the communities benefitting from this guideline.

4.03P.13

Biotransformation and blood flow: Does the well-stirred liver model underestimate in vivo clearance?

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Most commonly, the so called well-stirred liver model is used for in vitro-in vivo extrapolation of hepatic biotransformation data. This model represents the whole liver as a single well-stirred compartment and, by this, is a great simplification of the actual in vivo situation. The most prominent alternative, that represents the in vivo situation more accurately, is the so called parallel tube model. The parallel tube model considers the liver as an aggregation of identical cylindrical tubes. Mathematically, the well-stirred model is simpler than the parallel tube model. However, the well-stirred liver model neglects the concentration gradient that develops for well metabolized chemicals during passage through the liver capillaries, the so called sinusoids, and is known to underestimate hepatic biotransformation. In the poster, we present a comparison between both models and evaluate how much the in vivo predictions differ. For doing so, we calculate the whole-body in vivo biotransformation from given intrinsic hepatocyte biotransformation for varying input parameters (i.e. different fish sizes, different hepatic blood flows) with both models. The analysis reveals that the results of both models differ by roughly 20 % maximum. Furthermore, this maximum difference occurs for scenarios in which the chemicals are cleared efficiently from blood. Precisely, the maximum difference is observed when hepatic extraction ratio (sometimes also called “hepatic elimination efficiency”) is bigger than 0.6, meaning that the blood concentration is reduced by more than half during a single passage through the liver. For these cases, biotransformation is so efficient that bioaccumulation becomes rather unlikely. Accordingly, the use of the parallel tube liver model will usually not be needed in regulatory context.

4.03P.14

Quantifying the Biomagnification Potential of Polychlorinated Biphenyls in Arctic Wolf and Domestic Dog by Equilibrium Sampling

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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 that they eat. The underlying mechanism is the decrease in the fugacity capacity (*Z*) of the food for the biomagnifying contaminants during digestion and assimilation in the gastrointestinal tract (GIT). Traditionally biomagnification is quantified by measuring contaminant concentrations in tissues collected from dead animals. Here we propose a non-invasive way to quantify the Maximum BioMagnification Factor (MBMF) by determining the ratio of the *Z*-values of the undigested and digested food using equilibrium sampling. We quantify the *Z*-values by equilibrating homogenized food and feces samples with silicone films of variable thickness coated on the inside of glass vials that are being rotated for several days. Contaminants that have migrated to the silicone are extracted and quantified using gas chromatography-mass spectrometry. Equilibration is confirmed if the amount of contaminant in the film is proportional to the volume of silicone in a vial. The content of contaminants in food and feces samples is also quantified. We demonstrate the feasibility of this method for wolf (*Canis lupus hudsonicus*) and domestic dog (*Canis lupus familiaris*). For adult wolf eating a relatively lean meat diet, MBMFs of approximately 41 were observed, whereas the MBMFs reached 81 for an adult domestic dog eating a lipid-rich diet. Beside the dietary lipid content which strongly affects the *Z*-value of the diet, the capability of an animal to digest its diet also influences the MBMF by controlling the *Z*-values of their feces and the volume reduction of the foods in the GIT. Less efficient digestion leads to a lower MBMFs in a juvenile dog (approximately 35) compared to its older self, even though their diets had similar lipid contents.

Bioremediation and Phytoremediation of Contaminated Ecosystems (P)

4.04P.1

Hexachlorocyclohexane bioaccumulation in Alnus glutinosa - height, age, season and isomer specificity

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Groundwater phytoscreening is an established technique for the indication of volatile pollutants (VOC). Its main advantage is quick and non-expensive mining of VOC presence data. Over 30 studies deal with specific aspects of phytoscreening application, e.g. with VOC contamination plume delineation, with seasonal and directional specificities of VOC in-plant variability, with the tree sap monitoring or with fast growing species VOC uptake. The presence of hexachlorocyclohexane (HCH) in plants was also indicated by some authors, but to our knowledge, no study dealt with HCH tree monitoring for phytoscreening purposes. HCH are not considered belonging to VOC. They are less volatile and less water-soluble (units of mg/l) than most of the VOC. On the other hand, HCH isomers are more water-soluble than any other persistent organic pollutants (POPs). Our preliminary field data from the year 2014 were promising. Ever since we have developed HCH phytoscreening methodology and data interpretation. In this study, we report on our progress in phytoscreening of HCH isomers in Alnus glutinosa. Data for this study were gathered at HCH dumpsite Hájek, Czech Republic. Seasonal harvesting of Alnus glutinosa trees was performed at the site. Pieces of the trunk were sub-sampled in the laboratory providing altogether 355 samples throughout the year. After extraction, GC-MS/MS quantification of HCH isomers and their transformation products chlorobenzenes (ClB) was performed. A reference (clean) Alnus glutinosa tree individual was harvested the same day to provide material for matrix-matched calibration. The results of our study bring detailed information on the distribution of individual HCH isomers and ClB in alder trunks. As for ClB, values under LOQ were usually found in tree biomass, with few exceptions. A significant change was found in isomeric profile in groundwater and in alder biomass. Isomer δ prevailed in groundwater, forming over 76 % of total HCH. In contrast, isomer δ was a minority in alder trunk, where β and ε isomers dominated (52.5 % and 43 % resp.). Isomer δ presence was significantly higher in winter samples. A decreasing gradient in total HCH concentrations with above-ground height was indicated. Similarly, total HCH concentrations were highest in the oldest wood and decreased towards tree surface. Seasonal variability, wood age and height specificities have to be taken into account for proper data interpretation of HCH groundwater phytoscreening.

4.04P.2

Bioaugmentation of a consortium isolate for the degradation of Sodium Lauryl Ether Sulphate in soil

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Bioaugmentation is a green technology used for removing contaminants from soil and water. The addition of selected microbial strains promotes the catabolic potential of soil microbial communities for the degradation of pollutants. The anionic surfactant Sodium Lauryl Ether Sulphate (SLES) is the principal component of several commercial foaming products for soil conditioning in the tunneling industry. Huge amounts of soil debris are produced during the excavation process and the presence of SLES can affect the re-use of the spoil material as a by-product. A prompt SLES biodegradation is a key point for the re-use of the excavated soil. A bacterial consortium capable to degrade this anionic surfactant was isolated and characterized from an excavated soil. Then the bioaugmentation, using the bacterial consortium isolated, has been performed in soil microcosm experiments. For this purpose, microcosms containing uncontaminated soil and soil treated with a foaming agent containing SLES, both derived from a tunneling construction site, were set-up. Half of the soils were inoculated with the SLES degrading bacterial consortium. Soil sub-samples were collected from each replicate microcosms at selected time to determine the SLES residual concentrations (Pressurized Liquid Extraction (PLE) and Methylene Blue Active Substances (MBAS) method), the microbial abundance (DAPI counts method) and cell vitality (live/dead method). The main results of the experiment will be shown and discussed.

4.04P.3

Terrestrial Microbial fuel cells for degrading persistent organic pollutants

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Terrestrial Microbial Fuel Cells (TMFC) are bioelectrochemical systems (BES) which exploit the microbial activity of exo-electrogen bacteria for reducing pollutants, recycling useful elements and producing electricity. In the framework of bioremediation BES technologies have attracted a lot of interest in the recent years, for their effectiveness in environmental depollution. DDE [1,1-dichloro-2,2-bis(chlorophenyl)ethylene] is the main metabolite of the pesticide DDT [2,2-bis(chlorophenyl)-1,1,1-trichloroethane], a common and persistent organic pollutant. A cause for concern is its toxicity (mutagenicity, estrogenicity, carcinogenicity) and biomagnification through aquatic and terrestrial food chains. In this study TMFCs were used for testing their effectiveness in promoting DDE degradation in a contaminated soil. The microbial fuel cells were set up in presence or absence of compost and using an open (OCV) and closed circuit (CCV). The pesticide concentration and the structure (microbial abundance and characterization by NGS) and functioning (cell viability and activity) of the microbial community were evaluated over the experimental time. Moreover, electric measurements for assessing power generation were also carried out. The overall results show that the DDE degradation was promoted by the TMFCs, on the contrary no decrease in DDE concentration was observed in the control soil. Interestingly, the microbial activity in TMFCs was positively correlated with the voltage values showing the key role of bacteria in this technology.

4.04P.4

Waste Water Treatment Plants?- Phytoremediation by submerged macrophytes

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Nowadays there are almost limitless options for creating new products to make us healthier, strengthen our buildings and make our everyday life easier. The key to all these is the use of chemicals in all sorts of combinations and amounts. Due to their widespread use, the advantages of such chemicals as part of the modern society can turn into a disadvantage for the environment and eventually into a disadvantage for human and wildlife health. Many man-made substances find their way into wastewater and some are only poorly removed by wastewater treatment plants (WWTPs). This issue is receiving growing attention due to the possible ecotoxicological effects of organic micropollutant (OMP) mixtures when they enter the environment. Here, different approaches are being tested to deal with the complex mixture of OMPs remaining after conventional WWTPs. Technologies such as ozonation are promising approaches, but because of their high financial costs not always affordable for all municipalities or countries. A more cost-effective and technically simpler way of removing such OMPs from wastewater is phytoremediation (PHR), which already has shown positive effects. However, in most of these studies the fast-growing species *Elodea canadiensis* has been used. This species is an invasive neophyte from North America and can harm native ecosystems. Therefore, in order to find a more sustainable PHR solution for ecosystems in Germany and Europe, three native species of submerged macrophytes (*Lemna minor, Ceratophyllum demersum* and *Myriophyllum verticillatum*) have been investigated for their removal efficiency of selected OMPs. In an initial laboratory batch-experiment over 25 days, a cleaning effect by the plant for 16 out of 44 tested substances could been determined. PHR by itself or in combination with other approaches could be a chance for removing OMPs more efficiently from wastewater. This effect is confirmed in a semi-field study currently underway at the Soers WWTP in Aachen. Here, the removal efficiency of the plants is being tested with effluent of the secondary clarifier of the WWTP in a flow-through set-up. Over a period of 14 days the three plant species either alone, or in combination, are being exposed to the wastewater while the inflow and outflow concentrations of a range of wastewater OMPs are being measured. Finally, a radiolabelled fate study using one of the OMPs is planned to look in detail at possible metabolite production and fate within the plants.

4.04P.5

Estimation of key micropollutants present in different wastewater settings across New Delhi and development of treatment system for their mitigation

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Sewage (treated and untreated) is one of the major sources of micropollutants in fresh water ecosystem. A comprehensive screening of micropollutants was performed in different environmental settings such as sewage treatment plants (STP), major hospitals and sewer drains across New Delhi considering 21 most abundant PPCP residues (pharmaceutical and personal care products). Modified trickling filter (MTF) and advance oxidation process (Ozone) are used to remove these micropollutants from wastewater. Wastewater from the second largest sewer drain of Delhi, Barapullah drain was used to assess the performance of the treatment systems. In the wastewater from Barapullah drain, 8 prominent PPCPs (metformin, acetaminophen, trimethoprim, atenolol, sulfamethazine, erythromycin, carbamazepine and mefenamic acid) were found. The MTF was operated on different HRTs for more than one year and its performance was assessed for the removal of conventional as well as emerging pollutants. Significant removal (>80%) of the targeted emerging pollutants was observed during the operation of the MTF reactor. No removal of carbamazepine was observed in the treated effluent from MTF, whereas sulfamethazine and erythromycin were not detected in the influent as well as in the effluent of the reactor. The secondary treated effluent from the MTF was passed through the ozone system for tertiary treatment to observe further removal of targeted compounds. Significant removal of the targeted emerging pollutants was observed during tertiary treatment and overall, results indicate MTF reactor with ozone is a promising for decentralised wastewater treatment.

4.04P.6

MOLECULAR BIOLOGY TOOLS (MBTs) COUPLED WITH COMPOUND-SPECIFIC ISOTOPE ANALYSIS (CSIA) TO DEFINE A RECLAMATION PLAN, BASED ON ENHANCED BIOREMEDIATION, FOR A CONTAMINATED AQUIFER

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An aquifer contaminated mainly by monochlorobenzene (MCB), benzene and the three isomers of nitrochlorobenzenes (i.e. orto-nitrochlorobenzene_ o-NCB, meta-nitrochlorobenzene_ m-NCB and para-nitrochlorobenzene_ p-NCB) was fully characterized by the use of Molecular Biology Tools (MBTs) combined to Compound-Specific Isotope Analysis (CSIA). Given the type of contaminants, potentially biodegradable both in the presence and in the absence of oxygen, the genetic markers for the aerobic biodegradation and the anaerobic reductive dehalogenation were investigated. The presence of good levels of gene markers for aerobic degradation (i.e. toluene dioxygenase_*todC* for MCB, toluene monooxygenase_*tmoA* for benzene and *ncb* for NCBs_ new genetic probes, not yet described in the literature, were designed for the identification of a dioxygenases active on their biodegradation) in three of the five points investigated is associated with possible oxidative reactions of the substituted benzene ring, catalyzed by mono or di-oxygenase. Based on these values, the supposed aerobic Natural Attenuation (NA) processes were investigated through different microcosm tests in laboratory. The results showed that an effective aerobic degradation, excepting for o-NCB, can be stimulated by oxygenation of the aquifer in the three points characterized by the higher values of the genes identified as degradation markers. In the remaining two points, characterized by a lower concentration of those markers, this process was further stimulated with the addition of inorganic salts. The presence of an aerobic degradative pathway was further strengthened by an isotopic investigation (i.e. $^{13}C/^{12}C$) on residual contaminants. The results showed similar values, even temporally, for all the contaminants examined with not evidences of isotopic enrichments, confirming the aerobic rather than reductive capacity, certainly for MCB and benzene. Concerning the NCBs, even it is not possible to obtain a precise indication of the potential degradation processes because of the lack in literature studies; the absence of fractionation suggests that, similarly to MCB and benzene, there are not reductive degradation processes ongoing. Moving from all these evidences, a reclamation plan based on an enhanced bioremediation with oxygenation of the aquifer and the addition of inorganic salts in low concentration was approved.

4.04P.7

Bacterial degradation of organophosphorus and organochlorine pesticides in contaminated agricultural soils of the Colombian Caribbean

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Among main environmental problems facing humanity today, is the impact of contamination with organic pesticides of synthetic origin. The high demand for agricultural products, are some of the factors that cause the use of pesticides. Even when their toxicological effects on ecosystems, organisms and human health are known, they are still used in accordance government regulations. These chemical substances used in crop protection are adsorbed in soils, where they bioaccumulate and biomagnify. Among the most used pesticides in Colombia, we find organophosphorus (OP) and some organochlorine (OC), these groups of pesticides are characterized by their high degree of toxicity and persistence. For these reasons, the objective of this research was to degrade organochlorine and organophosphorus pesticides using bacterial strains native to agricultural soils in the Colombian Caribbean. The methodology used consisted of the isolation and identification of native bacterial colonies with the ability to degrade the pesticides evaluated. Malathion, chlorpyrifos and coumaphos were used for OP pesticides. For OC, a mixture of lindane, metolachlor, endrin and p, p'-DDT was used. Bacillus cereus, with degrading capacity of OP and Paenibacillus lautus, with degrading capacity of OC was obtained from the identification of bacteria. Finally, the kinetics of bacterial growth were studied by optical density in a UV-VIS spectrophotometer and the quantification of pesticide degradation in a gas chromatograph coupled to a mass spectrometer (GC-MS). After twelve days of bioassays, the bacterial colonies B. cereus degraded the pesticides malathion, chlorpyrifos and cumaphos, in percentages of 52.37%, 78.79% and 79.55%. And the P. lautus degraded the pesticides lindane, metolachlor, endrin and p, p'-DDT, in percentages of 65.04%, 60.80%, 55.74% and 65.14%, respectively. Considered as very good results when compared with other report research, thus meeting the objectives proposed in this project.

4.04P.8

Pyrene co-metabolism in soil by Pseudomonas putida G7 and metabolite uptake and distribution by sunflower crops.

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Polycyclic aromatic hydrocarbons (PAHs) are a recalcitrant group of contaminants, known to be highly persistent in the environment. Most of these compounds, such as pyrene (PYR), are persistent pollutants in the soil. Actually, motility and dispersal are key ecological drivers that allow bacteria to move toward places of beneficial environmental conditions and to maintain significant ecosystem functions. Few studies have been carried out on the use of plant growth regulators and their influence on the removal of PAHs from the soil. However, no studies have been conducted on the effect of 14C on crops with sunflowers and its effect on PYR removal efficiency in the soil. The present study aims to investigate the effects of the simultaneous application of 14C PYR by passive dosing (O-rings) and inoculation with *Pseudomonas putida* G7 on the enhancement of PYR removal in the presence of sunflowers. The experiment was carried out in a greenhouse at 23 ± 1 ºC. The treatments included planted soil with the simultaneous application of bacteria and pyrene (T1), planted soil with pyrene (T2) and unplanted soil with pyrene and bacteria (T3). Planted soil (C1) and planted soil with bacteria (C2) were included as positive controls. Furthermore, in order to identify the metabolites formed during the experiment a preliminary study under laboratory conditions was achieved. Samples of leachates, soil and plant were obtained at different times from cultivation until harvest for the investigation of uptake, transport, and accumulation of the microbially-processed PYR in sunflowers. The results showed that planted soil with simultaneous application of pyrene and bacteria treatments enhanced the efficiency of pyrene transformation. The roots presented the highest levels of uptake and greatest bioconcentration factors. GC–MS analyses of the extracts from the preliminary studies revealed the presence of 3 metabolites due to pyrene cometabolism by *P. putida* G7. From the data acquired, we suggest that the combined phytoremediation and bioaugmentation can be a suitable alternative for reducing risks during remediation of PAH-contaminated soils.

4.04P.9

Chemoeffectors influence on the motile behavior and dispersion of pollutant-degrading bacteria in membrane bioreactors with restricted porosities.

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The controlled dispersal of motile bacteria through contaminated soils is currently considered as one of the most promising aspects in bioremediation. The difficulty caused by the heterogeneous distribution of the pollutants and the degrading bacteria can be overcome by the use of different chemoefectors. These compounds provide the control over bacterial tactic motility, what results in an increase in the efficiency of the microorganisms dispersion and subsequently, enhanced biodegradation rates (Environ. Sci. Technol. 42: 1131-1137, 2008; Environ. Sci. Technol. 46: 6790-6797, 2012). The objetive of this study was to evaluate the dispersal capacity of the chemotactic bacterium Pseudomonas putida G7 (cell dimensions: 1 µm x 3 µm) through bioreactors assays. The innovative experimental design consisted in a bioreactor system with two chambers separated by a membrane with specific pore sizes that restrict the bacterial dispersal (Environ. Sci. Technol. 49: 14368-14375, 2015). Different chemoffectors (salicylate, glucose, zero-valent iron nanoparticles [Environ. Pollut. 213:438-445, 2016] and the root exudates components:γ-aminobutyric acid (GABA) and citrate) were used to modulate the motile bacterial behavior throught restricted porosities (1 µm to 12 µm). The initial results were promising. The transport of P. putida G7 through the membrane occurred at higher rates than the control for the majority of chemoeffectors. Progress in this field will undoubtedly open up new possibilities for bioremediation processes in contaminated soils, improving the existing techniques for the treatment of poorly bioaccessible contaminants.

4.04P.10

Use of a standardized desorption extraction method to study the effect of enhanced phase exchange in bioremediation

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This work investigated the effect of bioremediation on bioavailability of native PAHs present in contaminated soils. Bioavailability has been measured with a single-time point Tenax extraction at 20 h because this method has been recently

proposed by the International Organization for Standarization as one of the chemical methods to measure environmental bioavailability of nonionic pollutants (ISO/TS 16751). In addition, as a quality assurance, we also characterized the desorption kinetics of the full profile of PAHs in some of the studied samples to highlight the importance of ensuring the 20 h time window to capture the fast desorption when using this standarized method. This work is one of the first ones that use this ISO method systematically in the field of bioremediation, and shows its advantages when used in combination with total concentrations determined with conventional, exhaustive solvent extraction. The method was applied to different PAHs contaminated soils which had different level of total PAHs (66-4370 mg Kg^{-1}), and dissimilar origin and bioremediation approaches. Some samples were from traditional remediation approaches such as biostimulation and phytoremediation, and others were from bioavailiability oriented treatments such as involving either biosurfactants or bioaugmentation with specialized microbial inocula. In the samples which were from biosurfactants treatments, the effect of a rhamnolipid biosurfactant in solubilization and biodegradation of PAHs was studied previously. To do it, the desorption extraction with Tenax was also used succesfully and the results showed that rhamnolipid can constitute a valid alternative to chemical surfactants in promoting the biodegradation of slow-desorption PAHs, which is one of the most important problems in bioremediation. Furthermore, the results from the complete set of the studied samples in this work showed that the pollutant fractions extracted with Tenax during 20 h (D20) decreased after traditional bioremediation (biostimulation and phytoremediation), but they often increased in bioavailability-oriented treatments. Therefore, the application of D20 assessment provided us information on the bioremediation performance, not directly evident through the measurement of total PAH concentrations.

4.04P.11
Ecological modelling in support of soil remediation in metal polluted nature areas
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Nature based remediation (NBR) techniques offer less disruptive alternatives to traditional soil remediation. This is especially relevant for pollution in nature areas. To make an informed choice on the optimal remediation technique, quantitative measures are critical. It is, however, often hard to quantify the potential benefit of a NBR technique over conventional techniques. Within the EU-LIFE project NARMENA (Nature-based Remediation of Metal pollutants in Nature Areas), ecological modelling is used as a tool to assess the ecological impact of several remediation techniques and help guide the remediation process. Three target sites in Flanders with historical metal soil and sediment pollution were chosen: Grote Calie, Winterbeek and Grote Laak. These sites are situated in valuable nature areas and low-impact remediation techniques such as NBR are thus preferred options here. It is however unclear how effective such NBR techniques would be to reduce the actual ecological risk. Therefore, the ecological risk of the current metal pollution will be determined using a combination of environmental monitoring, ecotoxicity testing and ecological modelling. The impact of different remediation scenarios will be assessed using ecological modelling. Remediation scenarios include no action, conventional techniques (i.e. excavation) or nature-based remediation (i.e. phytoremediation and artificial wetlands). To assess their ecological impact, population models for indicator species per compartment (water column, sediment and soil) will be used. Environmental monitoring will provide insight into the main toxicants and data on their effects will be gathered based on literature and dedicated toxicity tests. The population models allow to extrapolate these effects observed in the laboratory to population effects in the field. This allows to evaluate the expected ecological impact of each remediation technique and select the least disruptive option.

4.04P.12
Bioaccumulation of α-, β-, and δ-HCH isomers by Alnus glutinosa
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The hexachlorocyclohexane (HCH) and its isomers are long-banned pesticides. Even though their use has been prohibited for decades, their presence in the environment is still reported worldwide. HCH is a cyclic chlorinated compound and has five major isomers α-HCH, β-HCH, δ-HCH, γ-HCH and ε-HCH. In this exterior study, we aimed to determine the bioaccumulation potential of α, β, and δ isomers of HCH by Alnus glutinosa (Alder tree) seedlings. Each HCH isomer alone was mixed with soil to reach a final concentration of cca 5 mg/kg in one pot. Control was prepared with the same soil without HCH. All variants were set in triplicate and had instant access to water for three months. HCH was analyzed in soil and sections of seedlings (roots, trunks, branches, leaves) on GC/MS. Amplicon 16S rRNA sequencing was applied to study the microbial community in rhizosphere samples. The highest concentrations of HCH isomers were detected in the roots with a decreasing trend towards the branches and the leaves. The δ-HCH isomer was taken up in highest quantities (14.7 µg/g in roots, 7.2 µg/g in trunks, 1.53 µg/g in branches and 1.88 µg/g in leaves) while α-HCH and β-HCH were found in much lower concentrations. Most interestingly, in the β-HCH treatment, we detected high concentrations of α-HCH as well. The abundance of rhizosphere populations was similar in all HCH isomer samples with some exceptions. For example, Pseudomonas sp. significantly decreased in all HCH-amended samples, the lowest abundance was found in the δ-HCH – the isomer that was detected in highest quantities. In contrast to Pseudomonas, the abundance of an anaerobic Opitutus sp. was the highest. In samples treated with β- and δ-HCH, Rhizobacter sp. appeared, while not present in control samples. To conclude, the A. glutinosa seedlings were able to accumulate all HCH isomers. The reason why was the δ-HCH most easily bioaccumulated will be subjected to further study.

4.04P.13
Differential transport of BTEX in groundwater - Experimental and computational data to predict its removal by bioremediation
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Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX) are present in almost petroleum-derived fuels. BTEX is one of the most common contaminants found in European soils, after heavy metals and mineral oils. Although these volatile organic compounds are often treated as a whole contaminant, they behave distinctly in a porous medium as the soil is. The possibility of remediating BTEX (in admixture or separately) contaminated natural soils was evaluated by applying bioaugmentation and biostimulation techniques. Laboratory work took place in a controlled temperature environment, using two soil types (granitic and calcareous), previously sterilized and artificially contaminated. Results from soil characterization, bioremediation in steel columns, and sorption tests constituted input data to mathematical models to predict the distribution and transport and fate of the contaminants in real field conditions. Mackay's concept of fugacity was adopted to develop mathematical models able to describe and reproduce the behavior of the two different soils contaminated with the compounds of BTEX, before and after bioremediation actions. Predictions of scale-up from laboratory to field resorted to the two-dimensional advective and diffusive contaminant transport model in groundwater, as a complement to Mackay's compartmental models.The computational implementation of the mathematical models allowed the evaluation of the retardation coefficients and the differential transport of the separate components of the BTEX forming in situ zoning leading to different remediation kinetics. On the other hand, the spatiotemporal simulation of the contaminant concentrations studied allows establishing doses and exposure scenarios for a future environmental risk assessment.

4.04P.15
Integrated biological approaches for the remediation and the requalification of a hydrocarbon polluted urban area
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In this study, the design of a multipurpose phyto-bio-remediation project and the results of the first test activities are presented. The site was a former small plant for coal gasification, located in an already dense urbanised area in the city of Pesaro (Italy). The contamination (hot spots) of top and deep soil is characterised by light (C< 12) and heavy (C>12) hydrocarbons, PAH, PCE. Similarly, hydrocarbons polluted the groundwater with peaks of some aromatics ones like, benzene, m,p-xylene, toluene, naphthalene, fluorene; additionally, organochlorides like vinyl chloride, PCE and TCE are also over the limits. Finally, the inorganic elements in excess in the groundwater are boron, iron and manganese. Now the site is kept safe, with a pump & treat system and a gas-proof covering part of the area. The remediation approach is an integration of bioaugmentation for deep soil and ground water (realising bio-reactive permeable barriers), and a phytoremediation and site requalification for the top-soil. To develop and validate the bioaugmentation approach, the indigenous microbial community (bacterial and fungal) has been abalysed either both with traditional cultivation-dependent and metagenomic approaches. Metagenomic profile of the polluted area retrieve prevalence of Operational Taxonomic Unit (OUT) belonging to *Cladosporium*, *Alternaria*, *Simplicillium* and *Fusarium* genera for fungi and *Solibacter*, *Longilinea*, *Dehalogemimonas*, *Hypomicrobium*, *Rhodoplanes* and *Rhodovibrio* for bacteria most of which have been associated from literature with hydrocarbon degradation even in anaerobic conditions. After an enrichment procedure, several autochthonous strains have been isolated and screened for their capability to utilize and degrade aromatic and linear hydrocarbons using the method based on the redox indicator 2,6-dichlorophenol indophenol (DCPIP). Positive strains have been following identified based on 16S rRNA gene and Internal transcribed Spacer (ITS) sequencing for bacteria and fungi, respectively. Among all the isolates, strains belonging to *Acinetobacter* and *Pseudomonas* genera and *Purpureocilium lilacinum* have been selected for consortia development. Laboratory scale microcosm tests have been carried to evaluate degradation performance of different inoculum suitable for the bioaugmentation (soil, groundwater). At the end treatment period (30 and 90 days) pollutants degradation, microbial load and microbial community composition

have been evaluated.

4.04P.16

Rhizobacteria inoculants for phytoremediation of oil-sludge-contaminated soil: from lab formulation to pilot production

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Formulation of inoculants based on plant growth-promoting rhizobacteria (PGPR) is the crucial issue for commercial inoculants. Successful commercialization of these strains is dependent on improvements and advancements in interdisciplinary research, large-scale production, formulation methods etc. [Tabassum et al., 2017]. This industrial process can determine the commercial success or failure of a biological agent that has outstanding performance in a research facility. Formulation is the industrial “secret art” of converting a promising laboratory-proven microorganism, carefully-cultivated by skilled specialists in carefully designed and supervised experiments into a commercial product used by common growers under uncontrolled field conditions [Bashan et al., 2014]. This study is focused on practical aspects of inoculations technology development based on rhizobacteria Bacillus RB15 and Pseudomonas RB43. Technology including cultivation, concentration and drying of PGPR to establish dry inoculants using beneficial PGPR has been developed. Application of contact drying was shown the most appropriate in compare with spray drying method in terms of survivability of the thermolabile microorganisms. Special emphasis was given to formulations drying using natural organic materials. Shortly after suspensions of bacteria are inoculated into the soil without a proper carrier, the bacteria population declines rapidly. This phenomenon, combined with poor production of bacterial biomass, difficulty sustaining activity in the rhizosphere, and the physiological state of the bacteria at application time, can prevent the buildup of a sufficiently large PGPB population in the rhizosphere. Humic substances, peat, and montmorillonite were used as carriers of thermolabile PGPR cells at drying and long-term storage. All components have proved to be an excellent matrix for immobilization of cells due to its biocompatibility, non-toxic nature, and reduced vulnerability to environment stresses which will provide prolonged action of bacterial preparation in field conditions. References: 1. Tabassum B., Khan A., Tariq M, Ramzan M., Khan M., Shahid N., Aaliya K. Bottlenecks in commercialization and future prospects of PGPR. Applied Soil Ecology (2017) 121: 102. 2. Bashan, Y., de-Bashan, L.E., Prabhu, S.R. et al. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil (2014) 378: 1.

4.04P.17

DIFFERENCES IN THE SENSITIVITY OF PLANT SPECIES IN STANDARD PHYTOTESTS

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The phytotoxicity test is widely used to evaluate various environmental objects: for screening phytotoxicity of chemicals, soil filtrates or solid waste, pesticides, biocides, fertilizers and other research applications. This test measures the decrease (or the absence) of seed germination and growth of plants “in contaminated soils” in comparison to germination and growth in a “reference” soil. It can also be used to determine the impact of chemicals on the plants by “spiking” of the reference soil with a chemical or a water sample. Acute express phytotests as well as chronic phytotests are used to assess the effects of soil remediation after the application of sorbents or other soil improvers. According to the standard methodology, several species with mandatory presentation of monocotyledonous and dicotyledonous plants are recommended. The most commonly used are monocotyl Sorghum saccharatum (sorghum), Avena sativa (oats) and dicotyledonous Lepidium sativum (watercress), Sativa radish (radish) and Sinapis alba (mustard) known for their rapid seed germination and root growth. It is known that living systems differ in sensitivity to different impact. And the result depends on the set of test species. Can siderate species which are resistant to chemical contamination be used for adequate soil quality screening? In terms of sensitivity, we compared tree species of plants in experiments with metal polluted soils and with applying fertilizers. Seed germination, root and shoot length of Sinapis alba, Avena sativa, Raphanus sativus seedlings were estimated in acute phytotests using eluate and applicative experimental design. Several types of soils were used in phytotoxicity tests :artificial soil according ISO 11268-2 (as a “reference” soil), poor-humus agrozem and rich-humus chernozem (European part of Russia) and urbanozem (soil samples from the territory of Moscow) Control and metal-spiked (650 mg/kg Pb + 1100 mg/kg Zn + 660 mg/kg Cu) soil samples were analyzed as well as soil samples with amendments - additions of lignohumate and a mineral-humate-bacterial preparation (Eminekst, LLC Biosphere company) In decreasing sensitivity in experiments with different substrates, the order of test plants can be represented as follows: metal polluted rich humus soil (chernozem): Avena sativa> Raphanus sativus> Sinapis alba; metal polluted poor humus soil (agrozem): Raphanus sativus> Avena sativa >Sinapis alba; urbanozem and artificial soil with amendments: Raphanus sativus> Avena sativa >Sinapis alba . Comparing the sensitivity of standard test cultures is important for optimizing sets of sensitive plant species. According to obtained data mustard it is quite resistant to high doses of heavy metals and excessive concentrations of fertilizers. And, therefore, this must be considered when choosing the seeds to be tested. According to current methods, a set of test plants is formed arbitrarily, which leads to ambiguous conclusions.

Complex Mixtures in User Products and the Environment: Chemical and Toxicity Profiling, and Modelling to Identify Risk Drivers and Estimate Footprints (P)

4.05P.1

Conceptual models for the identification and prioritisation of chemical mixtures in environmental risk assessment for bees and other terrestrial invertebrates

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In 2019, EFSA published the MIXTOX guidance on “harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals”. As a follow up, a set of technical reports have been prepared. Defining the problem formulation and conceptual model is key. While monitoring tools can be used for post-marketing assessments and retrospective assessments, premarketing and prospective assessments require complementary approaches. Here, frameworks and models based on use patterns and ecological behaviour are proposed for the identification and prioritisation of the chemicals to be included in the mixture assessments for different taxonomic groups, covering simultaneous and successive co-exposures. The proposal covers a set of conceptual approaches for the identification and prioritisation of relevant mixtures focusing on bees, exemplified through a comparison with other terrestrial invertebrates covering the soil and above ground compartments. The proposal focuses on the agricultural environment, and covers marketed mixtures, chemical combinations intentionally mixed during agricultural practices, as well as the most relevant unintentional exposures; considering the links between environmental risk and impact assessments as suggested by Streissl et al (doi: 10.1007/s10646-018-1962-0). The conceptual prioritisation schemes combine a) information on the key use patterns/emission routes for agrochemicals/industrial pollutants into the agricultural environment with b) the biology and ecology of representative species within each selected taxonomic group. Spatial and temporal variability are combined for identifying and prioritising relevant mixtures for short-term co-exposures requiring the assessment of acute effects, and those leading longer-term assessments, including scenarios for repeated exposures, relevant for chronic assessments. For honey bees, the concept of the colony as a superorganism is further explored regarding the assessment of combined toxicity.

4.05P.2

Identification of chemical-gene-associations applying network inference and association rule mining

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Chemical monitoring has been widely used to assess exposure to environmental contaminants. Recent methodological advances now allow for concurrent monitoring of a wide array of biological responses. When collected concurrently, elaborate chemistry and bio-effects data could enhance chemical prioritization, but methodologies for integration of these data are poorly developed. To identify and prioritize contaminants of emerging concern in Minnesota’s streams and rivers, surface water samples from 50 randomly selected locations across Minnesota were analyzed for a total of 146 chemicals (pharmaceuticals, personal care products and variety of other chemicals). 48h-exposure of adult fathead minnows to surface waters from 10 sites (n=5-7 fish per location) was conducted; liver gene expression was analyzed using a custom 60K feature fathead minnow microarray. Twenty-nine out of 146 measured chemicals were detected and 10 occur at single compound concentrations sufficient to initiate molecular responses in vitro. Although only a small subset of compounds was detected, we identified transcriptomic effects associated to anthropogenic chemicals. A differential expression analysis was performed based on chemical presence (detected chemicals vs. control).

Network inference was applied to cluster genes by their coexpression as modules and to correlate the modules to chemical exposure. Within significant chemically correlated modules, hubgene-like genes were identified, that were additionally significantly differentially expressed dependent on chemical presence.

Association rule mining - a machine learning approach - was applied as a second computational approach. Transaction sets of all microarray samples were generated and expanded by the respective set of detected chemicals. Based on

approach-specific metrics, the interesting chemical-gene-interactions were mined. Again significantly differentially expressed genes associated with chemicals could be identified.
For both approaches, identified associations were reviewed for plausibility using the reference databases that curate chemical-gene-interactions (ToxCast and Comparative Toxicogenomic Database). Narrowing down to the chemical-gene-interactions that were examined by both approaches and consider differentially expressed genes will help to identify potential chemical drivers. The presented workflow can be of utility for prioritization of chemicals and biological effects.

4.05P.3
Development of national land use regression models for atmospheric concentrations of PAHs, PCBs, and DDT across the Czech Republic
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Despite the success of existing passive sampler-based monitoring networks in capturing global distributions of semi-volatile organic compounds (SVOCs) in outdoor air, the limited spatial resolution of individual networks remains a challenge. Adequate spatial coverage is necessary to better characterize concentration gradients, identify point sources, estimate human exposure, and evaluate the effectiveness of chemical regulations such as the Stockholm Convention on Persistent Organic Pollutants. With sufficiently large datasets, land use regression (LUR) models can integrate land use characteristics and other predictor variables (industrial emissions, traffic intensity, demographics, etc.) to describe or predict the distribution of air concentrations at unmeasured locations across a region or country. While LUR models are frequently applied to conventional and data-rich air quality pollutants such as particulate matter and nitrogen/sulfur oxides, they are rarely applied to SVOCs. The national Czech MONET network consists of 29 passive samplers deployed across the entire country that measure air concentrations every 3 months. Using data from a two-year period (2015–2017), we were able to develop national-scale LUR models and evaluate their ability to describe air concentration profiles and identify sources of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and DDT across the Czech Republic. LUR models successfully described air concentrations for $\sum_{14}$PAHs and $\sum_{7}$PCBs. The PAH model included three predictor variables – total fuel consumption representing emissions from domestic heating, PAH industrial point sources, and the hill:valley index, a measure of site topography – and explained 68% of the variance in air concentrations. The PCB model included two predictor variables – site elevation, and secondary sources of PCBs represented by soil concentrations – and explained 62% of the variance in air concentrations. These predictor relationships were then used to create national SVOC distribution maps which highlight the spatial variability of air concentrations across the Czech Republic and can be used to optimize sampling network site locations and evaluate human and environmental exposure to these compounds. Thus, LUR can be a valuable application of monitoring network data to improve understanding of the links between sources, environmental distribution and exposure to air pollutants.

4.05P.5
Chemical safety of food packaging: (HPTLC-)bioassays for detection and identification of toxic non-intentionally added substances
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Humans are exposed to potentially hazardous chemicals from food packaging materials. European regulations require that food packaging does not contribute toxicity, in particular mutagenic, carcinogenic, or reproductive toxicants, to food. However, beyond providing migration limits for known additives or other chemicals likely to occur in food packaging, methods for assessing non-intentionally added substances remain inadequate to follow regulations and therefore to protect human health. Bioassays are increasingly used to assess complex mixtures migrating from food packaging materials, but they are slow to be accepted due to concerns about sensitivity, robustness, or level of standardization. In the context of food packaging materials, we have evaluated yeast and bacteria based *in vitro* bioassays for estrogenicity and genotoxicity in microtiter and on high-performance thin layer chromatography (HPTLC) plates. In general, bioassays on HPTLC plates proved to be more sensitive than their microtiter counterparts, although both platforms were sensitive enough to detect, for example, bisphenol A below the specific migration limit set by the European Commission. Importantly, separation with HPTLC revealed effects obscured by reduced cell growth in microtiter bioassays. In the HPTLC bioassays, we observed patterns of toxicity in food packaging materials. Alone, these patterns are useful in identifying problematic materials. We also use those patterns to prioritize pervasive substances for toxicant identification in effect-directed analysis by coupling HPTLC with HR-MS/MS. We present, with case studies, findings from our experiences adapting *in vitro* bioassays on HPTLC plates for assessment of food packaging.

4.05P.6
Bridging the gap between chemical fingerprinting and site-specific hazard and risk assessment
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Surface waters contain complex mixtures of inorganic and organic pollutants that may affect the health of humans and wildlife. As the composition of these complex mixtures are highly variable, both in temporal and spatial scales, their cumulative hazard and risk are thus often poorly characterized. Efforts to develop approaches to assess cumulative impact of pollutants on organisms and identifying "Archetypical" mixtures being representative for different emission scenarios are highly warranted. The present work aimed to develop a conceptual approach to identify such mixtures on basis of their cumulative potential for perturbing relevant toxicity pathways and causing adverse effects on aquatic species of regulatory relevance. A combination of taking advantage of exposure information, available effect data representative for selected Adverse Outcome Pathways (AOP) and multi-variate statistics to decipher common patterns between chemical exposures, hazard and risk predictions will be used to demonstrate the approaches on monitoring data from the 3rd Joint Danube survey (JDS3). Special emphasis will be given the identification of ecologically relevant reference compounds and mixtures of these that can inform future efforts in chemical monitoring and experimental effect assessment. This approach represents the initial step in designing computational and experimental approaches to decipher complex chemical interactions associated with combined toxicity of complex mixtures, and to bridge the gaps between chemical monitoring and bioassay testing of surface waters. **Acknowledgement:** FP7-ENV-2013 project SOLUTIONS (grant agreement no. 603437), RCN 268294 "Cumulative hazard and risk assessment of complex mixtures and multiple stressors (MixRisk)", and NCTP: NIVA's Computational Toxicology Program, NCTP (www.niva.no/nctp).

4.05P.8
Environmental risks posed by PAHs and PCBs in seawater from Northwest Iberian Peninsula (Portugal)
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The industrial development and human occupation of seashores in the northwest of Portugal is producing anthropogenic pressures in aquatic habitats, including in estuaries and coastlines. Despite the numerous monitoring surveys accomplished by our group (and others), embracing xenobiotics from distinct natures, up to this moment, no information exists about the levels of both the 16 priority PAHs and of 7 indicator PCBs in the Portuguese Atlantic seacoast. For this propose, during 2017-2018, seawater samples weres taken from the sea at four areas located in Porto coastline (Northwest Portugal), including areas adjacent to a bird reserve. Water extracts were obtained by solid-phase extraction and analysed by gas chromatography-mass spectroscopy (GC-MS). Results revealed that Σ16 PAHs ranged from ≈ 77 ng/L to 84 ng/L and Σ7 PCBs ≈ 45 ng/L to 58 ng/L. Concerning the origins of these pollutants several ratios between key PAHs, e.g. phenanthrene (Phe) /anthracene (A) = 3.3 – 5.2, A / (A + Phe) = 0.16 -0.23, among others, revealed that the primary source of PAHs in this area is mainly pyrolytic despite the occurrence of petrogenic contamination caused by both urban and maritime traffics. The calculation of the toxicity equivalent quotient (TEQ) for PAHs in these samples was on average ≈ 5.3 ng/L revealing that, according to the Directive 2008/105/EC, PAHs in these levels do not pose risks for local biota. The origins of the 7 indicator PCBs (PCB28, PCB52, PCB101, PCB108, PCB138, PCB153, PCB180) are challenging to establish, since their input into the aquatic environment is principally due to discharges from sewage and industrial effluents, leachates from landfill sites, atmospheric deposition and, agricultural run-off, which exist close to the sampling area. Also, the global TEQs for PCBs was ≈ 0.0015 ng/L, revealing that regardless of the high concentrations of these compounds in surface waters PCBs do not seem to pose risks for the aquatic organisms. Moreover, the toxicity test using Artemia salina (ARC-test) using the above-referred levels of PAHs + PCBs revealed no differences between exposed and control groups. Further assays using both groups of pollutants and concentrations measured in particulate suspended matter and sand will allow unveiling other possible effects of these pollutants in seashores. Keywords: organic pollution, TEQ, petrogenic, pyrogenic Acknowledgements: Partially supported by FCT and ERDF (UID/Multi/04423/2019).

4.05P.9
S. aurata primary hepatocytes as a tool to assess PAH mixtures cytotoxic effects

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Both *in vivo* and *in vitro* models have been widely used in the context of Ecotoxicology. Among *in vitro* models, primary cells is well known as the best alternative to attain the realism of *in vivo*, yet respecting the 3R's policy. In the present work, we used primary hepatocytes isolated from *Sparus aurata* to investigate the toxic effects of PAHs and their mixtures. PAHs are compounds composed of two or more benzene rings, being that the high molecular weight PAHs, with four or more aromatic rings, have high mutagenic and carcinogenic potentials. Due to their carcinogenic effects, they are listed as priority substances on Annex II of Directive 2008/105/EC. In general, PAHs are ubiquitously present in the environment compartments (atmosphere, soil, water, sediment) as persistent pollutant. *S. aurata* primary hepatocytes were isolated by an enzymatic-based dissociation method – pancreatin digestion - and cultured in L-15 medium at 18 ± 1 °C. At these conditions, hepatocytes in primary cultures were found to be stable for up to 6 days. The applicability of these cultures to assess PAHs effects was evaluated. Primary hepatocytes were exposure for 24, 48 and 72 h to 0.1-100 µM of Phenanthrene, Benzo[a]pyrene, Benzo[b]fluoranthene and Fluoranthene and their respective binary mixture. The toxic effect of both individual PAHs and their mixture was assessed through the evaluation of their cytotoxicity, EROD induction and ROS production. The highest EROD induction was observed by low concentrations of Benzo[a]pyrene. In general, there was ROS production, being this more accentuated after cells exposure to Phenanthrene. The results suggest that *S. aurata* hepatocytes in primary cultures are useful tool for the assessment of PAHs effects.

Dealing with and Communicating Uncertainties in Environmental Risk Assessment While Ensuring Trust Among Stakeholders: Mission Impossible? (P)

4.06P.1

A Bayesian Network approach for probabilistic risk assessment of pesticides: expanding the binary outcome of risk assessment

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Conventional Environmental Risk Assessment is carried out by evaluating predicted or measured environmental concentrations (PEC) in relation to the predicted no-effect concentrations (PNEC) to organisms or taxa-specific effect thresholds. This deterministic approach is using single values of PEC and PNEC to quantify the risk a substance poses to the environment. A more informative approach is carried out by probabilistic risk assessment, where both PEC and PNEC (and taxa-specific effects) are expressed as probability distributions to account for variability and uncertainty. In this research, an alternative approach to probabilistic risk assessment is explored with Bayesian Network (BN) modelling that focuses on chemical emission path by rural-land use, such as pesticides. BNs can serve as meta-models that link selected input and output variables from several other project outputs. In the early stage of this research, the NIVA Risk Assessment database (RAdb, www.niva.no/radb) is used as a toolbox that aggregates exposure and effect data, identifies assessment criteria on basis of international recommendations and provides transparent and harmonized cumulative risk estimates. The Norwegian Agricultural Environmental Monitoring Programme (JOVA) will provide exposure data. The case study focuses on the risk assessment of two pesticides: Azoxystrobin and Metribuzin. A conceptual model of the BN is presented, that is adapted to a specific case study region being representative of northern Europe. The developed BN can be used as a set of tools to communicate environmental risk and uncertainties to industry and policy makers.

4.06P.2

Differences in physiology and enzyme activity of two genetically distinct Daphnia magna laboratory lineages related phylogeny

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The filter feeding, planktonic crustacean, *Daphnia magna* (Crustacea: Branchiopoda: Cladocera) is a much-favoured model organism, not at least in ecotoxicology. Their wide natural distribution and their extensive application in laboratory testing globally, makes genetic variation between the applied clonal lineages likely. Furthermore, some *D. magna* clones have been maintained in laboratories for decades, likely resulting in some degree of adaptation to laboratory conditions. This may further enhance genetic differences between lineages and induce variation in studies conducted in different laboratories. Clonal differences in sensitivity to toxicants has for instance been observed in multiple experimental setups. In the current study we investigated clonal differences in growth and reproduction in a in a 21-day setup incorporating two different food sources. General cytochrome P450 (CYP) biotransformation capacity was also quantified and finally total DNA was isolated and mapped to be able to relate the parameters to phylogenetic differences between the two *Daphnia magna* clones: DK and F, originating from Denmark and UK, respectively. Results showed that the two clones responded in a similar way to the two food sources, growing faster when fed with the algae *Raphidocelis subcapitata* compared to *Chlorella vulgaris*. When grown under the better feeding regime, they grew to similar size, but the reproductive parameters differed as the F-clone had more, smaller neonates, while the DK-clone had fewer but larger neonates. The absence of size difference between mothers of the two clones indicates that their allocation of energy to general maintenance did not differ and it is likely that they were able to allocate the same amount of energy to reproduction, while the reproductive strategy simply differed between the clones. The two clones also differed in baseline CYP-activity, which could indicate a difference in biotransformation capacity of xenobiotics. These parameters were subsequently related to the genetic landscape in a phylogenetic analysis.

4.06P.3

From linear to circular - concept for a simplified and dynamic risk assessment for pesticides

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The complexity of ecotoxicological risk-assessments (RA) of plant protection products (PPP) is not reflected in the simple binary result of safe/unsafe use. We argue that valuable information about uncertainties in the RA and levels of risk accepted between different authorized PPP, are insufficiently utilized and communicated. Uncertainties in RA are mostly dealt with by applying safety factors, which decrease with each step of the tiered approach, implying that the uncertainty in the assessments decreases likewise with each step. The call for more guidance in higher tier RA indicates, however, that uncertainties remain high also at higher tiers due to the reliance on expert judgement. Substances considered safe only after refinement and application of risk-management measures possess a higher risk-potential compared to substances with acceptable risk at tier-1. While for the latter, deviation in actual use and immission from predicted scenarios or authorized application may have little environmental impact, the opposite might be true for substances with high risk-potential. The scarcity of feedback loops, to validate effect and exposure assessments systematically, aggravates the situation. Therefore, we suggest a change from the complex and mostly linear higher tier approach to a simplified and focused tier-1 RA based on comparative RA and a feedback loop from monitoring. We demonstrate here a simple comparative evaluation of the aquatic risk-potential of > 200 active substances on a tier-1 basis, coupled with an uncertainty analysis that shows, for each active substance, the range of risk-potentials in different evaluation scenarios, from generic to more realistic. Following this analysis, general areas on the overall scale of risk-potentials for all active substances were identified: (i) unsafe, (ii) safe with risk management if used correctly, (iii) safe if used correctly, and (iv) most likely safe under all use scenarios. The proposed scheme allows for new active substances or possibly also new uses to be easily and swiftly assigned to one of the four categories. Resources can then be used to focus monitoring on substances with high risk-potential, which in turn can guide refinement to ultimately create a dynamic RA scheme.

4.06P.4

Is a blog helpful for communicating risk assessment's subtleties to the administration and to the society ?

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Working in the field of aquatic risk assessment of chemicals for more than 20 years, I'm regularly questioned by journalists, administrations or the public about water quality and risk assessment procedures. They consider water quality criteria as « sure ». They are not aware of the procedure underlying the determination of these criteria and, therefore, do not imagine that these criteria derive from models. The consequence being that water quality criteria are subject to uncertainties. Two years ago, I was invited, among other reseachers, to write a blog in the journal Le Temps. This is a swiss french journal that targets a high middle-class audience. It is not politically affiliated and has a daily written edition as well as an on-line edition. The blog is only on-line available. I write a post each month, reacting to the news or not. I also try to use this forum to communicate on the risk assessment procedure. One post was for example on the lack of ecotoxicological data and therefore the inability to define environmental criteria for many chemicals (for example cosmetics). The blog is well-read with a total of more than 108'000 visits (42'000 visitors) from January 2018. Each post reachs at least 1000 visits. The commentars show that people appreciate when we explain clearly the facts. They can for example easily understand uncertainties linked with the risk assessment procedures, even if they deplore the lack of data. But they are more interested in human than environmental health. The hotest words are climate change, pesticides, drinking water and agriculture. In conclusion, I consider that this kind of communication is a valuable tool to exchange with the society on subjects like risk assessment that seem difficult to understand in a first glance. A large audience is easily reached. But it is time consuming and a certain time is needed to construct the readership.

4.06P.5

NOEC verses EC10: An impact analysis based on the decision scheme presented in EFSA (2019) Pesticide Peer Review Meeting on general recurring issues in ecotoxicology

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Commission Regulations (EU) No 283/2013 and 284/2013 established that for all chronic/long term/reproductive toxicity studies, it is necessary to provide EC_{10} and EC_{20} values together with the NOEC. For chronic and long-term risk assessments, it is a widely held view the EC_{10} is considered the most appropriate ECx value to be used in the risk assessment. Therefore, to provide further clarity to Member States as to when it is appropriate to use the EC_{10} as opposed to the NOEC in the risk assessment, EFSA published a technical report (2015). The report contained a decision scheme used to analyse the reliability of the EC_{10} and recommends the use of lowest endpoint between the NOEC and EC_{10}, or the EC_{10} lower limit in instances where the EC_{10} is the lowest endpoint but is not considered reliable. A modified decision scheme was subsequently agreed in the EFSA (2019) Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. To determine the appropriateness EFSA's decision scheme to aid in regulatory decision making, an impact analysis was conducted based on chronic studies comprised of: soil organisms, daphnia, algal, and fish. The results of the impact analysis highlight deficiencies in the EFSA decision scheme and the use of the EC_{10} lower limit as an endpoint for use in the risk assessments. Finally, recommendations are made on how to determine whether an EC_{10} is considered reliable and when the NOEC instead of the EC_{10} should be used in the risk assessment.

4.06P.6

On the influence of uncertainty on SSD analyses - A case study with non-terrestrial target plants

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Today, SSD analyses are established as a key tool for ERA of chemicals, providing a reliable assessment of sensitivity ranges within plant or animal communities thus allowing to estimate HC_5 i.e., hazardous concentrations prone to affect 5% of the species. Estimating the HC_5 means fitting a probability distribution on a collection of toxicity values, usually derived from a regression model, itself fitted on toxicity test data observed at several concentrations at a given target time. Toxicity values are classically considered as point estimates within SSD analyses, while an uncertainty can be associated to them, rarely accounted for in ERA. With non-terrestrial target plants (NTTP), treatment levels are called tested rates. Data analyses lead to estimate 50% Effective Rates (ER_{50}) used as inputs in SSD analyses. The used tested rates are selected prior to the experiments with sometimes little consideration of species sensitivity to the herbicide of interest. Consequently, unbound ER_{50} values (namely ER_{50} < lowest tested rate or > highest tested rate) occur when the tested rate range does not match the observed sensitivity of a species or when this species is not affected at the highest tested rate. Such unbound ER_{50} are produced under standard protocols, so that they are not questionable. The Guidance Document on Terrestrial Ecotoxicology (GD) provides no advice on how to deal with unbound ER_{50} values or with uncertainty around ER_{50} values within SSD analyses. As a consequence, the common practice is to ignore the uncertainty by considering point estimates only, and to discard unbound ER_{50} values from the analyses or to substitute them with arbitrary values. Nevertheless, this is a clear loss of valuable information with drawbacks: (i) the range of remaining ER_{50} values may not cover the full range of sensitivities among studied species; (ii) if unbound ER_{50} values occur for many species, after discarding them, the data set might not be sufficient enough to allow SSD analyses. Since SSD analyses are currently the only higher tier option prescribed by the GD and accepted by authorities, being unable to finalize them may prevent refining ERA of some chemical substances. Based on a case study, we will show how it is possible to account for both the uncertainty on ER_{50} values and unbound ER_{50} values into SSD analyses. In particular, we will explore how they can influence the estimation of the HC_5.

4.06P.7

Statistical framework for regulatory risk assessment of field studies for soil organisms

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In the frame of the European regulation 1107/2009, an ecotoxicological regulatory risk assessment for Plant Protection Products (PPP) is mandatory. It typically follows a tiered approach, based on a large range of organism tests, following the latest guidelines. Regarding the soil organisms, risk assessment covers from worst-case situations (i.e. conservative estimates and toxicity laboratory studies) to more realistic assessment (i.e. field studies). A recent EFSA scientific opinion [1] proposed a new framework for risk assessment of soil organisms and a revised protection goals defined for soil organism groups (i.e., enchytraeids, arthropods etc…). Yet, no key element is given about how data retrieved from field study should be properly analysed [2]. The aim of the present work is to suggest a statistical framework of existing tools already in use for higher tier aquatic studies at population and communities scale. The statistical framework includes community analysis based on multivariate analysis or generalized linear models, by combining the overall soil community described in the field. The population density and/or biomass are compared date-by-date using univariate test. The percent Minimum Detectable Difference (%MDD) [3] should also be included to ensure the reliability of core data for the statistical analysis of differences between the treated and the control populations. The ISO guidance for earthworm field studies [3] provides methodological recommendation for selecting the field, plots size and margin between them, as well as sampling and extraction of earthworm. ISO guidance also set some validity criteria, especially regarding the minimum earthworm density (number of individual /m²) required for testing substances determined in a pre-treatment sampling. Thus, the %MDD may also be used to adapt the sampling strategy during the preliminary investigation of the chosen site, in order to obtain a relevant number of earthworm. This work could help to fill the current lack of harmonized statistical framework for field studies of soil organisms in the context of regulation of PPP. [1] EFSA Journal 2017;15(2):4690 [225 pp.]. [2] Bayona et al., SETAC Europe 29th Annual Meeting, 26-30 May 2019, Helsinki [3] Brock TCM et.al. 2015. Environ Sci Pollut Res 22:1160-1174. [4] ISO 2014. Soil quality: effects of pollutants on earthworms. Part 3: guidance on the determination of effects in field situations (ISO 11268-3).

4.06P.8

Stereo noise: acknowledging uncertainties and generalizing both sides of environmental risk assessment

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Environmental risk assessment (ERA) can be characterized as an evaluation of measured environmental exposures and observed laboratory effects. One of the current challenges in ERA is the generalization of exposure and effect to incorporate uncertainty in subsequent risk assessment. The risk quotient (RQ; exposure divided by effect) is still the "gold standard" for regulatory purposes, where a RQ value > 1 is considered as a risk scenario. By expressing exposure and effects as distributions, RQ distributions (RQDs) can be used to account for uncertainty of the RQ. For multiple stressors, currently no generalized framework for combined risk exists. We explore the use of probability distributions to estimate a combined environmental risk of multiple stressors. Our goals were (1) to generalize exposure and effect information into an environmental concentration distribution (ECD) and a laboratory effect distribution (LED), (2) to determine RQDs for each metal, and (3) to estimate a combined risk probability distribution (RPD) for all eight metals. A data set of 220 measured concentrations of eight metals based on exposure data in the Norwegian marine environment was extracted from NIVA's risk assessment database (RAdb). The metals were arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc. Relevant effect data was extracted from the EnviroTox Database. All inorganic forms of the metals were considered, encompassing a total of 12,966 effect values, 662 species in four trophic levels, 17 endpoints and 15 test statistics. Both ECD and LED were successfully expressed as lognormal models. The generalization allows for simulation of data based on all available evidence while maintaining full flexibility, e.g., for exploration of date or site-specific scenarios. This also made it possible to simulate chronic saltwater LEDs even in the absence of reported effect data. Species sensitivity distributions were based on the LED, and distributions of HC5 values were extracted, from which RQDs for each metal were determined. The RQDs indicate a generally low risk scenario, with the majority of the predicted RQs having values ≤ 1. The risk probability of each metal was used to model a combined RPD with uncertainty. A median risk probability of 0.02% (95% credible interval: 0.00–2.3%) was estimated. Our results provide an example of how data generalization can be utilized for a combined environmental risk assessment, without ignoring uncertainties.

4.06P.9

Take two - Uncertainties with ECx calculations are reduced by assessing confidence limits as well as performing the "good old" visual inspection of data

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The use of EC_x (Effect Concentration with an effect of x %) instead of the No-Observed Effect Concentration/Lowest Effect Concentration (NOEC/LOEC)-approach is highly recommended by the authorities and already the preferred determination of the respective endpoint in many OECD guidelines (e.g. OECD 201 (2011)). For the assessment of the quality of the calculated EC_x and the reliability of the used models many parameters are suitable, e.g. chi^2-test for the correspondence between the distribution of the calculated values and the experimental data, F-test for the statistical significance of the concentration-effect curve and the coefficient of determination for the goodness of fit of the concentration-effect curve. Additionally, the assessment of confidence limits of the EC_x-values comes into focus following EFSA (2019). In some cases however, all these parameters can demonstrate a strong reliability of the one selected concentration-effect curve but a careful visual inspection of this curve sometimes reveals an unsatisfactory fit for the whole range of tested concentrations. This can be especially observed when the effect increases slowly at the lower test concentrations and increases rapidly at the highest test concentrations. By using only one concentration-effect curve, the effect levels at lower test concentrations might be overestimated with possible implications for the risk assessment. This presentation gives an example how two different concentration-effect curves for one data set can result in reliable EC_{10} and EC_{20} as well as EC_{50} with an acceptable representation of the experimental data for the whole data set.

4.06P.10

Towards a More Reliable and Appropriate ECx Endpoint Derivation Scheme in Environmental Risk Assessments within Regulatory Context

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In July 2019 EFSA released the outcome of the "Pesticides Peer Review Meeting on general recurring issues in ecotoxicology". According to this report, EC_{10}, EC_{20}, and EC_{50} should be routinely provided for associated test guidelines together with 95% confidence interval (CI). It is good practice to assess the reliability of EC_x both by the width of confidence interval around the median and by certainty level of protection classified by the overlapping relationship of EC_x CIs. When to use EC_{10} (prescribed by Guidences or < NOEC) with **scare "certainty"** on the level of protection, then the lower limit of 95% CI should be reported as the endpoint for deriving Predicted No-Effect Concentration (PNEC) or Regulatory Accepted Concentration (RAC). However, the classification of certainty by normalized width and the relationship between EC_{10} , EC_{20} , EC_{50} is based on the database compiled by ICPS/Wageningen University using arbitrary thresholds, lacking in-depth discussions on questions like whether an estimated endpoint with a smaller NW is always more reliable, and whether the lower limit of EC_{10} CI is always more suitable for risk assessment when the CI of EC_{20} includes EC_{10}. In this study simulation examples and real data examples will be presented to illustrate the indications and impacts of the reliability criteria. We also propose procedures and corresponding software tools to improve the current standard dose-response analysis to fit to the new recommendations of EFSA.

References[1] "Pesticides Peer Review Meeting on general recurring issues in ecotoxicology"

4.06P.11

Towards a non-toxic environment: an analysis of current EU chemicals legislation and recommendations for a harmonised risk assessment framework

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Chemicals provide essential services to society. However, their use results in emission to the environment where they affect water quality and aquatic ecosystem functioning, among others. European legislation aims to protect human and environmental health. Current legislations might however not be fit for purpose as chemicals are increasingly being detected in surface waters and more than half of EU surface waters are in poor ecological condition. Because chemical consumption is increasing and the EU wants to develop a strategy for a 'non-toxic environment', current EU legislation needs to be updated. We analysed the overlap and differences in underlying risk assessment approaches and shortcomings of the legislation for industrial chemicals (REACH), biocides, pesticides, pharmaceuticals and veterinary pharmaceuticals, covering the majority of chemicals on the EU market. These legislations require the screening of chemicals on a substance-per-substance and use-per-use basis for their risks. As chemicals can have multiple uses, their actual emission are cumulative. Diverging risk assessment schemes of the different frameworks also result in incoherent assessment of similar chemicals. Overlapping chemicals under different frameworks were identified by retrieving and analysing CAS-numbers. Up to 70% of currently approved biocidal active substances are also registered under another framework. Assessment factors applied on ecotoxicity endpoints to derive PNEC values for the aquatic environment can differ up to a factor of 100 between regulatory frameworks. 18% of banned pesticides and 62% of banned biocides were found to be still available on the market under a different framework. The overall function and goals of the legal frameworks are comparable but we identified some major differences. These result in incoherent assessment and underestimation of the risks associated with using the chemicals. We raise concerns regarding the identification of possible hazardous chemicals during the registration and provide recommendations to shift towards a more harmonized and integrated framework for the assessment of chemicals, in order to close the gap towards a non-toxic environment.

4.06P.12

Uncertainty in acute fish testing data - a meta analysis

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We performed a meta-analysis of nearly 70,000 acute fish toxicity tests for a wide ranging variety of chemicals with equally wide ranging toxicity values. The surprising results were that with the possible exception of metals, very little biologically relevant diversity exists across a number of chemical categories. The high number of observations, however, accounted for a narrow "confidence interval", notwithstanding the acknowledged variability. Data were obtained from a variety of sources, including USEPA, HSDB, USGS and ECHA. A total of 69999 records were initially obtained. RAW data were represented by all 66573 assays with recordable data. A data cleanup scheme reduced the number of records to CURATED, n= 47694. CURATED data were then split into a number of databases including ORGANICS, METALS and CAS>50, the latter of which describes individual chemicals (more than one hundred) for which at least 50 assay results were available. CURATED LC50 data contain only those data points definitively identified as LC50 values. The data were not normally distributed but rather log-normallly distributed as evidenced by the fact that geometric mean and median lay in close proximity whereas the arithmatic mean was several orders of magnitude greater than the median. TTC's (Toxicological Threshold of Concern) were derived both graphically and mathematically. Very little (and definitely no biologically relevant) distinction was found between RAW, CURATED and ORGANICS data – the geometric means and medians are within approximately 20% of each other. METALS data on the other hand appear distinct from the other data sets with an approximate three to six fold difference in geometric mean and median, respectively. Arithmatic means are orders of magnitude higher than either medians or geometric means. Even for single chemical datasets log-normal data distribution persists. Curation makes very little difference in overall data magnitude, especially surprising in light of the amount of effort expended on such activities. Metals data were different from all other groups. Quite unexpectedly, P. promelas was at least an order of magnitude more sensitive than D. rerio in overall acute fish toxicity testing. O. mykiss data showed intermediate sensitivity. TTC data was extremely congruent across classes/datasets, even for metals.

4.06P.13

Using historical control data to contextualise the variability in ecotoxicity studies

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Ecotoxicity studies, undertaken to address the risks from potential exposure to chemicals, vary in their design e.g. duration of exposure, effect types and endpoints measured. The biological responses measured can be highly variable, with limited opportunity for control of extrinsic sources of variability. It is critical to distinguish between treatment-related effects and background 'normal variability' when interpreting results. This is particularly difficult for studies with low replication and/or reduced concentration ranges (e.g. vertebrate studies, field or semi-field studies) where statistical power can be low due to limitations in study design. For these types of studies, historical control data (HCD) can be a valuable tool in contextualising results from single studies against previous studies performed under similar conditions. This poster will discuss the case for better use of HCD in ecotoxicology assessments, illustrating with case studies the value and difficulties of using HCD in interpretation of results of standard and higher-tier study designs. The possible reasons for HCD being routinely used in mammalian toxicology for human health assessments but not directly in ecotoxicology will also be discussed e.g., different data types, the potential to mask effects, and the lack of guidance. We aim to raise awareness regarding this topic and to encourage organisations such as OECD, EFSA and USEPA to develop guidance on the principles of HCD collection so better use can be made of this potentially valuable tool.

Effect Modelling for Regulatory Environmental Risk Assessment: Current Applications and Future Directions (P)

4.07P.1
Exploring GUTS as a tool for marine environmental risk assessment: a case study on metal toxicity to marine copepod Tisbe battagliai
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Mechanism-based effect modelling has long been discussed as a potential replacement for causality-agnostic statistical procedures. In (eco-)toxicology, toxicokinetic–toxicodynamic (TKTD) modelling approaches are used to causally link exposure to effect, taking both uptake of substances and their organism-internal effect propagation into account. The General Unified Threshold model for Survival (GUTS) for water-borne toxicity has emerged as the most promising TKTD modelling framework to gain regulatory acceptance from authorities, such as the European Food and Safety Agency (EFSA). We present the case study of the Southern Norwegian Kaldvellfjord, which has experienced increased metal contamination after unfinished and unattended highway construction work. The aim of our study was to provide a mechanistic-based risk assessment of this fjord system, in order to support regional authorities in the evaluation of the environmental health status and to support the mitigation of regulatory action to improve ecosystem health. We focused on potential risk scenarios for crustaceans, as they play a key role in the ecosystem functioning and hold value as a food source. However, GUTS-relevant data for marine crustacean species and metal toxicity was not readily available. Based on an initial PNEC-based risk assessment within NIVA's Risk Assessment database (RAdb), the four potentially most impactful metals were chosen to be examined in laboratory studies: aluminium, copper, nickel, and zinc. As marine test species, juveniles and adults of marine copepod Tisbe battagliai were used in acute exposure experiments. Using geometric gradients of metal concentrations, the survival was tracked over the course of 48 hours and metal concentrations were verified through ICP-MS analysis. Reduced GUTS models were fit for each metal and life stage, assuming stochastic death as death mechanism. For both adults and juveniles, copper was the most toxic out of the four metals. Models for juveniles contained considerably more uncertainty than models for adults. The distributions of the effect threshold concentration of adult models was thus used in further risk assessment procedures, together with monitoring data from Kaldvellfjord. We present the first metal-toxicity GUTS for marine copepod Tisbe battagliai, as well as a comparison of a GUTS-based risk assessment with classical PNEC-based risk assessment.

4.07P.2
How to reduce computation effort for GUTS modelling while retaining output reliability for risk assessment use?
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The GUTS framework (**G**eneral **U**nified **T**hreshold model for **S**urvival) is a modelling approach that addresses survival and is applicable in terrestrial and aquatic tiered acute and chronic risk assessments (ERA). GUTS modelling involves three steps: a) calibration to construct the model, b) validation to ensure its applicability, and c) projection to analyse survival responses to toxicants. The model calibration is realized with Bayesian or frequentist inference searching for most suitable parameter sets. This calibration can result in a large sample of e.g. 50,000 parameter sets. In ERA, GUTS is applied to predict survival in response to exposure profiles that can be obtained from environmental fate assessments. Currently, standard approaches following FOCUS recommendations result in profiles over a period of 12-16 months. It is expected that new versions of aquatic tools will follow a 'Multi-Year' approach. Consequently, in the near future, GUTS applications are expected to include survival projections using environmental fate exposure profiles from multi-year evaluations. For a proper propagation of parameter uncertainty, GUTS projections must ideally be conducted while considering all of the parameter sets in the sample obtained by calibration. The combination of exposure profiles over a much longer period and a large sample of parameter sets makes GUTS projections very time-consuming, due to intensive processing work. Although GUTS model applications in ERA are valuable, the considerably increased computational effort is a challenge for the practicability and common use of such applications. With the aim of reducing effort for GUTS projections, we investigated the dependence of GUTS projections on the completeness of exposure profiles and the number of used parameter sets. A suitable subsampling of parameter sets and exposure profiles could considerably reduce the processing effort without critically impairing projection reliability. Our analysis illustrates that reliable GUTS projections can be achieved, if relevant parts of an exposure profile are projected using a reasonable number of parameter sets. We propose the development of a standard procedure for subsampling exposure profiles and parameter sets as well as its inclusion in the standard methodologies of all Toxicokinetic/Toxicodynamic modelling in ERAs.

4.07P.3
Case study for DEBtox use in Environmental Risk Assessment
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Mechanistic modelling is gaining interest in regulatory environmental risk assessment. EFSA recently published a Scientific Opinion assessing the use of these models for survival and sublethal effects of plant protection products (PPP) on individual organisms. Whereas the toxicokinetic - toxicodynamics (TKTD) models for survival effects are deemed ready for use, TKTD models focusing on sublethal effects need further testing. DEBtox is the leading approach to assess sublethal effects of PPP on individuals. However, the use of DEBtox to analyse the effects of time varying exposure is extremely rare due to lack of coherent numerical methods and lack of suitable datasets. To tackle these issues, we developed numerical method to allow efficient calibration and uncertainty propagation of DEBtox models, and apply it in the context of regulatory risk assessment. To test the developed methods, we will expose three species to fungicide under a time-varying exposure. In this presentation, we outline the project aim, preliminary and expected outcomes. We discuss the usage of this model and its benefits to the regulatory risk assessment framework.

4.07P.4
OpenGUTS, user-friendly software for survival modelling
T. Jager, DEBtox Research / De Bilt; M. Wang, WSC Scientific GmbH / .; R. Ashauer, Syngenta Crop Protection AG / Environment
Mechanistic effect models are rapidly gaining interest in the context of environmental risk assessment. In 2018, this interest culminated in a dedicated EFSA opinion on toxicokinetic-toxicodynamic (TKTD) models for use in aquatic risk assessment of pesticides. This opinion focussed on three modelling frameworks; for the endpoint survival, the focus was on GUTS (the General Unified Threshold model for Survival). Since the EFSA opinion judged GUTS to be "ready to be used in risk assessment", there is now a pressing need for robust and user-friendly software to perform analyses with this model, following the proposed workflow in the opinion. In a Cefic-LRI funded project, we have developed such a software: openGUTS, which comprises a standalone Windows executable and a Matlab-based version (both open source and free). The software performs the procedures for calibration, testing and model predictions as outlined in the EFSA opinion. Furthermore, openGUTS performs these analyses with minimal demands on user experience in modelling and statistics. However, this software is not restricted to risk assessment of pesticides. It can also be used for other tasks, such as the derivation of classical LC50 values (as function of time) from standard and non-standard toxicity data, using all of the observation on survival over time. Specifically, openGUTS enables meaningful calibration to toxicity data that result from tests with time-varying exposure, which cannot be achieved using traditional dose-response curves. The software was released in December 2019, and in this contribution, we provide an overview of the software's layout, algorithm, functionality and look.

4.07P.5
Hydrogen peroxide effects on Northern shrimp: dynamic modelling of mortality
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Hydrogen peroxide (H2O2) is used as anti-parasitic veterinary medicine in salmon farming worldwide. Since the treatment water is discharged to the sea, there are concerns about the potential effects of H2O2 on populations of the Northern shrimp (*Pandalus borealis*), an economically and ecologically important species in Norwegian fjords. In the present study, adult shrimp were exposed to short pulses of H2O2, followed by a recovery period in clean seawater. The exposure concentrations represented 100, 1000 and 10000 times dilutions of the prescribed treatment concentration for salmon. In the two lowest dilutions, substantial and delayed mortality was observed. The complete data set (survival over time, in all treatments) was modelled using a toxicokinetic-toxicodynamic model from the GUTS framework. This modelling framework has several distinct advantages over descriptive dose-response curves. Firstly, it can be meaningfully parameterised on data with pulsed exposure. Secondly, it can make extrapolations to meaningful LC50s for constant exposure. And finally, it allows for meaningful predictions for untested exposure situations. In this contribution, we show how the model explains the toxicity patterns over time, and demonstrate how the parameterised model can be used, in conjunction with results from dispersion modelling, to predict mortality under representative field conditions, and hence to support environmental risk assessment. Special attention will be given to the propagation of parameter uncertainty from the model calibration to the model predictions.

4.07P.6

Ready-to-use tools to efficiently model effects and predict effect threshold in the ERA framework

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Among the consequences of the development of our societies, chemical pollutants and their impacts on living organisms have become a priority issue for regulatory agencies with guidelines reinforcing the requirements for the approval of active substances. Before any decision, information must be collected on their physico-chemical properties, toxicity and ecotoxicity, and the associated risks must be assessed. In this perspective, many toxicity tests are carried out within laboratories, according to standardised protocols whose data are then analysed by appropriate and reliable mathematical models and statistical inference methods. Classical outputs are critical effect concentrations for the biological traits of interest, that may be obtained by fitting an exposure-response/effect model. Such a model consists of a deterministic part describing the mean tendency of the data, and a stochastic part depending on the type of the data (namely, binary, count or continuous data). Choosing the most appropriate model may thus appear awkward, but can be supported by ready-to-use tools. Such tools should be specifically designed for ecotoxicologists and risk assessors, in order to help them in easily performing statistical analyses of standardised toxicity test data, in a user-friendly way, with a freely available graphical web interface and without requiring to invest in the underlying statistics and computer technologies. Faced with this statement of fact, our team developed several ready-to-use tools offering a collection of services for statistical inference and mathematical modelling applied to ecotoxicology. This presentation gives an overview of these tools: (1) the R-package 'morse'; (2) the web-platform MOSAIC, in particular its GUTS module (*General Unified Threshold model of Survival*) allowing to fit GUTS toxicokinetic-toicodynamic models directly online; (3) the GUTS Shiny Application to simulate GUTS models online and predict x% lethal concentration profils (LPx) as suggested by EFSA for time-variable exposure scenarios. All these tools give results associated with their uncertainty.

4.07P.7

Impact of different calibration methods for forward prediction and uncertainty propagation in environmental risk assessment

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The use of Toxicokinetic/Toxicodynamic (TKTD) models in the regulatory environmental risk assessment (ERA) of sub-lethal effects of chemical compounds requires the development of consistent and relevant modelling approaches. While the EFSA Scientific Opinion on the state of the art of TKTD effect models (EFSA PPR, 2018. EFSA Journal;16(8):5377) considers models that are based on the Dynamic Energy Budget theory adapted for ecotoxicology (DEBtox models) as valuable tools for ERA, their full acceptance by stake-holders still requires the standardization of their calibration/validation framework. In particular, EFSA recommended that the physiological part of the DEBtox model would be independently approved by a competent expert team, ahead of submission of DEBtox models for regulatory use. However, the possibilities of different approaches to allow for sound forward predictions and uncertainty propagation needs to be evaluated before a robust design could be chosen. Therefore, we tested to what extent separating calibration of physiological toxicological parameters from calibration of toxicological parameters may impact predictions of sub-lethal effects due to a chemical compound as well as the quantification of their associated uncertainty. To do so, we tested three different calibration frameworks for the standard DEB model coupled with toxic stress functions, using Bayesian inference methods: (i) The strict separation view: fix physiological parameters to their mean regulatory accepted values and estimate toxicological parameters only from experimental toxicity test data, then use fixed physiological parameters and estimated toxicological parameters with their uncertainty for predictions; (ii) the full estimation: use mean regulatory accepted values for physiological parameters as priors to perform Bayesian inference and get estimates of both physiological and toxicological parameters, then use them with their uncertainty for predictions; or (iii) a compromise: use mean regulatory accepted values for physiological parameters as priors to perform Bayesian inference and get estimates of both physiological and toxicological parameters, but use fixed mean regulatory accepted values of the physiological parameters together with the estimated toxicological parameters and their uncertainty for predictions. The results of these three calibration frameworks applied to toxicity test data will be compared and discussed.

4.07P.8

Evaluating model performance criteria for primary producer TKTD models

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The EFSA scientific opinion on Toxicokinetic/Toxicodynamic (TKTD) effect models (TKTD-SO) provides a detailed framework of how to perform TKTD modelling in a regulatory context. The evaluation of the quality of model predictions is a crucial step both in assessing the model. In addition to a qualitative check ('visual fit'), the TKTD-SO provides three quantitative model performance criteria: the Posterior Predictive Check, the Normalised Root Mean Square Error, and the Survival-Probability Prediction Error. These quantitative model performance criteria have been evaluated and tested for the use with GUTS models. The TKTD-SO suggests preliminarily using the same criteria also for other TKTD models, such as primary producer models. However, the TKTD-SO also recommends that the adequacy and performance of these criteria needs testing for models other than GUTS. Suitable adjustments to the criteria may then be made based on the experience gained. To stimulate this process, we have collected first experiences with applying the model performance criteria for primary producer TKTD models. Based on these examples, we present observations and proposals for discussion.

4.07P.9

Objectives and results of the Lemna Working Group in the SETAC Effect Modelling Interest Group

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Toxicokinetic–Toxicodynamic (TKTD) models can help to estimate effects of different and complex exposure patterns (e.g. from FOCUS exposure modelling) on organisms and populations. The EFSA PPR panel (2018) in its 'Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms' considered, the Lemna model by Schmitt et al. (2013) to be ready for use. This model describes the inhibition of population growth due to exposure to a toxicant by a TKTD model considering uptake, metabolism and elimination and the population dynamics in the field as affected by temperature, light, nutrient concentrations and density dependence. The model was implemented in R and the entire source code was provided as supplementary information with the publication of Schmitt et al. (2013). However, there are a few points in the source code and in the model description which need further clarification Therefore, an open Lemna working group was established in 2018 under the umbrella of the SETAC Interest Group on Effect Modelling with the following objectives: a) to agree on a description of the model equations; b) to agree on the default parameter set of the ecological model; c) to provide an open access reference implementation in accordance with a) & b); d) to recommend a procedure for calibration and validation; e) to define the types of outputs to be provided. The working group might decide at a later date to adopt also further objectives such as model extensions (e.g. mixture toxicity). The refined model description, the default parameter set and the reference implementation in R will be provided in an open access report. The poster will summarize the current status of the project.

4.07P.10

Pesticide mixtures in soil: Simulation of time-dependent effects on soil organisms

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Increased use of pesticides to protect crops leads to a decrease in species richness and diversity in agricultural landscapes (Robinson & Sutherland, 2002; Geiger et al., 2010). Pesticides are applied either as single substance, or, most often, as combination products or in tank mixtures, i.e., typically multiple substances are released to the environment. The repeated use of pesticides as a spray series is widespread practice (Roßberg & Harzer, 2015) resulting in pesticide mixtures in soil (Jablonowski et al., 2012; Chiaia-Hernandez et al., 2017) and organisms (Botias et al., 2017). Soil organisms, such as earthworms or collembolans, are directly exposed to the application of pesticides. As a result, soil organisms are immediately exposed to pesticide mixtures and assessing the effects of these mixtures on organisms is essential. Due to the high number of authorised pesticides not all possible mixture combinations can be covered by ecotoxicological tests. The current risk assessment already considers the risk for combination products and for those tank mixtures that are explicitly defined as "recommended" or "mandatory" by the applicant for authorisation (BVL, 2015). The risk of time-delayed pesticide applications is not yet considered however.We developed a model to estimate time dependent effects of pesticide mixtures on soil organisms. The model was developed using R (R-Core-Team, 2016) and the R-package morse (Baudrot et al., 2019) as well as assumptions from the German pesticide registration process. The concept of Independent Action (IA) (Bliss, 1939) is used to consider the time-dependent mixture effect of various pesticides from typical spray series. The model predicts time-dependent effects of single and mixed pesticides, as well as exposure and degradation of each substance over time (Sybertz et al., in press). The results of the developed model could serve as input parameters for a population model, for example. This would be a possibility to estimate time-dependent ecotoxicological effects on population level. In addition, the advantages, limitations and consequences for the risk assessment of pesticides are discussed.

4.07P.11
Developing a DEB-IBM to Predict Population Level Effects of Ni on the Great Pond Snail, Lymnaea stagnalis
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Ecological risk assessment (ERA) aims to preserve the structure and functioning of ecosystems. To this end, the potential effects of toxicant introduction to natural environments are estimated. However, such toxicity estimates are typically derived from individual level laboratory experiments. Despite the merits of apical testing, this methodology neglects the ecological context in which toxicant exposure unfolds, creating a disconnect between toxicity data and environmental scales of interest. Mechanistic population models can address these concerns by providing a mathematical framework connecting individual exposure effects with population dynamics. Currently, risk assessment of nickel (Ni) in freshwater is based on bioavailability-normalized species sensitivity distributions (SSDs), which do not account for population or community level processes. Previous work has demonstrated that the apical effects of Ni to *Daphnia magna* are not reflected in populations exposed to the same Ni concentrations. However, this discrepancy between individual and population level effects was correctly predicted using a dynamic energy budget individual-based model (DEB-IBM). The development and application of additional mechanistic population models for standard and sensitive species offers a promising strategy in optimizing ERA. *Lymnaea stagnalis* is a particularly sensitive species for a variety of metals. A DEB-IBM for Cu toxicity in *L. stagnalis* has already been developed. Building from existing DEB-IBMs, this work aims to implement a DEB-IBM to estimate the effects of Ni exposure for *L. stagnalis* at the population level. Using existing datasets, the model is calibrated to fit individual level dose response curves resulting from laboratory experiments at different pH values. The exact mechanism of Ni toxicity to *L. stagnalis* currently remains unclear. Under DEB theory, different physiological modes of action (PMoAs) result in the emergence of distinct exposure response patterns. Consequently, statistical comparison of the predictions of growth made under different PMoAs can be used to make inferences regarding the PMoA of Ni to *L. stagnalis*. Once calibrated with apical data, the DEB-IBM provides predictions for Ni toxicity effects on *L. stagnalis* within the context of intraspecific interactions. Overall, the results of this project demonstrate the potential of mechanistic modeling for interpreting laboratory toxicity data from the perspective of ERA.

4.07P.12
Pop-GUIDE: Population modeling Guidance, Use, Interpretation, and Development for Ecological Risk Assessment
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The assimilation of population models into the Ecological Risk Assessment (ERA) process has been hindered by their range of complexity, uncertainty, resource investment, and data availability. Likewise, translating model outputs into endpoints that can be used by risk assessors has been challenging. Recent research efforts have begun to tackle these challenges by creating an integrated Modeling Framework and Decision Guide to aid the development of population models with respect to ERA objectives and data availability. In the Framework, the trade-offs associated with the generality, realism, and precision of an assessment are used to guide the development of a population model commensurate with the protection goal. The Decision Guide provides risk assessors with a stepwise process to assist them in developing a conceptual model that is appropriate for the assessment objective and available data. We have merged the Decision Guide and Modeling Framework into Pop-GUIDE (Population modeling Guidance, Use, Interpretation, and Development for Ecological Risk Assessment), a comprehensive approach for the development of population models for ERA that is applicable across regulatory statutes, assessment objectives, and taxa. In Phase 1 of Pop-Guide, an approach is presented to guide assessors through the trade-offs of ERA generality, realism, and precision and translate these trade-offs into model objectives. In Phase 2, available data are assimilated and characterized as general, realistic, and/or precise. Phase 3 provides a series of dichotomous questions to develop a conceptual model that matches the complexity and uncertainty appropriate for the assessment that is in concordance with the available data. This phase guides model developers and users to ensure comprehension and transparency of the modeling process. In Phase 4, a conceptual model is developed to represent the trade-offs and uncertainties commensurate with the ERA objective. We demonstrate Pop-GUIDE using case studies of fish and pesticides.

4.07P.13
Towards a coupled exposure and effect model for earthworms
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The environmental risk assessment of plant protection products aims at the prevention of unacceptable adverse effects on the environment. Therefore, the risk assessment for soil organisms, such as earthworms, is based on two key elements: the exposure assessment and the effect assessment. As regards exposure, the behavior of an active ingredient in the soil is evaluated and, as a major output, predicted environmental concentrations are estimated. The effect assessment is based on toxicity endpoints derived from the investigation of the concentration–response relationships. In the current regulatory frameworks toxicity endpoints and predicted realistic worst case 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, effect estimates are only a snapshot in time, and organisms move in response to environmental factors. In order to address these shortcomings, a modelling framework is currently developed which relies on a modular approach linking exposure and effects: The spatio-temporal exposure pattern is calculated by a numerical simulation model. Based on advanced knowledge of the earthworm ecology, a movement module is programmed which directly translates the environmental exposure pattern into a time-series of exposure that each earthworm experiences. This individual exposure pattern serves as input for a toxicokinetic-toxicodynamic module which predicts the toxic effect on each individual. This approach allows for the prediction of effects on earthworms under a large range of environmental conditions under consideration of different application techniques. In this presentation we illustrate the concept of the modular modelling approach. Moreover, we present a study in which the exposure of an active ingredient was computed with FOCUS PEARL and in which the GUTS model was applied to simulate the resulting time course of lethal effects on earthworms. In order to consider different species, two already published earthworm models were applied to simulate the corresponding movement behavior. This example stresses how the effective exposure to a plant protection product is determined by the movement of earthworms. The ecological relevance of risk predictions will be further improved by targeted refinements of the model implementation of earthworm movement.

4.07P.15
Aquatic risk assessment at landscape scale - conceptual development and risk communication
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Risk assessment at landscape scales is currently discussed in Europe as a serious option to improve the realism and relevance of regulatory Environmental Risk Assessment (ERA) for plant protection products (PPP). Conceptually, geographical information can be used by spatially distributed models to predict the emission, exposure and environmental effects of the use of PPP at the region scale. This enables the assessment of local specific risks with a high level of realism. In practice, however, several challenges need to be solved before such landscape scale ERA can be used in a regulatory context. For example, there is a need for a proper framework for linking protection goals to landscape scale assessment endpoints. Also, the multitude of possible combinations and complexity of different exposure and effect assessment and modelling approaches needs to be organized and selection criteria identified. Our contribution shows preliminary results of multidisciplinary discussions that aim to provide a possible framework for organizing the challenges given above. In addition, suggestions will be given on how to define landscape scale assessment endpoints. Within the same project practical modelling tools for aquatic ERA at landscape scales are being developed and applied for example cases. These example cases are used to test and check the proposals for the risk assessment framework and endpoints.

4.07P.16

SimpleBoxTreat4REACH: simple and transparent method for chemical safety assessment under REACH

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Expected probabilities of exceeding critical effects concentrations in water can be predicted well by means of toxic pressure modeling. This has been known since the early 1990s. It is widely used in derivation of environmental quality standards, but not in Chemical Safety Assessment under REACH, most likely because uncertainty in model output has remained an unsolved problem. Recent studies in the framework of the EU project SOLUTIONS have convincingly demonstrated that uncertainties and variabilities of model parameters, and of modeling assumptions can be adequately dealt with in toxic pressure modeling. It has been shown that state-of-art environmental impact modeling by means of toxic pressure calculation can be carried out for all chemicals that are currently used in the EU. This paper focuses at the newly gained insight that toxic pressure modeling using the Van Straalen-Aldenberg convolution integral (i) intrinsically accounts for variances in model output that arise from uncertainty and variability, and (ii) intrinsically deals with simultaneous presence different chemicals. Results from earlier studies are revisited and are used to demonstrate how increased parameter uncertainty and variability automatically yield greater probability that exposure concentrations exceed critical effect concentrations, and how additive mixture toxic pressure automatically results from integration across all possible values of toxicologically standardized concentrations. It has become evident that the Van Straalen-Aldenberg integral yields uncertainty- and mixture-inclusive modeling results. These results also explain the often observed phenominon of having Pareto-distributed toxic impacts: overall (mixture) toxic pressure originates from a few chemicals only, namely those in right tail of the concentration distribution. For cW exceeding acute EC50, Pareto ratios are found to be generally (much) greater than the often-quoted '80/20 rule', namely > 95/5. This means that toxic pressures of chemicals in the environment, even those calculated under relatively great uncertainty and/or variability, can serve well as a scientific basis for regulatory decision making, notably for the REACH regulation, where, in order to allow a chemical on the market, 'safe use of chemicals' needs to be demonstrated by registrants. This can be understood entirely from the sensitivities of the Van Straalen-Aldenberg convolution integral to uncertainties and variabilities of model parameters.

4.07P.17

Statistical Analysis of Survival/Mortality/Emergence Data

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Survival data is a common type of response in many ecotoxicity studies. Estimation of EC50 or ECx for other choices of x is often done by probit analysis. Standard probit analysis ignores replicates and does not permit treatment of overdispersion. Background or control mortality must be taken into account to make a reasonable risk assessment. Abbott's formula is a common way to deal with that, but it can create large errors in both ECx point estimates and confidence or credible intervals. Some software packages force use of this technique. Simple, practical easily implemented unbiased alternatives in SAS, R and other software exist to analyze survival data that uses the replicate structure, adjusts for background mortality in a direct and mathematically sound manner, and also accommodates overdispersion where present. These include probit or other generalized nonlinear models with binomial error structure and reps treated as a random factor (with an overdispersion parameter when needed), analysis of replicate proportions using regression models for continuous responses. These approaches are compared to standard probit analysis and to each other and to standard survival models from other types of studies using data from regulatory guideline studies for non-target terrestrial plants, fish sexual development, and fish early life stage studies, as well as extensive computer simulations based on realistic assumptions. All approaches will include comparisons using two ways to treat background mortality (Abbott or direct). Recommendations are made for how these and most survival data from guideline ecotoxicity studies should be analyzed in the future. Standard models for survival data from other disciplines, such as proportional hazard, Kaplan-Meyer, and frailty models and sandwich estimators, are shown to be generally unhelpful in most ecotoxicity guideline studies.

4.07P.18

Domain Wide Effect Modelling to Support Read-Across in Hazard and Risk assessment

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Effect modelling is an important part of ecological risk assessment to provide environmental safety thresholds without excessive animal usage and resource-demanding experimental efforts. The current suite of QSAR models and other read-across approaches are efficient at filling data gaps in many cases, but lack the coverage required to predict diverse toxicity mechanisms, species/taxa and endpoints relevant for ecological exposure scenarios. We propose the use of background knowledge about species and chemicals to improve the reach of read-across techniques. We describe two methods, one deterministic and one probabilistic, to solve this problem. For the simplified case of binary prediction, the probabilistic machine learning method improves the results from 58% to 83% over the deterministic method. Therefore, we also expect improvement in metrics when applying the methodology to concentration prediction (regression).

Engineered Nanomaterials: Complex from Chemistry to Multi-stressor Situations (P)

4.08P.1

Bioactivities of polyligand Fe3O4 nanoparticles mediated by surface chemical states

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This study was aimed to estimate of the effect of hydrodynamic diameter and surface charge on bioactivity of Fe_3O_4 NPs modified by various ligands (humic acids, HA and 3-aminopropyltriethoxysilane, APTES). Behaviors of NPs were investigated in the aspects of their toxicity to *Sinapis alba* and *Paramecium caudatum.* Fe_3O_4 NPs were prepared by a chemical *coprecipitation in situ* from $FeCl_2$ and $FeCl_3$ in the presence of NH_4OH. The NPs formulated by sol-gel method were Fe_3O_4-APTES (Ar) and Fe_3O_4-APTES (air) (various formulation conditions: in argon or air atmosphere, drying in at 70 °C in vacuum or 150 °C). The zeta potential and hydrodynamic diameter data were determined by dynamic light scattering. According data of FTIR spectroscopy, there are free amino-groups onto the Fe_3O_4-APTES NPs surface. With HA increasing, the isoelectric point (IEP, pH at ξ=0) for Fe_3O_4 shifts to the acid region. The charge of Fe_3O_4-HA at pH=6 changes and becomes negative. Thus, at a Fe_3O_4-HA ratio as 1: 4 (full coverage of Fe_3O_4 NPs with HA), the surface charge at pH=6 equals −39 mV. At similar amount of HA for Fe_3O_4-APTES ξ~ equals +2 mV at pH 6. The EC_{20} parameter for NPs with positive charge (Fe_3O_4, Fe_3O_4-APTES (air) and Fe_3O_4-APTES (Ar), ξ= +10 mV and +17 mV, respectively) higher then EC_{20} for NPs with negative charge (HA, ξ= −37 mV). It means that NPs with positive charge surface are less toxic towards infusorians in compare with ones for negative charge surface, and Fe_3O_4-HA demonstrated stimulation towards infusorians. The greater toxicity of Fe_3O_4-APTES-HA in comparison with other negatively charged NPs can be explained by the insufficient coating of the surface with HA. So, HA has mitigation effect on toxicity of magnetite NPs. It has been shown that nanoparticles with zeta potential near IEP have greater toxicity. The primary amines on the surface of the Fe_3O_4-APTES give rise to the large positive surface charge and have previously been reported to cause a toxic effect. The reduction in toxicity observed in the humic acids coated particles arises due to the decreased surface charge. Higher plants turned out to be less sensitive to the selected nanoparticles at all range of concentrations, while the nanoparticles showed toxic effects in relation to the ciliates at concentrations of more than 0.1%.
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4.08P.3

Nanoscale zero valent iron-induced stress response in Pseudomonas putida: Transcriptome changes in gene expression

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Nanoscale zero-valent iron (nZVI) has gained notable recognition for its capability to reduce chlorinated ethenes, BTEX and many other harmful compounds in polluted aquifers. The remediation is more effective when applied in combination with subsequent enhanced biodegradation. Therefore, potential harmful effect of nZVI on indigenous bacteria has been widely explored, especially on those involved in dechlorination. Still, there is only a little knowledge on the response of bacterial cells on the gene expression level. The aim of this study was to describe the effect of nZVI on a common soil bacterium Pseudomonas putida. P. putida was exposed to 100 mg/L of nZVI suspension and its ionic forms up to 24 hours in physiological solution. The gene expression of several classical reactive oxygen species (ROS) detoxifying regulators were quantitated by real-time qPCR and transcriptomics analysis were done on an Illumina NextSeq platform. Apart from this, the behaviour of nZVI in the physiological solution was characterized by checking its pH, oxidative reactive potential and differential centrifugal sedimentation (DCS). Among the five tested

ROS detox genes, the expression of superoxide dismutase (sodB) showed the most distinct changes with approximate 3-fold up-regulation in the sample with dissolved Fe ions and a slight up-regulation in spiked nZVI conditions in comparison to the internal control. The differences in the gene expression profiles were further confirmed by the transcriptome analysis showing comparable gene expression to the real-time qPCR quantifications. The nZVI exposure has caused striking alterations in genes involved in response to oxidative stress, detoxification, and DNA repair. Subsequent data analysis had revealed several direct and indirect protection pathways against the superoxide radicals including efflux transport regulated by ynfM, coenzyme pyrroloquinoline quinone (pqqD-II) biosynthesis pathway and pentose phosphate pathway regulated by rpiA to eventually resolving the superoxide species. To conclude, P. putida effectively activated detoxifying genes to overcome oxidative stress caused by nZVI and ionic Fe(II)/Fe(III) to be able to restore cell functions.

4.08P.4

Toxicity and bioavailability of different forms of copper and copper nanoparticles to the earthworm E. fetida

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Copper nanoparticles (Cu NP) have a wide range of current industrial and agricultural applications. Both Cu and Cu NPs have potential as cost effective addition to fertilisers and as fungicides due Cu's role as a key macronutrient and its broad-spectrum antimicrobial properties. These applications utilise NPs enhanced with a variety of surface modifications, resulting in an increased diversity of Cu NP forms. These diverse Cu NPs are increasingly finding their way into eco-systems via practical applications and unintentionally through wastewaters and subsequent application to terrestrial environments through sewage sludge. Terrestrial ecosystems are sinks for both accidental and deliberately applied Cu NPs. This is leading to exposure of soil organisms to a variety of Cu forms. Although Cu is abundant as a trace element, elevated levels of bioavailable Cu in soils can have adverse effects on soil organisms, including earthworms. As important contributors to soil formation, amendment and nutrient cycling, earthworms are a critical indicator and facilitator of soil fertility. Previous studies of Cu and Cu Np toxicity to earthworms has focused on pristine NPs where, in general, they do not appear to be as acutely toxic as their metallic counterparts (Tatsi et al. 2018), there has been less work on earthworms exposed to augmented Cu based particles. In this study, we compare the exposure of the earthworm *Eisenia fetida* to ionic Cu, pristine Cu NPs and Cu NPs encapsulated in porous silica. The porous silica encapsulated copper has an anticipated slow release mechanism that releases Cu more gradually into the soil. This study utilised standard soil toxicity tests and observed several endpoints, including growth, mortality, reproduction, and gene expression. Examining gene expression patterns in the chloragogen/gut tissue allows us to investigate the underlying mechanistic effects of toxicity associated with Cu and specific Cu NP forms and compare these to changes in the expression patterns of genes involved in stress, ROS and metal-handling that have been reported in existing Cu toxicity studies.

4.08P.5

Toxicity and antioxidant activity of hydrated fullerene C-60.

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Hydrated fullerene C-60 is a hydrophilic supramolecular complex consisting of a fullerene C-60 macromolecule enclosed to a hydrate shell. It is the most known fullerene derivative, which already proved its pharmaceutical efficiency. However, biochemical aspect of the fullerene activity has not been studied in detail yet. The aim of the work was to consider toxic and antioxidant properties of hydrated fullerene using bioluminescence enzymatic assay which is based on the system of coupled enzymatic reactions of bacterial luciferase and NAD(P)H:FMN-oxidoreductase. Suppressing (inhibition) of bioluminescence intensity of the assay system characterizes quantitatively a toxicity of a sample under investigation. However, we did not find any bioluminescence suppression under the exposure to the fullerene in its concentration interval used. This result revealed the minimal toxicity of hydrated fullerene C-60 among all fullerene derivatives studied before under the comparable conditions: $C_{60,70}O_y(OH)_x$ (y=2–4,x=22–24); $C_{60}O_y(OH)_x$(y=2–4, x=22–24); $C_{60,70}O_y(OH)_x$(x+y=40–42), and others. Antioxidant activity of hydrated fullerene C-60 was studied in solutions of model inorganic and organic compounds of oxidative type: K_3 [Fe $(CN)_6$] and 1,4-benzoquinone. Antioxidant coefficients D_{OxT} were calculated, concentration intervals of antioxidant activity were determined. Values of D_{OxT} of the hydrated fullerene C-60 appeared to be higher than these for the other fullerene derivatives. Hence, the results show that the hydrated fullerene C-60 is characterized by maximal antioxidant activity and minimal toxicity among all fullerene derivatives studied earlier. This preparation can be recommended as a basis for drug synthesis and pharmaceutical application, being most friendly for ecology.

4.08P.6

Fullerenol toxicity via bioluminescence monitoring. Role of oxygen substituents

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Currently, synthesis and application of nanocomposites get ahead of investigation of their properties. We study toxic effects of a series of carbon nanoparticles, fullerenols, with different number of oxygen substituents , which form a perspective basis for drug development. Fullerenols are nanosized water-soluble polyhydroxylated derivatives of fullerenes, a specific allotropic form of carbon, and bioactive compounds. Two groups of fullerenols were under investigation: (1) C60Oy(OH)x, C60,70Oy(OH)x, where x+y = 24–28 and (2) C60,70Oy(OH)x, Fe0,5C60Oy(OH)x, where x+y = 40–42. Bioluminescent cellular and enzymatic assays (luminous marine bacteria and their enzymatic reactions, respectively) were applied to monitor toxicity in the model fullerenol solutions, and bioluminescence was applied as a signaling parameter. The inhibiting concentration ranges of the fullerenols were determined, revealing the fullerenol' toxic effects. All fullerenols produced toxic effect at higher concentrations (>0.01 g L-1). Quantitative toxic characteristics of the fullerenol toxicity (effective concentrations EC-50, concentration ranges) were found to depend on the number of oxygen substituents. Lower toxicity was determined in solutions of fullerenols with fewer oxygen substituents (x+y = 24–28). Content of reactive oxygen species (ROS) in fullerenol solutions was determined by luminol chemiluminescence method. Correlations between ROS content and bioluminescence intensity were found; a role of ROS in the toxic effects of the fullerenols was discussed. The differences in fullerenol toxicity were attributed to their catalytic activity due to reversible electron acceptance, radical trapping, and balance of ROS in aqueous solutions. The results provide pharmaceutical sciences with a basis for selection of carbon nanoparticles with proper toxic characteristics. We recommend fullerenols with lower number of oxygen substituents as components of nanocomposits with endohedral metal atoms involved into the carbon carcas. The potential of bioluminescence methods to compare toxic characteristics of carbon nanostructures was demonstrated. The biological activity of fullerenols should be taken into consideration due to risks of fullerenol intensive production for biomedical application in future. The work was supported by PRAN-32; grants of RFBR N18-29-19003mk, RFBR-Krasnoyarsk Regional Foundation N 18-44-240004, 18-44-242002,Tomsk Polytechnic University CE Program.

4.08P.7

Effects of boron and vanadium nanoparticles on Danio rerio embryos

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Engineered nanoparticles (NPs), as emerging environmental pollutants, demand for a better understanding of their environmental behaviour and potential harmful effects on organisms. Despite the various NPs developed and practical applications, there is still limited information available regarding the biological effects of certain nanomaterials, such as boron (BNPs) and vanadium (VNPs). Thus, the aim of the present work was to understand the effects of BNPs and VNPs (average size: 80-100 nm) to zebrafish developmental stages and at different levels of biological organization, e.g. biochemical and organismal (behaviour) levels. Gradient BNPs and VNPs concentrations was tested: 0-0.01-0.1-1-10 mg/L and the survival, hatching, heartbeat rate and malformations appearance of the organisms were assessed in a 96h exposure. The locomotor behaviour was also evaluated using an automated video tracking system at 120h. Additionally, embryos were exposed to 1 and 10 mg/L BNPs and VNPs, and a set of biomarker responses were assessed at 96h: cholinesterase, glutathione S-transferase, catalase, glutathione reductase and glutathione peroxidase activities, as well as lipid peroxidation levels, energy budgets and electron transport system. The physicochemical characterization of BNPs and VNPs on the test medium was also performed. BNPs, at 10 mg/L, induced malformations, decreased heartbeat rate and impaired locomotor behaviour. VNPs, at the same concentration, beyond the effects described above, also delayed hatching of the organisms. Biochemical responses, such as the ones involved in oxidative stress, were also altered by BNPs and VNPs exposure. The present work shows that BNPs and VNPs affect non-target aquatic organisms and reinforces the importance of the NPs risk assessment.

4.08P.8

Evaluating the toxicity of nanobiomaterials in different aquatic species

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Nanobiomaterials (NBMs) are receiving much attention because of their specific interaction with the cell membranes and their applications, which include drug delivery system, cell therapy, gene therapy, tissue regeneration, etc. 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 toxicity of two groups of NBMs based on hydroxyapatites (HA) or solid lipids (SLN), in different aquatic taxonomic groups. Also to assess the effect in three fish cell lines, to evaluate the similarity with the in vivo results and determine possible specific target organs. Two groups of NBMs were evaluated: 1) HA-NBMs manufactured and supplied as powder by ISTEC-CNR (Italy): Ca(HA)-P, Fe-HA, Ti-HA and TiHA-biopolimer; 2) SLN produced by Nanovector SRL (Italy): SLN-nutra-dis, SLN-nutra-sol (thickening agent) and LP-eye. The stock dispersion of HA NBMs were prepared using the NANOGENOTOX protocol. SLN suspensions were directly dispersed into the exposure media. The size distributions were measured by DLS (time 0 and every 24 h). In vitro toxicity assays were assessed in three fish cell lines (RTS-11, RTL-W1 and RTgill-W1). Cytotoxicity assays (Alamar Blue, CFDA-AM, NRU) were conducted after a 24h exposure period. Acute toxicity was studied in Daphnia magna (OECD TG202) and rainbow trout (OECD TG203). Dispersions of HA NBMs showed good stability for 24-48 h (PdI < 0.7; Z-ave: 100-400 nm) in the three exposure media. After this time, the polidispersion was too high to obtain good measures and appeared aggregates >1000 nm. The SLN suspensions were stable in the exposure media during the exposure periods. They presented sizes of 100-400 nm in water, being in the cell media 100-200 nm for LP-eye and < 60 nm for both SLN-nutra. All the NBMs, except TiHA-biopol, presented EC20 < 100 mg/L in the RTS11. LP-eye was toxic for the RTgill-W1. In D. magna, Ca(HA)P-NM provoked a significative immobilization at 100 mg/L (EC20, 42.46 mg/L). None of the NBMs showed toxicity for fish after 96 h exposure. The tested NBMs were not highly toxic to the exposed organisms (EC50 >100 mg/L). SLN showed effect in RTS11 and RTgill-W1 cell lines but not in the RTL-W1. The different effect of SLN exerted in cells with respect to in vivo assays could be linked to their smaller size in cell media in comparison with the size in water. Acknowledgement – Authors thank the H2020 project BIORIMA 760928 for its funding.

4.08P.10

Multiple Trophic Level Interspecies Studies on the Aquatic Ecotoxicity of Graphene Related Materials in Freshwater and Marine Environments.

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Currently, there is insufficient knowledge to make conclusions on the ecotoxicity of graphene and related materials and thus the impact of its release on aquatic environmental compartments remains unknown. Using key indicator species representing different trophic levels (bacteria, microalgae and water flea), from both freshwater and marine environmental compartments, we aim to characterise the hazard potential and identify any different species sensitivities. Recognising the need for tests to meet regulatory acceptance, as well as easy to apply testing platforms, we have used high-throughput alternative microbiotests that adhere to standardised and regulatory accepted guidelines and can be performed independently of culturing and maintenance of stocks of test species. We have payed particular attention to the applicability and necessary adaptations required when testing materials with unique physico-chemical properties and behaviours (e.g. graphene's absorbance, opacity, instability) in order to provide reliable data. The endpoints measured included bacterial luminescence inhibition, algal growth inhibition and Daphnia immobilisation and both graphene oxide (GO) and a reduced graphene oxide (rGO) were tested. The potential hazard to marine environmental compartments was assessed using the marine bacteria (*A fischeri*) and marine diatom (*Phaeodactylum tricornutum*). We have evidenced distinct behaviour of the graphene materials in marine water and thus effects cannot simply be extrapolated from freshwater environment species responses. Also a detailed investigation into the interaction of the graphene materials with the first point of contact (i.e. cell wall, membrane or in the case of Daphnia, the carapace) provided information on the mode of action/interaction of such materials with diverse species. Such an approach used facilitated the assessment of ecotoxicity of graphene related materials to multiple species from both freshwater and marine environments and provided valuable information towards an ecotoxicological risk assessment of graphene.

4.08P.11

Copper-doped silica nanoparticles impair the mechano-sensory based behavior of zebrafish larvae (D. rerio)

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The aquatic ecotoxicity of engineered nanomaterials (ENMs) and their release into aquatic environments has been of great interest and concern in the past decade. ENMs are used in a wide range of commercial products and applications. Herein, we investigated the impact of newly synthesized porous and non-porous Silica/Cu-ENMs by assessing physiological endpoints and behavioral responses of zebrafish (Danio rerio) larvae (ZFL). ENMs were suspended in a "Seasalt-BSA" medium containing a natural salt mixture with low ionic strength (6.7 mmol/L) and natural organic matter (0.05% BSA). This medium had a positive effect on particle stability compared to dH_2O or conventional media (i.e. OECD medium). The measured size differed slightly between three tested ENMs: ENM1 (porous silica 300nm) was 421 ±39nm, ENM2 (porous silica 100nm) was 439 ±165nm and ENM3 (non-porous silica 300nm) was 591 ±60nm. Physiological effects (lethal, sublethal) on different embryonic stages were determined using the Fish Embryo Acute Toxicity (FET) protocol at 10, 50, 100, 500 mg/L. No effects were observable for exposure periods < 72 hours post fertilization (hpf). Longer exposure times (96 and 120h) revealed effects on hatching success for ENM1 (EC50 = 242 mg/l at 96 hpf) and ENM3 (EC50 = 27 mg/l). Behavioral studies (96 hpf; 24 hours) showed neither effects on locomotion (swimming distance) nor the startle response (escape reaction upon vibration stimulus). However, exposure to ENM3 impacted the rheotactic behavior (counter flow swimming) which was measured in a flow-through system, integrating five individual flow channels. ZFE (50, 100 mg/l) spent significantly less time in the main zone and most larvae were located in the outlet zone. Reduced rheotaxis is likely due to the release of freely dissolved Cu-Ions, which was about four times higher for non-porous particles (ENM3) compared to porous particles (ENM1 & 2). Our data demonstrates that rheotaxis is a sensitive endpoint for Silica/Cu-ENMs and warrants further investigations to understand potential particle-specific effects.

4.08P.12

Transformation of zinc oxide nanoparticles in aquatic environment and its toxicity changes

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Zinc oxide nanoparticles (ZnO NPs) are known to be transformed by several ions in wastewater treatment plants. Changes in physicochemical properties of ZnO NPs by transformation was reported; however, little is known about its effect on toxicity changes. Sulfidation, phosphatation of ZnO NPs and zinc associated with ferric oxide-hydroxides were induced and characterized. Also, the toxicity changes were evaluated in zebrafish embryos. After addition of sulfide, phosphate, and ferric oxide-hydroxides, X-ray diffraction (XRD) and X-ray absorption fine structure (XAFS) was used to characterize surface composition of transfomred ZnO NPs. Particle size, zeta potential, and surface shape were measured by Particle size analyzer and transmission electron microscope (TEM), respectively. Toxicity of ZnO NPs significantly decreased as the ratio value (S/Zn and PO_4/Zn) increased whereas toxicity reduction was not observed in zinc associated with ferric oxide-hydroxides. We found that addition of ethylenediaminetetraacetic acid (EDTA) to solution of zinc associated with ferric oxide-hydroxides was strongly related to toxicity changes, indicating that decreased toxicity was dependent on reduction of dissolved zinc ions. In conclusion, our data indicate that changes in physicochemical properties in ZnO NPs through transformation should be carefully taken into consideration to determine potential ecological toxicity of ZnO NPs.

4.08P.13

Low concentrations of copper oxide nanoparticles are toxic in three loamy soils

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Copper oxide nanoparticles (CuO-NP) have already been applied as an efficient alternative to conventional Cu in agriculture and are expected to increase substantially, ending up in the soil environment. Previous studies revealed a high toxicity of CuO-NP towards cells and microorganisms, but no to low toxicity towards soil invertebrates was found. Soil ecotoxicity assays usually are conducted in a sandy reference soil and at comparably high test concentrations > 100 mg Cu/kg dry soil. The present study adressed the knowledge gap concerning the behavior of CuO-NP in loamy soils and at lower test concentrations. Two sandy reference soils (clay content = 6%) and three loamy soils (clay content 19-25%) were spiked with CuO-NP at 5 - 158 mg Cu/kg dry soil. 28-day reproduction and growth as well as 14-day bioaccumulation and elimination of Cu were investigated for the springtail Folsomia candida. Ecotoxicological effects were only observed in loamy soils and were always strongest at the lowest test concentration of the reproduction assays (-31 to -61%) . Most pronounced effects were found in a loamy-acidic soil with a strong impact on reproduction (-61%) and a moderate impact on growth (-28%), both again at the lowest test concentration. In the same soil and concentration, a significant accumulation of Cu in F. candida was measured after 14 days, whereas in subsequent 14 days nearly no elimination of Cu took place, hinting at a possible cause of the observed toxic effects. Interactions between NP and clay minerals with impact on their bioavailability and ecotoxicity were already proven in other studies. As soil ecotoxicity assays are usually conducted in sandy soils, we suggest considering loamy soils especially for studying nanoparticle ecotoxicology, and a focus on low, field-realistic concentration ranges.

4.08P.15

Effects of graphene oxide alone and with sorbed PAHs on zebrafish*

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Graphene is a carbon based nanomaterial showing a 2D flat sheet conformation. Due to its properties, it is increasingly being introduced in multiple fields. Because of its high hydrophobicity and specific area, once in the aquatic environment, graphene is expected to act as a carrier of pollutants to aquatic organisms, especially of hydrophobic compounds such as polycyclic aromatic hydrocarbons (PAHs). This phenomenon is known as Trojan horse effect. In this study we aimed to (1) assess the capacity of graphene oxide (GO) to sorb PAHs and (2) evaluate the toxicity of GO alone and with sorbed PAHs on adult zebrafish. Graphene oxide showed a high sorption capacity for B(a)P (98% of B(a)P sorbed from an initial concentration of 100 µg/L) and for other PAHs present in the water accommodated fraction (WAF) of a naphthenic North Sea crude oil, depending on their log K_{ow} (95.7% of phenanthrene, 84.4% of fluorene and 51.5% of acenaphthene). Transmission electron microscopy and atomic force microscopy (AFM) showed that the procedure used to contaminate GO did not affect the stability or stacking of the GO platelets. According to AFM images, GO thickness (0.410-0.738 nm) was similar to GO-B(a)P thickness (0.594-0.924 nm). To assess toxicity, adult zebrafish were exposed for 21 days to GO (2 mg/L), to B(a)P-contaminated GO, to WAF-contaminated GO and to 100 µg/L B(a)P. Fish exposed to GO or contaminated GO presented GO in the lumen of the intestine and liver vacuolization. No significant alterations were detected in the transcription level of genes related to cell cycle regulation and oxidative stress, but a slight up-regulation of *cyp1a* was measured in fish exposed to B(a)P for 3 days that resulted in a significant increase of EROD activity.). At 3 days, fishes exposed to GO-B(a)P and B(a)P showed significantly higher catalase activity in the gills than control fish. Neurotoxic effects (significantly lower acetylcholinesterase activity) were also observed in all fish treated for 21 days. These results demonstrate the capacity of GO to carry PAHs and to exert sublethal effects in zebrafish. *Funded by UPV/EHU (grant to IMA), Basque Government (consolidated research group IT810-13 & IT1302-19), Spanish MINECO (NACE, CTM2016-81130-R), French ANR (No.–10–IDEX-03-02) & Cluster of Excellence COTE (ANR-10-LABX 45). Thanks to staff at Driftslaboratoriet Mongstad, Equinor (former Statoil) for supplying the oil. SGIker technical and human support (UPV/EHU, MICINN, GV/EJ, ESF) is acknowledged.

4.08P.16

Mixture toxicity of nanomaterials and antibiotics in freshwater microcosms

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Applications of copper oxide nanoparticles (CuONP) recently have substantially increased, accompanied by expected rising environmental concentrations. Single species tests have shown toxic effects on limnic algae, daphnids, snails and sediment worms, among others. The community structure and diversity of sediment microbial communities is also affected by CuONP exposure. Under natural conditions, multiple species, trophic levels and functional groups interact and toxic effects of multiple chemical compounds can potentially influence and change an entire ecosystem. To mimic realistic exposure scenarios we combined CuONP with a widely spread antibiotic. Sulfamethoxazole (SMX) is an antibiotic that is applied in human medicine on a global scale. The removal rate in wastewater treatment plants is low, and therefore SMX has been detected in surface, ground and drinking water world-wide. In freshwater systems, especially algae react sensitively to SMX, and toxic effects on the bacteria *Vibrio fischeri* and daphnids have also been documented. We set up outdoor freshwater microcosms with a sediment and a water phase. Algae, daphnids and sediment worms, *Lumbriculus variegatus*, were added to represent several trophic and functional groups. Their population sizes have been monitored throughout the study (duration at present: three months). Treatments are an untreated control, SMX, CuONP and SMX+CuONP with 5 replicates each (in total 20 microcosms). The chemicals are added on a monthly basis at low concentrations to mimic a long-term exposure scenario, and chemical analyses are conducted to assess bioavailability. Currently we are preparing analyses to compare microbial communities and activity between treatments. We aim to answer the following research questions: 1) Do effects of SMX and CuONP on freshwater algae, daphnid and *L. variegatus* populations differ in single and mixture exposure under natural conditions? 2) Do aquatic animals accumulate CuONP differently when SMX is present? 3) Does the community composition of algae differ between treatments? 4) Do abundance and composition of the sediment microbial community change with treatments? First results are currently being analyzed and will be presented.

4.08P.17

Assessing the risk of oral exposure to nanomaterials for vertebrates in the environment. Extrapolation from EFSA human health guidance

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EFSA published in 2018 the guidance (Part 1) for assessing the risk of nanomaterials to humans. Part 2, covering the environmental assessment for pesticides and feed additives, has been initiated through a contract with RIVM and first results will be presented in a complementary piece (see Quick et al. abstract). The EFSA human health risk assessment profile focuses on oral exposure and fate of particles in the gastrointestinal track, including potential for transformation. We explore the adaptation of the guidance for assessing food chain wildlife exposures. Direct application of nanoformulated plant protection products or atmospheric/dust deposition on vegetation following industrial emissions may lead to the presence of nanoparticles on plants and direct oral exposure of herbivorous. The digestion of cellulose requires relative large digestion timelines, and may lead to the accumulation of the ingested nanoparticles in the digestive tract of herbivorous and the exposure of carnivorous species. EFSA animal toxicokinetic models provide information for adapting the human conditions to birds and mammals with different digestion approaches. For aquatic trophic chains, hetero-agglomeration with suspended particles and adsorption on algae may constitute a first step for accumulation in plankton and organisms feeding through filtration, followed by secondary consumers, and then humans and wildlife. Regarding the hazard characterization, mammalian toxicity data can be used for assessing risk for vertebrates in the environment. Environmental assessments require the extrapolation from a limited number of species to a broad diversity of taxa at population level. Local inflammatory effects are considered of low relevance at population level, while effects on the microbiome could affect growth. The additional focus should be on systemic toxicity. In vitro systems are suggested as a first step for human risk assessment and could be also applied to wildlife. Dissolution should be assessed first for the gastrointestinal conditions, and then at lysosomal level. When in vivo testing is needed, the guidance recommends the integration of toxicokinetics in both, the dose range finding and the final studies. We will explore how this approach could consider interspecies differences for the assessment for wildlife.

Environmental Risk Assessment of Pharmaceuticals: Connecting across Disciplines and Continents (P)

4.09P.1

A risk assessment framework for recycling of diapers and incontinence materials, focussing on pharmaceuticals and pathogens.

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Every year, more than 160 million kilos of used diapers for babies and incontinence material for adults end up in waste in the Netherlands. To reduce the amount of diaper waste, materials can be recycled and new products can be made. For example, plastic from diapers can be converted into plastic bottles for cleaning products. It is important that these new products and materials are safe for people and the environment. RIVM has developed a risk assessment framework to assess these concerns. This allows recyclers of these materials to collect the necessary data to perform a risk assessment. Diapers and incontinence material contain pathogens and medicinal residues that people excrete through their urine and faeces. The diaper material itself contains plastics, cellulose and granules that absorb moisture. The step-by-step plan also provides a method for licensing authorities to assess the risk of new products and materials. In addition to the risk assessment, licensing authorities and policy makers can look at other advantages and disadvantages, including sustainability. The producer remains responsible for the safety of his product. Two waste processors tested the step-by-step plan to make it practicable. The first step in the risk assessment is a general and relatively strict assessment of potential risks. Meeting these strict criteria means that a choice can be made for a broad range of applications of recovered materials from the diapers. If a material or waste stream is potentially not safe to begin with, the next step tests whether the risks of pathogens or medicinal residues are sufficiently reduced during the recycling process. For example, pathogens can be killed by heating. If risks cannot be ruled out, a check is done to determine whether unwanted substances are released from the materials produced. If that is the case, specific products may be designed, from which the unwanted substances cannot be released. The risk assessment framework will be presented as a step by step approach.

4.09P.2

How far does the apple fall from the tree? An approach for a standardized multigenerational toxicity testing with Daphnia magna

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Surface waters are experiencing exposure to a variety of permanently present (pseudo-persistent) micropollutants. As a consequence, it seems reasonable to consider an extension of available standardized ecotoxicological test methods, taking realistic long-term exposure scenarios over multiple generations into account. Based on its key role in aquatic food webs and the parthenogenetic reproduction, *Daphnia magna* seems ideal for testing effects of micropollutants over multiple generations. As a first step, we extended the OECD 211 reproduction test procedure by considering published protocols, which were supplemented by additional endpoints such as the fitness of the offspring. As a second step, the influence of the insecticide pirimicarb was tested over three generations (F_0, F_1 and F_2). In accordance to previous studies, the reproduction performance decreased with increasing toxicant concentration and subsequent generations. Contrary, the offspring showed an increased tolerance towards pirimicarb in acute toxicity tests. We are currently also testing dexamethasone, a corticosteroid, and plan to extend the database to further micropollutants representing different substance classes, to ascertain the necessity and practicability of such a test procedure. These experiments ultimately have the potential to broaden our understanding of environmental consequences induced by long-term exposures improving the environmental risk assessment of micropollutants.

4.09P.3

Prioritisation of environmentally hazardous pharmaceuticals

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Many active ingredients of human and veterinary pharmaceuticals enter the environment in treated sewage or manure after their application. There they can pose a risk to organisms in the environment or in the groundwater due to their substance properties such as poor degradability, accumulation or transport capacity as well as ecotoxicity. The objective of our project was to compile a broad overview of active substances in human and veterinary medicinal products available in the Swiss market. Proceeding from the data we established a prioritisation of potentially environmentally hazardous substances. In Switzerland 3,160 substances are listed as active pharmaceutical ingredients. In a first step, we deselected 1,449 substances due to their low consumption (< 3kg cumulative consumption from 2016-2018) in Switzerland. 486 substances were identified as plants or phytopharmaceuticals and excluded. Another 255 could generally be discounted from analysis because they are natural products or more specifically elements, animals or viruses. Additionally, 155 substances could be classified as dietary supplements, vitamins or food and again were deselected. Furthermore, there were 206 peptides, proteins and antibodies classified which were excluded. In a second step, different criteria for substance identification and authorisation were collected. In detail 13 criteria for physicochemical properties (e.g. log Kow, log Koc, vapour pressure, pK, water solubility), three criteria for degradability (elimination in WWTP, soils and biodegradability) and four criteria for bioaccumulation were determined. Criteria for consumption and expected environmental relevance were also considered. Additionally, twelve criteria for toxicity; for example, acute, chronic, aquatic, terrestrial, PNECs or high specific toxicities were compiled. Our hazard based prioritisation identified mainly substances with high specific toxicity; biocidal products, hormone active substances, substances triggering antimicrobial resistance, antihypertensive drugs and a painkiller. Ivermectin, an anti-parasitic agent, was prioritised at the highest level. The second highest prioritisation product was calculated for the biocide triclosan. The antibiotic rifabutin, amiodarone hydrochloride, which is used to treat cardiac arrhythmias and deltamethrin, also used as a pesticide and biocide, are listed on the following priorities.

4.09P.4

The effects of diclofenac on fertilisation in echinoderms and polychaete worms

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The chronic marine ecotoxicity dataset for diclofenac covers crustaceans, echinoderms, polychaete worms and molluscs. Of particular note, is a study by Zanuri et al. (2017)[4] which reports No Observed Effect Concentrations (NOECs) for fertilisation on sea urchins, starfish and polychaete worms. Zanuri et al. (2017) investigated fertilisation success based on the exposure of sperm and oocytes, prior to mixing them together to allow fertilisation. The gametes were collected from wild-caught animals, which were not pre-exposed to diclofenac. A clear dose-response was observed for fertilisation, and NOEC values were reported as 0.1 µg diclofenac L^{-1}, for all three test organisms (when both sperm and oocytes were exposed). The experimental method is well described, but we considered there to be a number of potential issues in terms of the methodology employed: · The study applied a wide spacing factor between test concentrations (x 10); · The study lacked confirmatory analysis of exposure concentrations; · The adult organisms from which the gametes were obtained prior to fertilisation were not exposed to diclofenac; · The gametes (eggs and sperm) were pre-exposed to diclofenac individually, but not during the fertilisation phase (i.e. when mixed together); · The temperature of the gamete exposure and fertilisation phases was 4°C higher than the temperature at which the adult organisms were held; and · The apparent degree of fertilisation at the lowest exposure concentrations was only very slightly reduced compared to control exposures, but was nevertheless reported to be statistically significantly different from the control responses. Taken together the above methodological factors cast doubt on the ecological relevance of the study results because; · The exposure of gametes individually without any exposure of adults or during fertilisation is not environmentally realistic; · Echinoderms have been shown to be especially sensitive to sudden temperature changes; · The statistical significance at the lowest exposure concentrations could potentially be caused by large intra-treatment variability, and the application of more appropriate statistical tests could have resulted in different outcomes (raw data requested but is no longer available), and · The actual exposure concentrations of diclofenac to which gametes were exposed is not clear. Therefore, in order to further investigate these issues, it was decided to repeat elements of this study in a more robust manner, addressing the above issues. The new studies outlined above are currently in progress, with results expected at the end of 2019. A comparison between the original study and the results of the new studies will be presented.

4.09P.5

Impacts of sediment-associated diclofenac and citalopram to a sediment-living amphipod: single compound and mixture toxicity tests

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Wastewater treatment plants (WWTPs) are handling increasing amounts of various pharmaceutic compounds due to an increased usage and consumption globally. WWTPs are, however, not designed to handle pharmaceutic compounds, which generally result in low removing efficiencies. This could potentially pose an environmental risk, due to most pharmaceuticals having effects at low concentrations. Thus, pharmaceuticals are considered an environmental concern, which have enhanced the monitoring of compounds (i.a. antibiotics, antiparasitics, hormones, analgesics and psychotropic medicines) in the aquatic environment measured primarily in water and a lesser extend in sediments. Many pharmaceuticals are hydrophobic (log K_{OW} of 2-6) and are therefore likely to accumulate in the sediment compartment, if not captured in the sludge fraction of the WWPT. We conducted sediment-associated toxicity tests of diclofenac (DCF) and citalopram (CP) as both single compound and in a mixture on the sediment-living amphipod *Corophium volutator*. This presentation will provide 1) an example of how compounds as DCF and CP partitions between sediment and water after a 10-day exposure period, and 2) examples of the usage of both dose-response modelling and the toxicokinetic-toxicodynamic (TKTD) model GUTS (general unified threshold model for survival) describe data and how well the mixture toxicity is predicted by the mixture models Concentration Addition (CA) and Independent Action (IA). We found that applying a 1st order decay model to the actual sediment concentrations, the predictions of GUTS-RED-SD seemed to accurately describe the relation between sediment-associated DCF and CP and their mixture on the survival of *C. volutator* over a 10-day period. The models, however, seemed to underestimate the survival close to LC_{50}. Mixture toxicity was well predicted by CA, but was synergistic relative to IA.

4.09P.6

Environmental risk assessment of the anti-inflammatory diclofenac for marine organisms under ocean acidification scenarios

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The ease involved with the purchase of over-the-counter drugs and the unconscious use of these compounds endangers not only the health of the human population, but also the marine environment and the organisms that live in it, as a consequence there is an increasing problem which involves the presence of emerging chemical compounds in the oceans mainly through the disposal of domestic effluents. Since the last decade, many studies have reported the presence and effects of these substances on marine organisms, especially the class of anti-inflammatory drugs such as Diclofenac, which in addition to the recurrent use by the population, is also widely used in veterinary medicine, the consumption exceeds 1000 tons per year. However, little is known about the effects of these compounds in a scenario of decreasing oceanic pH, characteristic of the increase in carbon dioxide (CO2) emissions in the atmosphere since the time of the Industrial Revolution, causing changes in the physical and chemical parameters of water, affecting directly marine ecosystems. In Brazil, as a mitigating measure, deep oil exploration activities include the injection of CO2 increase the pressure to remove oil from the wells, giving greater efficiency to the extraction system; this technique lowers the concentrations of this gas in the atmosphere, but accidental leakage can result in several effects on marine environments, especially those related to the bioavailability of emerging pollutes. Given the context described, the aim of this study was to evaluate the occurrence and effects related to the cellular stress (Lysosomal Membrane Stability – LMS of Diclofenac on *Perna perna* and *Mytella guyanensis*, considering the influence of predicted marine acidification until the end of the century (pH 8.0 – 7.6 – 7.3). The pH values were achieved using injection of carbon dioxide. Water and sediment samples were collected at 5 points around the Santos submarine sewageoutfall (SSO). Diclofenac was observed at all sampling points, with concentrations ranging from 5.2 to 762 ng/L in water and from >0,1 to 3.39 ng/g in sediments. With regard to water cytotoxicity assays, after 96 hours of exposure, the LMS of *Perna perna* significantly decreased from the concentration of 200 ng/L when compared to the control group at pH 8.0 and 7.6. Already in the results of the *Mytella guyanensis* sediment trials under exposure to Diclofenac after 96h of exposure, the LMS decreased significantly at 30 ng/g at pH 8.0 and 7.6. However, the toxic effects were more severe in organisms exposed to pH 7.3, with effects from 3 ng/g. It was possible to calculate the EpH50 value too (pH: 7.43), which demonstrates the pH that decreases the LMS by 50%. It was observed that the marine acidification process increases the effects of Diclofenac in the different matrices and cause effects at environmentally relevant concentrations. The present study demonstrated the importance of drug-related toxicological research and pH changes in the oceans, as increased drug toxicity was observed when subjected to the acidification process.

4.09P.7

Environmental risk assessment for genetically modified live vaccines: Critical issues for clinical trial and market authorisation applications

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The environmental risk assessment (ERA) forms a significant part of clinical trial and market authorization applications for vaccines or therapeutics containing or consisting of genetically modified organisms (GMO). An ERA is required regardless of the type of therapy, i.e. *in vivo* or *ex vivo* gene therapy, oncotherapy or vaccine. A case study using a recently approved vaccine for Ebola virus disease, ERVEBO^{TM}, will be presented. In order to prepare a comprehensive and compelling risk assessment document, an ERA should cover all characteristics of the GMO having a potential adverse effect in case of exposure of people other than the treated persons or the environment. Most importantly, a rationale has to be given on a) how exposure is avoided due to safe application of the drug substance or b) how negative consequences from exposure are prevented due to safety features of the GMO or through post administration measures. To this end, information is derived from specific data gained from research and development, from manufacturing processes as well as from pre-clinical and clinical trial studies. All information is compiled in a step-wise risk assessment approach per EU Guidelines. Key issues and areas for collaboration across disciplines will be presented.

4.09P.8

ORPHENADRINE BIOLOGICAL EFFECTS UNDER DIFFERENT OCEANIC ACIDIFICATION SCENARIOS

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ABSTRACT The use of marine geological structures in the carbon dioxide capture and storage (CCS) process as a global warming mitigation strategy was proposed in the London Protocol, which was aimed at preventing marine contamination. Despite the undisputed advantages of the strategy, CO2 leaks may occasionally occur, contributing to the ocean acidification process. Among the various pollutants that may change due to lower water pH, drugs and personal care products (FPCP) are of major concern. Currently the amount of data on the effects and environmental risk of FPCP on marine organisms is still thin. Given this scenario the present study aims to analyze the occurrence, behavior and bioavailability of the drug orphenadrine under different scenarios of ocean acidification. The drug orphenadrine, used as a muscle relaxant and widely consumed was observed in all sampling points of the influence areas of the outfalls of Santos and Guarujá - SP, with concentrations ranging < LOQ to 2.14 ng / L a in water and < LOQ at 0.5 ng / g in sediments. The results of the water toxicity test using sea urchins (Echinometra lucunter) at different pH 8.0; 7.6; 7.3 had CEO values of 0.05mg / L and EpH50 was set at 7.30. As for assays with Perna perna mussels effects were observed at environmentally relevant concentrations, with CEO of 200 ng / g. Test results for the evaluation of embryolarval development in water indicated that both the acidification process and the increase in concentration affect the development of sea urchin embryos. In the tests with P. perna, it was possible to verify that the presence of the basic drug reduced the effects of ocean acidification, indicating the possibility that the interaction between these two may influence the toxicity of the compound and the effects of acidification. In this sense, the need to deepen the studies on drug related toxicology under marine acidification scenarios is evident.

4.09P.9

PERK: an interactive web-based tool to model environmental risk assessment

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The presence of active pharmaceutical ingredients (APIs) in the aquatic environment has been known for decades. Recent advancement in the scientific instrumentation and analytical methods, enabled to identify and quantify wide range of APIs in the aquatic and terrestrial environment, and helped to evaluate their impacts on exposed aquatic environmental species and humans. Still, quantification and environmental risk assessment of many APIs are very limited, this is partly because of the limitations in analytical method development, and costly and time-consuming toxicity tests. Therefore, environmental risk assessment modelling is an important alternative. Specific guidelines for the environmental risk assessment of the APIs for human use were developed by European Agency for the Evaluation of Medicinal Products and United States Food and Drug Administration. These guidelines formulate equations to calculate and model the predicted environmental concentration (PEC) of a drug, based on human consumption data and the eco-toxicity, and have been demonstrated to be useful in approximating different APIs exposures to the environment. Large number of studies have proposed different PEC models to assess the risk of APIs in the environment using different criteria. The aim of this study is to build a software application to facilitate automated modelling and reporting PEC of a comprehensive set of pharmaceuticals derived from a wide range of therapeutic classes with different modes of action and prescription in England. Information on the consumption of pharmaceuticals, along with data on metabolism, dilution data, flow data and removal during wastewater treatment, were used to predict the concentrations of the selected APIs. The concepts of the calculation procedure will be discussed, and the software will be demonstrated with attention for the features used to compare predicted concentrations with measured concentration from monitoring data in South West England. **Acknowledgement:** This work is a part of the Wastewater Fingerprinting for Public Health Assessment (ENTRUST) project funded by Wessex Water and EPSRC IAA (grant no. EP/R51164X/1).

4.09P.10

PrAna: an interactive web-based tool to explore prescribing data in England with spatiotemporal trends

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During the last decade, wide range of active pharmaceutical ingredients (APIs) have been identified and quantified in aquatic environment across several studies and indicated their impacts on exposed environmental species and humans. For the prediction of total amount of the APIs released to the environment, the predicted environmental concentration (PEC), and for the estimation of the expected introduction concentration (EIC), information about APIs consumption data is vital. Several methods were reported globally to estimate the APIs consumption data from the national prescription data, manufacturers, importers and dispenser's data. However, the methods reported to predict the APIs consumption data are limited to a geographical region and/or to a small group of selected drugs. We aim to collect the data for all groups of APIs to the different geographical regions in England with spatiotemporal trends and to use the data to calculate PEC. In England, national level prescription data is provided by National Health Service (NHS) at general practice level, reporting the item counts, quantity and cost of prescriptions aggregated by British National Formulary (BNF) code. This data is freely accessible and consist of individual files for each month. With the large file with over 10 million records every month, the data from the NHS cannot be used for the direct calculation of the prescription levels of different APIs. Re-organisation and processing of the files is required before to do any exploration or analysis and to speed up the data reading. We made the decision to carry out the aggregation and analysis using open source statistical software R.

The processed dataset is visualised in an online web-based dashboard layout developed using the R Shiny framework. The tool helps to calculate and visualise total prescribed quantity of different APIs for the period 2015 to 2018 for England, and to facilitate spatiotemporal trends. Total prescribed quantity is calculated from monthly prescribing data files published by NHS Digital for the period 2015 to 2018 and NHS Data Model and Dictionary (dm+d). Data normalisation carried out based on individual pharmaceutical compound with current classification based on BNF code. The tool showed some interesting insights, especially showed a strong difference between the antibiotic prescription quantity over summer and winter season, in different CCG regions in England. The concepts of the calculation procedure will be discussed, and the software will be demonstrated with attention for the spatiotemporal features used to compare antibiotics prescription quantity over different CCG regions in England.
Acknowledgement: This work is a part of the Wastewater Fingerprinting for Public Health Assessment (ENTRUST) project funded by Wessex Water and EPSRC IAA (grant no. EP/R51164X/1).

4.09P.11
Development of initial environmental risk assessment methods for pharmaceuticals by using Eco-QSAR system

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It is important to evaluate environmental risk of pharmaceuticals as well as human health risk, before marketing. Recently, medical regulatory agencies require pharmaceutical companies to assess environmental impacts of the submitted pharmaceutical products. Hence, it would be valuable to predict environmental risk of a new pharmaceutical product at the developing stage. In order to develop the predicting system for environmental risk, we need to construct a kind of eco-toxicity QSAR model and a prediction system for environmental dynamics for pharmaceuticals. In this study, we constructed ecotoxicity database of about 350 human pharmaceuticals by gathering the data of toxicity studies for algae, daphnia and fish in compliance with the standard OECD test guidelines for supporting to develop QSAR models. Also, we have developed a database of environmental fate including physico-chemical properties of approximately 300 pharmaceuticals for developing the environmental dynamics predicting system, and have developed monitoring database of pharmaceuticals in Japanese rivers as well. However, focusing on pharmaceuticals frequently detected in Japanese water environment, it is elucidated that the environmental toxicity test data of pharmaceuticals is limited. Based on the structural analysis of the human pharmaceuticals whose calculated values by the QSAR models were underestimated, possible toxicophores unique to human pharmaceuticals were identified in relation to environmental toxicity. Comparison of the monitoring database and toxicity databases revealed a large data gap for the development of ecotoxicity risk assessment system of pharmaceuticals. As further studies, we need to conduct more ecotoxicity studies for pharmaceuticals. (Acknowledgment: This work was supported by the Research on Regulatory Science of Pharmaceuticals and Medical Devices from Japan Agency for Medical Research and Development.)

4.09P.12
FLUOXETIN HYDROCHLORIDE TOXICITY ASSESSMENT USING VIBRIO FISCHERI BACTERIA

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Considering the current concern of the scientific community about the presence of drugs in the environment, the uncertainty about the deleterious effects on organisms and the lack of proper drug elimination procedures are being discussed worldwide. The variety of pharmacotherapeutic chemical specialties, as well as the metabolites generated, unchanged or conjugated excreted drugs via urine and faeces, as well as improper disposal and expired drugs, indicate the need for studies related to ecotoxicological aspects, quantification and identification as to the presence of these compounds in the various environmental matrices and their removal efficiency. The aim of the present study was to evaluate the toxicity of fluoxetine hydrochloride. Tests were performed to evaluate the potential for biodegradation of the compound based on respirometry according to Gledhill-modified 1988-IBAMA using the commercial dosage form that was used. quantified by HPLC / MS. The acute toxicity test was conducted using the marine bacteria Vibrio fischeri (Microtox System). Method 15411-2 (ABNT, 2006a) using three different formulations of FH (fluoxetine hydrochloride): the pure compound (Sigma content> 90%), a pharmaceutical brand and the generic. The degradation test showed a partial degradation of FH with approximately 27% reduction. Toxicity results showed not only the toxicity of fluoxetine hydrochloride, but also the problem inherent in the type of excipient used in various dosage forms. The generic drug was as toxic as the pharmaceutical brand (pure drug trade content < 90%). Sigma FH was slightly less toxic than the other two forms. The results demonstrate the need for procedures to minimize the presence of these compounds and reduce the effects.

4.09P.13
EVALUATION OF PHYSICAL-CHEMICAL PROPERTIES OF PHARMACEUTICALS, IN ORDER TO IDENTIFY THE SOLID PHASE SORTION POTENTIAL AND TOXICITY OF THESE ENVIRONMENTAL SUBSTANCES

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Considering the need to find alternatives for the beneficial use of sludge generated in sewage treatment plants (TEEs), mainly agricultural use, the present work aims to evaluate the structure and behavior of the drugs in sludge from TEE, submitted to composting process. The physico-chemical properties of selected drugs and their behavior in TEEs, considering the tendency of persistence in the sludge, and the ecotoxicological potential when disposed in the soil, using the QSAR-ECOTOX-EPA model. Sludge composting line installation: • Characterization of crude and composted sludge; • Quantification of drugs by HPLC and their behavior in composting. The evaluated drugs are in the most consumed compounds list in Brazil 2016/17 (ANVISA, 2018) The preliminary results regarding the octanol / water partition coefficient, we have: log Kow < 2.5: high hydrofibicity and low absorption tendency in biomass and lipid fractions of suspended solids. 2.5 ≤ log Kow ≤ 4.0: moderate tendency of absorption in these matrices. log Kow> 4.0: highly hydrophobic and high sorption potential in the solids present in the sewage Preliminary results show that atenolol, carbamazepine, paracetamol and clonazepam show Log Kow of 0.16; 2.30; 0.46 and 2.53, respectively, with low sorption tendency, and have no bioaccumulation potential. The Ibuprofen (3.79) has a moderate tendency and simvastatin, a high tendency of sorption (4.68), both potentially bioaccumulative. Agricultural use of biosolids is a controversial issue because of the potential adverse impacts on human health. In the name of safety, stringent standards, high quality criteria and restrictions of use are imposed, standards should be based on risk assessment, but data on the subject are lacking, and there is still no clarity as to what constitutes a tolerable risk in the context for the composition of a standard. Keywords: pharmaceuticals, toxicity, contamination environmental

4.09P.14
The impact of medicinal compounds on the physiology of Daphnia magna

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There is a significant concern over the impact to the aquatic environment resulting from human activities. Specifically, in relation to active pharmaceutical ingredients that can affect wildlife, there is extremely low amount of information in Irish water, and this uncertainty highlights the importance of monitoring and controlling the presence of active pharmaceutical ingredients in the environment. Modern approaches emphasize on the use of molecular endpoints in search of mechanistic insight for pollution and risk assessment with several key species used as bioindicators. Among the aquatic organisms, daphniids have acquired a central position in pollution monitoring. In this study, we examined the toxic impact of active pharmaceutical ingredients ranging from antibiotics, to oestrogen hormones, anticonvulsive drugs and painkillers, on daphniids. Using phenotypic endpoints (i.e. lethality) and molecular markers of oxidative stress (i.e. antioxidant enzymes, thiols redox state markers, detoxification enzymes) the underlying mechanisms of actions for specific active pharmaceutical ingredients was investigated. Keywords: Daphnia magna, active pharmaceutical ingredients, toxicity, oxidative stress

4.09P.15
Oxidative stress-related effects induced by independent and combined exposure to cocaine and benzoylecgonine on the Mediterranean mussel Mytilus galloprovincialis

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Illicit drugs have been recognized as emerging aquatic pollutants. After human use, illicit drugs are excreted in their native form or as metabolites and enter the aquatic ecosystems, where they are commonly measured in concentrations that might represent a threat to the health status of non-target organisms. Despite the current levels of these molecules are quite low (in the ng/L range), the risk for the environment cannot be neglected. Among these molecules, cocaine (COC) and its main metabolite, the benzoylecgonine (BE) are the most abundant illicit drugs found in surface waters and seawater worldwide. At present the information regarding the toxicity of these molecules, both independently and in mixture, towards marine species is still scant. Thus, the aim of the present study was to investigate the adverse effects induced by 96-hours exposure to an environmental concentration of COC (500 ng/L) and BE (20 ng/L), as well as their mixture (500 ng/L of COC and 20 ng/L of BE) on the Mediterranean mussel *Mytilus galloprovincialis*. After 48 and 96 hours of exposure, the gills and the digestive gland of mussels were isolated and used to apply a suite of oxidative stress biomarkers. In detail, the modulation of oxidative status, including changes in the amount of reactive oxygen species (ROS) and in the activity of antioxidant (SOD, CAT and GPx) and detoxifying (GST) enzymes, and the onset of oxidative damage (i.e., lipid peroxidation) were investigated. Our results showed that both COC and BE induced significant responses both independently and in mixture, suggesting that environmental concentrations of these drugs might affect the health status of marine organisms.

4.09P.16

Data-Derived Predicted No Effect Concentrations for Antibiotics used in Human and Animal Health

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Industry and literature data were compiled in order to establish a database of appropriate predicted no effect concentrations (PNECs) for a variety of antibacterial pharmaceuticals used in human and animal health. A specific focus was placed on Active Pharmaceutical Ingredients (APIs) classified as having antibacterial properties for which blue-green algae (cyanobacteria) studies following OECD 201 or equivalent guidelines were available (or justification for why cyanobacteria are not the most sensitive species and data for the most sensitive species in its place). GLP studies following standard testing guidelines were preferentially used, as well as the EC_{10} in lieu of a NOEC value if a dose-response was observed in the study. In addition, if chronic fish (OECD 210) or chronic daphnid (OECD 211) studies were available, these were also integrated into the dataset. The lowest EC_{10} or NOEC of all species tested was divided by a factor of 10 representing extrapolation to ecosystem effects in order to derive a PNEC value. In cases where multiple EC_{10} or NOEC values were available for the same species for the same API and they were within the one order of magnitude, the geometric mean of the two values was used to determine the PNEC. Over 60 PNECs were ultimately derived, which will help add to the growing body of literature used to develop a framework of minimum environmental expectations for antibiotics discharged from manufacturing operations.

4.09P.17

Investigating Selection for Antimicrobial Resistance by Non-Antibiotic Drugs (NADs)

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Non-antibiotic pharmaceuticals have recently been shown to have antimicrobial effects on representative strains of gut bacteria. Antibiotics and non-antibiotic drugs (NADs) are present in freshwater systems as micropollutants due to wastewater treatment plants discharging into freshwater systems. Antibiotics have been shown to select for resistance at concentrations in the range of those found in the environment, which are considerably lower than the clinical breakpoint and minimal inhibitory concentration, in both simple and complex bacterial communities. This project aims to determine if NADs select for antimicrobial resistance, by investigating effects in a complex bacterial community. Pharmaceuticals from 7 different drug classes have been screened for antimicrobial activity. The most potent compounds will be used in exposure experiments to identify any existing antimicrobial resistance genes that confer cross-resistance to both antibiotics and NADs using a metagenomics approach. In addition, changes in community composition will be investigated to determine if there is enrichment for opportunistic pathogens. This project aims to inform water quality standards and environmental risk assessment of wastewater treatment effluent in order to mitigate the growing problem of environmental antimicrobial resistance.

4.09P.18

Algal and Cyanobacterial Toxicity of Four Antibiotics for Human Clinical Use against Gram-Positive Bacteria

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In environmental waters, the primary producers are potentially being affected by low concentrations of antibiotics, but show significant species-specific differences in sensitivity. This makes it difficult to select the best representative organisms for the regulatory Environmental Risk Assessment (ERA) testing. Due to the large interspecies differences, the European Medicines Agency (EMA) guidance draft of 2018 foresees newly a tailored risk assessment testing course for antibiotics consisting of growth inhibition tests (OECD Testing Guideline (TG) 201, 2011) with two cyanobacterial species (Anabaena flos-aquae and Synechococcus leopoliensis) and one green algal species (Raphidocelis subcapitata). Also a daphnid reproduction test (OECD TG 211, 2012) is still foreseen, while experimental data from the fish are not any more considered necessary. A literature survey performed by the authors did not retrieve full datasets in agreement with the new guidance draft for the majority of antibiotic substances, selected by their use volume. As such overview would be particularly interesting in borderline antibiotic cases, where due to the clinical spectrum no indication for (gram-negative) cyanobacterial toxicity is given, the lacking endpoints of four such antibiotics, i.e. Clindamycin, Flucloxacillin, Linezolid and Metronidazole, were determined experimentally. This poster presents the full data sets of these four antibiotics as a compilation of literature and new experimental data such as considered acceptable for Environmental Risk Assessments (ERA) of Human Medicinal Products (HMP) for marketing authorisation application. On the basis of these datasets the new guideline approach is discussed.

4.09P.20

Challenges in the one-size fits all approach to the Environmental Risk Assessment of pharmaceuticals

E. Nfon, Smithers / Department of Regulatory Affairs

On November 15, 2018, the EMA published a draft revision to the Guideline that remained open to comments from stakeholders for a period of 6 months (for consultation until June 30, 2019). The revision proposes notable changes to the tiered testing approach and environmental risk assessment (ERA) for human pharmaceuticals, more technical detail on the required environmental tests, clarifies the specific requirements for addressing endocrine active substances (EAS) and the assessment of the persistence, bioaccumulation and toxicity of active substances. Furthermore, guidance is also provided on the assessment of indirect exposure pharmaceuticals (secondary poisoning) via the food chain. These changes, it is hoped, will provide a more consistent approach to ERA's by both assessors and applicants. However pharmaceutical research is moving away from mainly small molecules into novel chemistries e.g. antibody drug conjugates (with over 100-150 currently in preclinical stage), PEGylated biopharmaceuticals (over 10 approved and many more are in nonclinical or clinical development), deuterated drugs, nanocrystals technology for pharmaceuticals (nanocrystal drugs). The OECD standard tests are still recommended, guidance although these standard tests were developed for testing industrial chemicals with a focus on traditional toxicological end points including acute effects, organ toxicity, and reproductive toxicity. Many of the new chemistries are not expected to be toxic in the traditional sense and the common tests used in the context of chemical registration could be therefore be unsuitable. This poster will discuss the positives in the revised guideline but also illuminate the shortcomings of the apparent focus on small molecules.

4.09P.21

Science-Based Trigger for the Terrestrial Assessment of Human Pharmaceuticals

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The main route of environmental exposure to human pharmaceuticals is via discharge from waste water treatment plants (WWTP), as a consequence of patient use. The environmental risk assessment, therefore, focuses primarily on the risks to the aquatic environment, utilising a PECsw action limit ($\geq$0.01 µg/L) to trigger testing and assessment. Sewage sludge from WWTPs is often used as fertiliser in an agricultural setting; therefore, there may also be exposure to terrestrial environments for active pharmaceutical ingredients which adsorb significantly to sewage sludge. Current EMA guidance recommends a trigger, based on adsorption, for testing and assessment in the terrestrial compartment, utilising a Koc value in sewage sludge $\geq$10000 (equivalent to Kd $\geq$3700). In the recently updated draft guidance document, the EMA recognise the potential for high concentrations in the WWTP to also drive potential risk in the terrestrial environment by proposing a combined trigger incorporating both PECsw (as a surrogate for concentrations entering WWTPs) and Koc/Kd. However, little scientific evidence was provided to justify why such an approach was required, the level of protection it provides or how many additional terrestrial assessments may be required as a result of the proposed change. Terrestrial toxicity data generated under the current EMA environmental risk assessment guidance has been collated. These data will be analysed and presented to determine the scientific basis for a PECsoil action limit, in line with the current approach to triggering aquatic testing in the EMA guideline. This would allow for consideration of substances present at high concentrations in WWTPs and those with highly adsorptive properties, as well as helping move towards a more harmonised approach for exposure-based triggers for the terrestrial assessment of human and veterinary medicines (where a PECsoil action limit is currently applied). The distribution of existing terrestrial PNECs will be presented to determine whether a PECsoil action limit of ≥0.1 mg/kg would be protective of the risks presented by human pharmaceuticals in the terrestrial environment.

4.09P.22

Evaluation of the EMA trigger for fish BCF testing and secondary poisoning assessment: Evaluation of log D and fish BCF data for several pharmaceuticals

I. Van Ginneken, Janssen Pharmaceutical Companies of Johnson & Johnson; L.A. Constantine, Pfizer, Inc. / PDM; J.G. Tell, Merck & Co., Inc. / Global Safety and the Environment; T. Davidson, Bristol-Myers Squibb / EHS; A.N. Perkins, Eli Lilly and Company; M. Lee, Eli Lilly and Company / Environmental Risk Assessment; S. Maynard, Astrazeneca UK Ltd; A. Häner, F. Hoffmann-La Roche Ltd / Group SHE (LSR); B. Hoeger, Novartis Pharma AG / Health, Safety and Environment; J.J. Ryan, GlaxoSmithKline; P.W. Wilson, Sanofi U.S., Inc. / Health, Safety and Environment; J. Vestel, Merck & Co., Inc. / Global Safety and the Environment; J. Snape, AstraZeneca UK Ltd. / Global Sustainability; N. Burden, NC3Rs

As per the EMA Guideline for Environmental Risk Assessment of Medicinal Products for Human Use, a fish bioconcentration study is triggered in Phase I for pharmaceuticals having a log K_{ow}>4.5 to support the Persistence, Bioaccumulation and Toxicity (PBT) assessment and in Phase II, Tier A for pharmaceuticals having a log K_{ow}>3 to support the secondary poisoning assessment. The recommended protocol for bioaccumulation is OECD Test Guideline 305: Bioaccumulation in Fish, Aqueous and Dietary Exposure. Based on the standard 305 sampling schedule, approximately 200–300 fish per study may be required to determine steady-state (BCF_{ss}) and kinetic (BCF_k) bioconcentration factors following exposure to a low and high concentration of the test substance. The draft revision of the EMA guideline, released for consultation in 2018, maintains this trigger for BCF testing stating that where the resulting BCF is ≥100 L/kg, a secondary poisoning assessment is required. Considering the potential for fish to metabolise and excrete xenobiotics and the number of animals required for a standard BCF test, the BCF values for several pharmaceuticals were evaluated to understand whether existing data support the current log K_{ow} trigger of 3 for BCF testing. In the interest of animal welfare, BCF values were also evaluated to determine if study designs could be limited to a single exposure concentration and still provide a good estimate while reducing the number of fish required. Confirmation of an appropriate, data driven log K_{ow} trigger for BCF testing and a recommendation for evaluating bioaccumulation potential of pharmaceuticals in a single concentration or a low and high concentration will be presented. In addition, the proposed EMA BCF trigger for a secondary poisoning assessment will be evaluated.

4.09P.23

Critical Environmental Concentrations of Pharmaceuticals: Read-Across from Human Therapeutic Concentrations or Mammalian Toxicology Endpoints

A.N. Perkins, Eli Lilly and Company; N. Klüver, Helmholtz centre for environmental research - UFZ / Department of Bioanalytical Ecotoxicology; S. Konradi, German Federal Environment Agency UBA

Active pharmaceutical ingredients (APIs) are data-rich molecules in terms of mammalian safety and human efficacy. However, many pharmaceuticals do not have a corresponding dataset regarding environmental risks. In the absence of ecotoxicology data, leveraging the available mammalian data is one way to prioritize the environmental risk potential of APIs. Human therapeutic plasma concentrations of APIs (HtPC) can be used to predict the critical environmental concentration (CEC) at which pharmacological effects may occur in fish. Used in a similar way, plasma concentrations associated with toxicological endpoints in preclinical mammalian toxicity studies (MtoxPC) may provide a better parameter to predict a CEC that results in toxicological effects in fish. We have used a refined fish plasma model to predict plasma concentrations based on the parameters D_{lipw} or D_{ow} in order to account for ionization state of APIs. Critical environmental concentrations (CECs) were calculated for a suite of APIs based on either HtPC or MtoxPC. The predicted CEC values from these two datasets were compared with the experimental ecotoxicity data (LOECs from chronic fish tests). The approaches are discussed with respect to APIs of differing therapeutic classes and with respect to factors of the preclinical dataset including species, endpoint used (lowest adverse effect level, change in body weight, mortality), and study length.

4.09P.24

Antidepressant citalopram stimulates population growth of the harpacticoid copepod Nitocra spinipes

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Like many pharmaceuticals, antidepressants are designed in such a way that they do not degrade easily. Due to their limited breakdown capabilities, they often enter sewage systems in their active form and may even end up in the environment. Significant concentrations of the commonly prescribed antidepressant citalopram have been measured in freshwater systems in the past. Moreover, recent experiments in our laboratory revealed effects on the life history of the harpacticoid copepod *Nitocra spinipes* caused by exposure to citalopram at concentrations of 100 ng/L and upward. It is, however, unclear how these effects on individuals propagate to the population level.In this study, freshly initialized populations of *N. spinipes* were exposed to citalopram hydrobromide at concentrations of 0 (control), 100, 1000 µg/L (18 populations per treatment). After 1, 3, 5, 6, 7, and 8 weeks, 3 replicate populations per treatment were permanently removed from the setup and preserved in 70% ethanol. All samples were first counted manually under a light microscope (excluding the larval stages which were too small) and subsequently photo-documented using a FlowCam. Manual counts showed no effects on the population abundance at 100 µg/L. At 1000 µg/L, population abundances were slightly reduced, at first, but strongly exceeded the control at weeks 7 and 8. This supposed stimulation effect may be attributed to an increased reproduction rate which had been observed earlier in individual females exposed to citalopram. At the time of abstract submission, the FlowCam pictures are still being processed. They are, however, expected to allow for a more thorough evaluation of the population dynamics, including high-resolution size-distributions of each sample, over time. The results of this study indicate no immediate threat of citalopram to *N. spinipes* at concentrations found in the environment (< 1 µg/L). However, they provide valuable insights into the form and timing of stressor-induced population-level effects on *N. spinipes*.

Environmental Risk Assessment of Polymers (P)

4.10P.1

Development of an exposure assessment framework for polymeric materials

H.C. Brunning, University of York / Environment and Geography; J.B. Sallach, University of York / Environment; A. Boxall, University of York / Department of Environment and Geography

Exposure assessments are key for development of environmental risk assessments, and require data from emissions, structure-activity relationships and experimental fate studies to feed into exposure models, that can be validated directly with data from monitoring studies. This poster will provide recommendations for development of an exposure assessment framework for polymeric materials, as well as how this can be applied to environmental risk assessment. The challenges associated with conducting an exposure assessment for polymeric materials compared with typical chemical pollutants will also be presented. A key difficulty is that in development and analysis of fate and monitoring studies for assessing risk, both an emitted macro-polymer and its degradation products must be accounted for, including micro- and nano-scale particles and chemical products. In order to incorporate a polymer and all of its degradates into an environmental exposure assessment, environmentally relevant degradation studies and extensive analyses of the fate behaviour of both polymer and product are required; however, analytical methods for detection and characterisation of polymeric materials and their degradation products have proven challenging to develop. There is currently a deficiency in well-established techniques for analysis of nano-polymers, and a lack of standardisation and validation of techniques for each stage of the polymer degradation process, limiting our understanding of polymer deterioration and environmental fate. The current techniques available for such analyses will be reviewed in this poster, including spectroscopic techniques such as FTIR and Raman, optical and electron microscopy methods, chromatographic and spectrometric techniques, and tandem combinations. The current limitations of the methodology will also be presented, along with recommendations for further development and adaptation of techniques. The application of these techniques, both in characterising polymeric materials and their fate processes to inform exposure models and in direct field measurements for monitoring studies, will be presented along with recommendations for development of the resulting exposure assessment for use in environmental risk assessments of polymeric materials.

4.10P.2

How to test algal toxicity of polyquaterniums?

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Little is known of cationic polymers and their effect in the environment, due to their exemption of regulatory frameworks and hence their lack of public available data. Literature suggests that water soluble cationic polymers may have adverse effects on aquatic organisms such as algae. Algae are often the most sensitive test organisms and primary producers are of vital importance to maintain a healthy ecosystem. Guideline algal toxicity test such as the OECD 201 are routinely used within regulatory frameworks for risk assessment of chemicals. This guideline was designed with soluble compounds in mind but testing of difficult substances challenge the traditional test setup. Cationic polymers can be inherently difficult to test given their “sticky” nature. Additionally, given their large molecular size, it is unclear if traditional test methods are appropriate for characterizing their hazard potential. This research investigated how to perform algal toxicity tests in accordance with the OECD test guideline and at the same time minimize inter-laboratory variation for cationic polymers. The polymer investigated was polyquaterinum-10, which is often applied in consumer- and house-hold products. While the tests generally followed the OECD 201, a number of factors were varied to identify what could be acceptable to vary between laboratories and what must be kept as standard procedure. Factors like for example test vessels, volume to surface-ratio of test solution and variation of the test were varied. Our findings points towards a number of factors that are important and will influence the toxicity measures in algal toxicity tests. This is crucial knowledge to apply for further research in the polymer area, and should be taken into consideration for developing registration needs of cationic polymers.

4.10P.3

The debate on the future of cationic polymers in REACH and on the EU market

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The discussion about the incorporation of polymers into the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) framework has been ongoing on for years. The current direction is to prioritize some polymers, recognizing those of potential concern for the environment and human health. Cationic polymers fall into this category. REACH operates with the "no data, no market"-principle and collecting of this data is a major challenge for scientists, policymakers and industry. Guidelines, designed for testing in the risk assessment processes, were developed targeting well-defined substances. A well-defined structure is also needed for developing QSAR models. Since polymers vary in size and structure, how should they be prioritized in REACH? Are the existing legal tools applicable to manage potential risks from cationic polymers? This study analyses the stakeholders' insights, collected through the semi-structured interviews resulting in their forecasts for the polymers place in REACH. The purpose of this research is to ensure the communication between the iTAP project (Improved aquatic Testing and Assessment of Cationic Polymers) with the external stakeholders' viewpoints. Stakeholders' recommendations for the cationic polymers testing methods, technical set up and opinions on the 'polymers of low concern concept' provide a picture as to how these polymers are currently assessed in laboratory conditions and how to implement them into the excising REACH framework. This process is by no means complete, however, the call for decisions is now. Policymakers need a scientific input, which currently faces challenges of the available tests' applicability. Legislation ought to remain pragmatic. Premises for the industry cannot hinder the innovation and competitiveness. This paper presents the mutual viewpoints of stakeholders, sources of uncertainty related to the future of decisions and matters considered important for other stakeholders in the same space. The direction taken in the future depends on open dialogue for understanding.

4.10P.4

Additional tests to OECD 303A to refine Risk Assessment for the cationic Polymer PHMB towards surface waters

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PolyHexaMethylene Biguanide hydrochloride (PHMB) is a cationic polymer used as a biocide active substance in a wide range of applications. As a polymer, PHMB meets the definition of UVCB, compound of "Unknown or Variable Composition". PHMB was evaluated as an active substance under the BPR. As a part of the review, an evaluation was performed to assess the risks for surface waters for intended uses leading emissions down to the drain and indirect emissions to the aquatic environment via the discharges of Sewage Treatment Plants (STP). For this purpose, a study according to guideline OECD 303A was performed to study PHMB behaviour in such systems and to provide mandatory data for the environmental risk assessment. In this STP simulation study (with Active Sludge sampled from a municipal STP), an isotopic stock solution of cold and [14C]-radiolabelled PHMB is injected. In this study, the applied radioactivity is distributed as 2-4% in air (CO2), 77-79% in the solid phase (sludge) and 19% in the supernatant (water). These values are inputted in the exposure assessment as the distribution of PHMB in STP compartment. However, the PHMB residues were not characterised for analytical profile and properties. This STP simulation study was therefore repeated with the same experimental conditions. Some analytical works was performed on the outputs to control whether the measured [14C] in the supernatant was actually PHMB or not. For this purpose, a HPLC trace, efficacy test on target organisms (2 bacteria strains) and toxicity test OECD 201 on non-target organisms (algae) were performed. The results of these analytical measurements revealed that measured radioactivity [14C] in the supernatant cannot be considered as being PHMB: HPLC signal was practically null and the trace found different. Besides, the residues present in the supernatant do no longer exhibit any biocide activity and acute toxicity decreased drastically. These results are consistent with the behaviour of PHMB, well known for its property to combine very quickly and very strongly to different substrates, soluble or not. In the case of very complex substance (e.g. UVCB), we conclude that more relevant data are needed to address more realistically the composition that the environmental compartments may be exposed to.

4.10P.5

Probabilistic environmental risk assessment of microplastics in marine and terrestrial habitats

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Microplastics (polymer particles smaller than 5 mm) have only recently become the focus of scientific researchers. Therefore, the environmental risks they could present are still poorly understood. This study aims at assessing the risks of microplastics specific to the marine environment and soils based on peer-reviewed empirical data. A probabilitic approach was used to account for data uncertainty and variability. Peer-reviewed experimental studies were selected to extract 1) environmental concentrations in seawater columns and soils and 2) exotoxicity values for marine and terrestrial organisms. Sets of criteria were applied to select the most relevant studies with acceptable quality. For each compartment, exposure data and their variabilities were combined in cumulative curves to produce probability distributions. Ecotoxicological data were used to build probabilistic species sensitivity distributions (PSSDs). The predicted no-effect concentrations (PNECs) were extracted as the fifth percentiles of these PSSDs, resulting in the final probability distribution for hazard assessment.The risks were then assessed by calculating the risk characterisation ratio (RCR) of each compartment, dividing the probability distribution of measured environmental concentrations (MECs) by that of the PNEC. Globally, the marine MECs ranged from 0 to $1.3 \cdot 10^6$ particles.m^{-3}, with a median value of 1.5 particles.m^{-3}. The most polluted regions were coastal waters (median MEC of 1.6 particles.m^{-3}) and the Pacific Ocean (median MEC of $1.8 \cdot 10^2$ particles.m^{-3}). The most sensitive species was a fish (*O. melastigma*), with a calculated NOEC of $3.9 \cdot 10^6$ particles.m^{-3}. The mean PNEC value was $3.64 \cdot 10^7$ particles.m^{-3}. Worldwide, the RCR spans between $2E \cdot 10^{-14}$ and $4 \cdot 10^{-3}$, with a modal value of $3 \cdot 10^{-4}$. These values are well below one, indicating no risk given current knowledge and concentrations. Urban and agricultural soils are more polluted than other soils, showing the influence of anthropogenic activities. The most sensitive species was a collembolan (*F. candida*). The resulting worldwide RCR probability distribution ranges from $4 \cdot 10^{-11}$ to 26 with a modal value of $2 \cdot 10^{-3}$. Therefore, given the current available data, risks cannot be excluded in soils. The assessment would be improved with more data available, especially in terrestrial habitats. In both compartments, ecotoxicity studies are encouraged that use microplastic sizes and compositions closer to those oserved in the environment.

4.10P.6

PISOX: a novel polyester family with relatively fast biodegradation

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In 2017, about 350 million tons of plastic was produced worldwide, of which at least 7 millions tons yearly accumulates in the environment[1]. The development of fast and slow degradable plastics could be an option to reduce the year-on-year accumulation of plastic in the environment. The biodegradability of a novel polyester, PolyIsosorbide Oxalate (PISOX), and its monomers was investigated in this study employing a respirometer, which measures continuous, real time CO2 production based on the conductance change of a compartmentalized KOH solution. PISOX demonstrates outstanding aerobic biodegradability in soil with (57.6 ± 4.0) % of carbon in the polymers converted to CO2 at 25 °C in 53 days. This is similar to to the final average percentage biodegradation of cellulose (66.8 ± 14.7) %[2],which is comprehensively used as positive reference for polymer biodegradation tests. As a comparison, commercialized compostable polylactic acid (PLA) showed limited biodegradation (less than 10%) in this condition, which is similar to other studies[3][4]. A long lag phase was observed for both PISOX polymer and isosorbide monomer (around 10 days), suggesting that adaption of microorganisms prior to biodegradation is required. Hydrolysis is suggested to be an important additional pathway of polyester degradation. Non-enzymatic hydrolysis of PISOX in aquatic incubations was tracked by 1H NMR spectroscopy to provide more insight into its hydrolytic degradation. The fact that water soluble monomers were not observed until 36 days at room temperature while the CO2 release (resulting from biodegradation of PISOX) started after 10 days, indicated that microorganisms promote PISOX degradation in soils (probably via enzymatic hydrolysis). Considering the high glass transition of PISOX, the relatively readily biodegradability of PISOX makes it an interesting and unique material for applications such as packaging. 2. References

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4.10P.7

Validation of the E-screen assay for toxicity testing of solar disinfected drinking water

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According to a recent report by WHO and UNICEF, 3 in 10 people worldwide, or 2.1 billion, lack access to safe, readily available water at home. SODIS (solar disinfection) is a cost-effective point of use technology recommended by WHO for water disinfection. In its simplest form it involves filling transparent plastic containers, usually 2L PET bottles, with water and exposing them to strong sunlight for 6 hours. Solar radiation and heat combine to disinfect the water. The use of SODIS to produce safe drinking water is practised worldwide and is a particularly valuable tool in regions where water is scarce and sunlight is plentiful. However, exposing plastic vessels to UV and heat runs the risk of leachables getting into the water. Leachables are chemicals which migrate from the plastic material to the water and at certain levels can be toxic to humans. According to the European Chemicals Bureau some of these substances can be carcinogenic, allergenic, mutagenic or endocrine disruptive.*Waterspoutt* (**Water** - **S**ustainable **P**oint-**O**f-**U**se **T**reatment **T**echnologies) is an EU Horizon 2020 funded research project. The aim of the project is to develop a range of large volume (≥20L) sustainable solar disinfection (SODIS) technologies that will provide affordable access to safe drinking water to remote and vulnerable communities throughout Sub-Saharan Africa and other resource-poor countries. The technologies include three different reactors made with different plastic polymers – a solar rainwater reactor made with polymethyl methacrylate (PMMA), a transparent 25L jerrycan made with polyethylene terephthalate (PET) and a transparent bucket made with polypropylene (PP). The E-screen assay, which measures oestrogenicity a type of endocrine disruptor, was validated to test the toxicity of the water produced by the three reactors.The reactors are based in four African countries – Ethiopia, Malawi, South Africa and Uganda. It was necessary to develop a method to facilitate transport of the water samples for toxicity testing at Dublin City University. An extraction protocol was developed to facilitate a sample size up to 2L. Investigations showed that Oasis cartridges performed best in the extraction procedure. The E-screen assay, using MCF-7 BUS cells, showed a dose dependent proliferative response to oestradiol and the inclusion of an oestradiol receptor antagonist confirmed the response as oestrogenic.

4.10P.8

Aquatic Toxicity of Polyquaternium-10 to Daphnia magna

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Polymers are exempt from EU regulation (REACH) based on the assumption that their significant molecular weight does not allow them to penetrate cell membranes and inherently renders them less toxicologically available. Some polymers, like cationic polymers, have been recently recognized as potential polymers of concern and have received increased regulatory attention. This study was focused on obtaining needed regulatory aquatic toxicity data for one class of cationic polymers: polyquaternium-10 (PQ-10). PQ10s are a range of polymeric quaternary ammonium salts of hydroxyethyl cellulose that are reacted with trimethyl ammonium substituted epoxide. These cationic water-soluble polymers, commonly used in personal care and cleaning products, can vary significantly in molecular weight and charge density. It is currently unknown if or how these properties may correlate with toxicity. The aim of this study was to further examine the acute and chronic toxicity of several PQ-10s to the freshwater invertebrate species, *Daphnia magna* through the utilization of the Acute Immobilization Test (OECD 202) and the *Daphnia magna* Reproduction Test (OECD 211). The results were used to quantify the aquatic toxicity of PQ-10 to *D. magna* as well as provide insights into the toxic mechanism PQ10 has on *D. magna* to be considered in a regulatory setting like REACH.

Fate, Effects and Risk Assessment Procedures for Chemicals in Tropical and Neotropical Regions (P)

4.11P.1

SILENT AMAZON: presence and risks of anthropogenic contaminants in the Amazon River

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The Amazon is the largest drainage basin in the world and contains about 40% of the world's remaining tropical rainforest, hosting an enormous diversity of terrestrial and aquatic organisms. Urban areas in the banks of the Amazon River have grown significantly during the last few years, and industrial and agricultural activities have been intensified. Almost all wastewaters produced at urban areas and agricultural fields are being dumped into the Amazon River untreated and may pose risks for freshwater biodiversity. The SILENT AMAZON project aims to provide the first comprehensive dataset on the occurrence and distribution of contaminants in the Amazon River. A monitoring campaign covering more than 1500 km of the Brazilian Amazon River has been carried out. Water, macrophyte and fish samples have been taken to analyse concentrations of pharmaceuticals and life-style compounds, fragrances, persistent organic pollutants, plastics and microplastics, metals and other industrial compounds (PFAS). By the time of this poster presentation, preliminary results of the monitoring campaign will be available. Measured concentrations will be displayed in maps to establish relationships between contamination patterns and anthropogenic land use. Furthermore, a risk assessment will be performed on the basis of available toxicity data to identify chemical compounds and mixtures that are potentially impacting Amazonian aquatic plants, invertebrates and fish. The SILENT AMAZON project is supported by the National Geographic Society and by the IMDEA Water Institute.

4.11P.2

Sargassum contamination by arsenic and chlordecone: fate ashore and in compost

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Massive sargassum beachings occur since 2011 on Caribbean shores. Sargassum involved in such events are S. fluitans and S. natans, two deriving species whom growth is observed in the south of North Atlantic Ocean. Sargassum ashore, piled up on beaches and decayed have to be studied because of the concerning content of pelagic arsenic reported in the literature and the sargassum contamination due to historical pollution in French West Indies due to chlordecone, a persistent organochlorine insecticide used against the banana's weevil Cosmopolites sordidus. The present study aims to state the level of arsenic and chlordecone contamination in intertidal, beached and piled up sargassum in Martinique and Guadeloupe (FWI), integrating the spatial and decay heterogeneities in order to support the decision making. Sargassum contamination by chlordecone is observed only close to contaminated river mouth: contamination heterogeneity is remarkable indifferently to algae decay when sargassum contamination by arsenic, due to phytoaccumulation offshore, is broadly homogeneous before decay then leaks lead rapidly to decrease the concentration into sargassum necromass, questioning the subsequent contamination of coastal environment. Retrieving it from beach translates the issue to pollution and storing places congestion -due to huge quantities beached and also sand co-retrieved from shoreline during shovelling. Sargassum leaks are polluted by micropollutants: Due to arsenic content, even chlordecone contamination, sargassum has to be considered as dangerous wastes and stored in corresponding landfills. Arsenic and chlordecone threats concur to the severe control of sargassum storage even valorisation. The present study assess the local industrial activity of organic amendment as a way of valorisation: arsenic and chlordecone fate under anaerobic and warm environment is unknown and, considering chlordecone, could induce its degradation (Dolfing et al., 2012). Arsenic and chlordecone content in windrow and in lixiviate were monitored to adjudicate. Dolfing et al., 2012. Gibbs Free Energy of Formation of Chlordecone and Potential Degradation… Environ. Sci. & Technol., 2012, 46, 8131-8139.

4.11P.3

Biochemical alterations as indicators of plasticity in Astyanax fasciatus (Teleostei: Characidae) females living polluted reservoirs

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The water quality from reservoirs close to city centers has decreased drastically over the decades due to environmental pollution. The effects of this pollution have been studied mainly in temperate regions, while in the tropical and neotropical regions the studies are scarce. In order to establish the effects of contamination, biochemical biomarkers were evaluated in the liver and muscle of the neotropical species *Astyanax fasciatus* females collected in two reservoirs located at Metropolitan Region of São Paulo: 1) Billings reservoir (Bil), located in a very impacted area, considered polluted, with frequent cyanobacterial blooms and metal concentrations above those recommended by resolution of the Special

Secretariat for the Environment; 2) a reference reservoir, Ponte Nova (PN), located in an environmental protected area. Samplings were carried out throughout one year at the four seasons (summer, autumn, winter and spring). The activity of oxidative stress enzymes, as superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT), were measured in liver, as well as, hepatic metallothionein (MT) and muscular acetylcholinesterase (Ach). Higher activity of hepatic CAT was observed in animals from Bill reservoir during summer and autumn than animals from PN reservoir. Enzymatic activities of GR and SOD did not change between environments, as well as, hepatic MT and muscular Ach. It is well established that some metals can alter the activity of oxidative stress enzymes, such as CAT, which in general presents elevated hepatic activity due to its function of biosynthesis and detoxification. The results presented in this study indicate that the females living in the Bill reservoir showed a few stress indicators and only during summer and autumn, however, SOD, which is the enzyme that provides the substrate for CAT functioning, did not increase, suggesting that hydrogen peroxide, the substrate used by CAT is providing from other processes. Regarding the polluted condition of Bill reservoir and the low number of living species, the results suggest that *A. fasciatus* present a higher degree of plasticity to live in such low water quality condition without triggering all the antioxidant stress responses.

4.11P.4

Evaluation of river waters in the development of Rhamdia quelen: biomarker approach.

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Excessive application of agrochemical compounds in South America has been justified by the growing demand for high agricultural productivities. However, the presence of pesticides residues in the environment is resulting in contamination of aquatic systems and promoting physiological alterations in non-target organisms. Therefore, the objective of this study was to carry out assessments of the health status of eggs and larvae of Rhamdia quelen fish under development in river waters, whose microbasins are in locations with different land use. One hundred and twenty litres of water were collected from two rivers belonging to the Baixo Iguaçu basin, in western Paraná - Brazil: Tormenta river, whose microbasin has intense agricultural use, containing only 26.5% of its original area with forest matrix, referred to herein as the impacted river (IMP); and Manoel Gomes River, located in the National Park of Iguazu, containing 96% of the forest matrix and considered here as a reference river (REF). The water collected was conditioned in 48 boilers, with 450mL, with 6 repetitions for each experimental time (12h, 24h, 48h and 72h), for each river. A total of 200 Rhamdia quelen eggs was added to each boiler, and organisms were taken at each sampling time for the analysis of energetic metabolism (e.g. LDH, PFK, HK, MDH), antioxidant defense (e.g. CAT) and detoxification enzymatic biomarkers (e.g. GST) and cell damage biomarker (LPO). It was possible to detect that the waters from the IMP, promoted differences in the health status of the developing animals when compared to the REF. There was higher activity of detoxification enzyme (GST) among the organisms from IMP. Within the first hours (12 and 24h) of development, a general activation of the pathways in the conduction of ATP (both aerobic and anaerobic) formation was observed. However, there was an increase on lipoperoxidation reactions in developing individuals in the IMP at all sampling times, when compared to the REF. The existence of cellular damage and therefore likely loss of fitness of individuals in these populations over time reinforces the vulnerability of these populations, in locations where agriculture activity is more intensive.

4.11P.5

Crab cells and hepatopancreas as biological model for assays in marine ecotoxicology: applications as metal bioaccumulator and oxidative stress quantifier

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Crustaceans are frequently used as bioindicators, providing data for variation in the availability of contaminants through cumulative concentrations in their body, tissues, organs, and cells. Contact between the crustaceans and the environment occurs through the gills and hepatopancreas (HP). HP can be indicating the stress level (lipid peroxidation – LPO) when it is submitted under lower and higher concentrations of metals, also it can indicate levels of stress when the animal is submitted for long time to air exposure with good sensitivity. The HP is composed of four cell types: embryonic cells (E), resorptive cells (R), fibrillar cells (F), and blister cells (B), which these can be used for studies for intracellular bioaccumulation and transport of metals and molecules. In this work we described the versatility of the HP (organ and cells) of different crab species *Ucides cordatus* (Brazil), *Menippe frontalis* (Peru), *Platyxanthus orbignyi* (Peru), *Callinectes danae* (Brazil) and *Callinectes sapidus* (United States) as model for indicate the contamination levels for metals and air exposure during acclimation process by means of LPO in HP, metal transport and bioaccumulation in HP cells by fluorescence, and as model membrane for fluorescent probes in HP nucleus. In *U. cordatus, C. danae. M. frontalis* and *P. orbignyi* were found high levels of LPO in hepatopancreas, when present in high contamination environments and air exposure. Besides, hepatopancreatic cells of U. cordatus displayed intracellular cadmium and iron compounds transport when they were submitted to concentrations of 0 - 1 mmol/L, keeping their viability intact. The nuclei of hepatopancreatic cells of *C. danae* and *C. sapidus* were detected using new fluorescent probes from natural sources. HP identifies stress levels due to the acclimatization process, air exposure, and metal contamination by the LPO biomarker. In HP cells, it is possible to identify the transport of toxic metals and quantify intracellular concentrations. Also, HP nuclei have shown versatility as a model for labeling assays for new fluorescent molecules. Crab HP is an effective model to indicate, through physiological responses, minimal changes in its surrounding environment. On the other hand, Hepatopancreas is a rich source of different cell models, with high viability, which can be used for membrane and intracellular physiological processes studies.

4.11P.6

The influence of expansion of sugarcane cultivation on macrophyte-associated macroinvertebrates of Salvinia auriculata

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The demand for renewable fuel production has grown exponentially in Brazil. Based on incentives to the sugarcane ethanol industry, there has been expansion of sugarcane cultivation areas, previously occupied by pasture, since 2005. This study evaluated the effects of this landscape change on the macroinvertebrate communities associated with the roots of the aquatic floating macrophyte *Salvinia auriculata*. Conducted in an experimental farm in Brotas (SP/Brazil), with 15 plots of 2,500 m^2, each containing a mesocosms of 9 m^3 in the center, the study had the following treatments in quintuple i) natural pasture (NT); ii) intensive pasture (IP) (NPK + herbicide (DMA806BR (ai 2,4D)), and iii) sugarcane (CA) (NPK + pesticides (Regent800WG (ai fipronil) and the herbicide). These treatments were based on standard management of the crop. 4 collects were performed: (Dec,18) 60d after NPK and 1 week after insecticide application; (Feb,19) 30d after insecticide and 1week after herbicide; (Mar,19) 30 and 90d after both pesticides, and, (Apr,19) to monitoring. Macrophytes were collects with a 210µm mesh net in a delimited area of 30 cm. A total of 12.165 individuals were collected and identified as belonging to 9 orders (Oligochaeta: 9.670; Odonata: 1.363; Diptera: 890; Hemiptera: 154; Ephemeroptera: 38; Coleoptera: 34; Gastropoda: 9; Lepidoptera: 6 and Hirundinea: 1), covering 32 families and/or functional feeding groups. Traditional ecological index (richness, Shannon and Simpson) did not show any statistical significance effects. EPT (Ephemeroptera-Plecoptera-Trichoptera) only occurred in NT and IP treatments and were solely made up of individuals of the Ephemeroptera order. The number of collector-gatherers (Oligochaeta) and predators (Odonata) were larger mainly in the CA treatments. This is discussed to be related with the measured fipronil concentration of 35 ng/L in this treatment as an indirect effect resulting from fipronil toxicity to sensitive orders like shredders. In NT and IP, the macroinvertebrate communities were similar. In conclusion, the macrophyte-associated macroinvertebrates were affected in CA treatments due to the presence of fipronil. The presence of 2,4D in CA and IP, however, did not show any direct or indirect effect on this fauna.

4.11P.7

The effects of landscape change (natural pasture to sugarcane) to the aquatic macrophyte Salvinia auriculata

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This study analyzed the effects of landscape change from natural pasture to sugarcane cultivation on the aquatic macrophyte *S. auriculata*. The study area is located in an experimental farm in Brotas (SP/Brazil), with 15 plots of 2,500 m^2, each containing a mesocosms of 9 m^3 in the center. The treatments (quintuple) are i) natural pasture (NT); ii) intensive pasture (IP) with NPK in the soil and herbicide DMA806BR (ai 2,4D), and iii) sugarcane (CA) with NPK and the pesticides Regent800WG (ai fipronil) and the herbicide, following the standard management of the crop. Macrophytes were collected from the mesocosms during several moments around fertilizer and pesticide applications. After the NPK and insecticide applications, no effects were identified in biomass (BM) and root length (RL) when the IP and CA treatments were compared with NT. The N and P quantification in macrophytes showed the highest levels of N (10% and 18%) and P (2 and 2.1 μg/g) in IP and CA as compared to 8% N and 1.7 μg P/g in NT, which is likely to be related with the NPK application. After the herbicide application, macrophytes in IP and CA showed a decreases in BM (122 and 92g), RL (9.7 and 10.4 cm) and N (4.8 and 5.4%) as comparing to NT (147 g and 8.1 %). No effects on P were denoted (1.1~1.7 μg/g). After 30d following its application, the 2,4D concentrations decreased in IP (113 ng/L) and CA (27 ng/L), which was accompanied with an increase in BM (IP 141/CA 134 g) and RL (IP 9.8/CA 10 cm). Even though the NT treatment showed a continuous increase of BM (up to 194 g), the final RL (24 cm) was below normal values reported in literature, which may be due to the low concentration of P in water (1.3 μg/L). The levels of N (3.6~3.8%) and P (1.3~1.9 μg/g) were similar in NT, IP and CA. The results shows that the presence of fipronil in water following an application of its recommended dose do not affect *S. auriculata*. Such an application of 2.4-D on the other hand, affected the BM and RL. NPK aided in maintaining N and P levels in water in the IP and CA treatments as compared to NT that not received NPK.

4.11P.8

Environmental risk assessment of fipronil and 2,4-D during the process of conversion of pasture to the sugarcane crops

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The dependence on fossil fuels and their damage to the environment resulted in the search for renewable energy sources and to reduce the emission of greenhouse gases. In this context, sugarcane proved to be an alternative in the production of biofuels (ethanol), in addition to its diverse applications. One of the factors that help to maintain several crops, including sugarcane, is the use of pesticides. Their use in the cultivation of sugarcane helps to maintain and grow the plantation, but its continuous application can result in harmful effects on the environment due to the contamination of water resources, soil, air, sediments and biota. This project evaluated the concentrations of fipronil and 2,4-D in mesocosms systems during the management of sugarcane in a pasture conversion area in sugarcane fields. Water samples from mesocosms located in control area as well as pasture and sugarcane crop areas were sampling in the farm located in the city of Brotas (São Paulo - Brazil). The target pesticides were extracted using 500 mL of sample by solid phase extraction (SPE) using HLB Oasis 500 mg cartridges (Waters). The quantification of the pesticides and their metabolites was performed Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) using chromatography Agilent 1200 coupled to a Triple Quadrupole mass spectrometer with an electrospray ionization (ESI) (Agilent 6410). The initial sampling in Brotas indicated that the mesocosms presented trace concentrations of 2,4-D and fipronil, possibly due to the residues of application in the agricultural regions around the experimental farm. After application of pesticides in the sugarcane crop and intensive pasture areas, the increase of pesticide concentrations in the mesocosms was observed, which was not observed in the control mesocosms (unmodified pasture). The average concentrations of 2,4-D and fipronil after application (1400 and 37 ng L^{-1}) were higher than water quality criteria (WQC) (200 e 12 ng L^{-1}, respectively), indicating potential risk to aquatic life. However, months after application the residual concentrations of pesticides in the sugarcane crop and intensive pasture areas are similar to those observed in the control mesocosms. Fapesp 2015/18790-3.

4.11P.9

Dietary intake of copper: a multi-biomarker approach in the neotropical carnivorous fish Hoplias malabaricus

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Dietary exposure is now recognized as a major pathway of metal poisoning. In light of this, the aim of this study was to evaluate the toxicity of Cu in the carnivorous neotropical fish trahira, *Hoplias malabaricus,* by dietary exposure. For this purpose, 20 individuals of *H. malabaricus* were divided into two experimental groups (n = 10): control group (CTR) and Cu group (EXP). Fish were fed every 96 h with live juvenile specimens of *Astyanax altiparanae,* corresponding approximately to 2% of the total trahira's weight per day. The EXP group was supplied with preys exposed to a nominal concentration of 20 μg L^{-1} of Cu, for 96 h, in a static system. The CTR group was fed with preys maintained in dechlorinated water only. After 10 doses, fish were anesthetized with benzocaine and blood samples were collected for the comet assay and hematological analyzes (hemoglobin content (Hb), hematocrit (Hct) and red blood cells number (RBC)) and, besides that, the concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, and glucose were determined in plasma. Following, gills, digestive tract, liver, kidney, brain, muscle, and bone were removed. Cu concentration was measured in all tissues. The activity of the biotransformation enzyme Glutathione S-transferase (GST), the antioxidant enzymes Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT) were analyzed in gills and liver as well as the quantification of reduced Glutathione (GSH) and the protein carbonyl content (PCC). Na^+/K^+-ATPase, H^+-ATPase, Ca^{2+}-ATPase and carbonic anhydrase (AC) activities were evaluated in gills and the acetylcholinesterase (AChE) activity in brain and muscle. Fish from EXP group showed an increase of Cu concentration in brain and blood, a decrease on hematological parameters (Hb and RBC) and higher DNA damage score in erythrocytes. Hypocalcemia was observed in the EXP group as well as an increase of Na^+ concentration. There were no significant changes on the other measured ions and glucose. In the gills of EXP group, a decrease in SOD, GPx, and CAT activities was observed. On the other hand, no significant alterations were noted on the ATPases and AC activities, GSH and PCC content or GST activity. Of all biomarkers evaluated in liver, only a decrease in SOD activity and PCC content was observed. No changes were observed on the AChE activity in brain and muscle. This study points out the trophic pathway as an important route of contamination to *H. malabaricus.*

4.11P.10

Effect of aluminum at environmental concentrations on the mitochondrial activity of Astyanax altiparanae spermatozoa.

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Alteration of the aquatic environment due to anthropogenic actions, such as the release of industrial, domestic and agricultural effluents, can negatively affect the local biodiversity, interfering with reproduction and other physiological processes of animals. Among these substances, aluminum (Al), a metal that is found in neotropical and tropical water bodies, can be highlighted, due to the adverse effects caused in the organisms and considering that it has no apparent role in animal physiology. However, there is no enough information about the effects of Al on sperm parameters that may interfere with the fertile potential of gametes. Therefore, this study aimed to evaluate the mitochondrial activity of *Astyanax altiparanae* sperm when submitted to different Al concentrations at different exposure times. Sexually mature *A. altiparanae* males were induced to spermiation with pituitary carp extract (5mg.kg^{-1}) and the semen collected was incubated in Beltsville Thawing Solution in five different concentrations of Al: 0mg.L^{-1}, 0.05mg.L^{-1}, 0.1mg.L^{-1}, 0.3mg.L^{-1} and 0.5mg.L^{-1} for 50sec, 10min and 30min. For the analysis of mitochondrial activity, 10μL of incubated semen solution added with 20μL of the probe (rhodamine/propidium iodide) were incubated for 10min. The final solution was deposited on slides for analysis under the fluorescence microscope (200x). A total of 100 sperm cells were analysed, in which midpiece fluorescent indicated mitochondrial activity and absence of fluorescence indicated no mitochondrial activity. Data were expressed as mean ± standard error of the mean and analysed by two-way ANOVA followed by the Holm-Sidak test ($P < 0.05$). By comparing different exposure times it was observed that the percentage of active mitochondria remained constant over 30min in the absence of Al in the solution ($P > 0.05$). When 0.05mg.L^{-1} of this metal was added, it was possible to verify a decrease of active cells between the 50s and 30min. In all other Al concentrations tested (0.1mg.L^{-1}, 0.3mg.L^{-1} and 0.5mg.L^{-1}) there was a reduction in mitochondrial activity as incubation time increased ($P < 0.05$), highlighting that at 0.5mg.L^{-1} there was a more expressive decrease (49.7%) after 30min. Therefore, it was possible to observe that the cellular viability, concerning mitochondrial activity decreases over time and Al increasing concentration. These results may support future studies on the reproductive physiology of *A. altiparanae*.

4.11P.11

Interaction of cadmium (Cd) and reproductive physiology in Callinectes danae in environments with different pollution levels

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Toxic metals, such as cadmium (Cd), are released into the environment as a result of mining processes and industries. These metals may adversely affect aquatic life, especially the physiology of animals, altering their reproductive cycle, as well as maturation of the reproductive organs and release / inhibition of reproductive and molting hormones, especially in crustaceans. Therefore, the objective of this study was to evaluate the influence of Cd on reproduction and molting processes in the crab *Callinectes danae*, considering animals from environments with different levels of pollution and Cd exposure. Females (N = 30 / region) were collected in three regions with different levels of contamination: uncontaminated (Region 1), low levels of contamination (Region 2) and contaminated region (Region 3). The animals were analyzed in the field (N = 10) and, after laboratory acclimatization, the remaining animals were exposed to 0.5 and 2 ppm of Cd. Organs related to metal accumulation were analyzed by atomic absorption and histology. Reproductive hormones, as crustacean hyperglycemic *hormone* (CHH) and molt-inhibiting *hormone (*MIH), were quantified by qPCR in eyestalks. 17β-estradiol (E2) was analyzed by ELISA and oxidative stress was analyzed by lipoperoxidation (LPX), comparing different environments and levels of pollution and exposure. The results did not show morphological alterations in hepatopancreatic cells of animals present in contaminated environments. In uncontaminated areas, CHH and MIH increased ($P< 0.05$) in relation to Cd exposure crabs, but decreased ($P< 0.05$) animals from contaminated areas. E2 levels decreased in crabs from uncontaminated areas ($P< 0.05$ and 0.01), comparing different levels of Cd exposure; and hepatopancreas, gills and ovaries increased LPX in relation to contaminated areas. In contaminated areas, gills and ovaries increased LPX ($P< 0.001$). In conclusion, animals from contaminated environments showed physiological differences compared to animals from uncontaminated environments. This fact could evidence a possible physiological adjust in organisms present in contaminated environments, which present lower LPX, lower levels of E2, CHH and MIH. On the contrary, animals from uncontaminated environments presented higher rates of LPX and higher levels of CHH and MIH, while E2 levels were reduced during Cd exposure, thus establishing a relationship between higher Cd levels and reduced reproductive process.

4.11P.12

Effects on gill surfaces of Poecilia reticulata promoted by iron oxide NPs associated glyphosate-based herbicide and glyphosate pattern

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Recent studies in nanotechnology demonstrates that iron oxide nanoparticles (IONPs) are used in nanomedicine and nanoremediation. However, its ecotoxicological effect to aquatic organisms remains unclear and the effects of the association with glyphosate are not completely described. In this study, the citrate-functionalized IONPs effect in environment shows relevant iron concentration (0.3mg L-1). Its association with GLY and Glyphosate-Based Herbicide (GBH) were investigated in Poecilia reticulata females by SEM and histologic approaches based on qualitative analysis and histopathological damage index for gills after a long-term exposure. The animals were collected at 7 and 21 days of exposure. The TEM results demonstrated rounded and crystalline IONPs with average size of 2,74nm ± 0,84nm. The DLS and ELS analysis showed that the IONPs has low hydrodynamic diameter and negative surface charge in ultrapure water (66.6 ± 0,17 r.nm; -55.4 ± 7.4 mV) compared to reconstituted water (21.4 ± 0.39 r.nm; 19.5 ± 6.5 mV). For guppies, the IONPs and IONPs+GLY induced high histopathological damage indexes associated with epithelial and circulatory disorders and peeling of squamous epithelial cells. The microridges were disorganized and secretions of mucous cells were altered indicating an increase of toxicity according to the exposure time. Besides, guppies exposed to IONPs+GLY or GBH showed higher numbers of chloride cells compared to the unexposed ones. The results indicated that toxicity estimated by qualitative parameters and histopathological index are important biomarkers to indicate the animal health and the environmental impact of IONPs+agrotoxic. Therefore, the datas of this study confirms that the guppy P. reticulata is an adequate model to test the toxicity of IONPs alone or associated and to indicate the quality of water after nanoremediation.

4.11P.13

Cell and tissue impacts of the surfactant polyoxyethylene amine (POEA) on behavior and histopathological parameters of liver, gills and testis in guppy Poecilia reticulata

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The glyphosate herbicides in several formulations contains the surfactant polyoxyethylene amine (POEA), in Brazil many types of them were recently approved to be widely used in agriculture and could consequently cause huge impacts in aquatic ecosystems, that will be observed in the next few years. In the present study, an integrated approach examining animal behavior, gonadosomatic and hepatosomatic indexs and histopathological parameters were applied to evaluate acute effects of POEA (24h to 96h). Males of Poecilia reticulata were exposed to 0,0µg.L-1 (Control); 1.0µg.L-1 (POEA 1); 1,5µg.L-1 (POEA 2); 2,0µg.L-1 (POEA 3) and 2,5 µg.L-1 (POEA 4), in triplicate. After 24 h and 96h exposure samples of testis, gills and liver were taken. Compared with the control group, liver of fish exposed to POEA 2 and POEA 3 showed increased cellular and tissue damages. In addition, fish of the group POEA 4 showed an increase in the natatory and respiratory activity and after alterations in proprioceptors, they died. Fishes exposed to POEA 3 presented increased hepatic activity of cells and alterations in testis. The histomorphometric assay used for analyzing damage in gills, hepatic and gonads cells indicated the toxicity of the surfactant at all concentrations tested. If taken together, these results show that POEA can cause effects in various levels, such as vasodilation, exudate reactions, hemolysis and alterations in cytoplasmic structure in all tissues studied. Therefore, is indicated that the rural properties take care of the waters of rivers and lakes, analysing them in order to pay attention to water quality and environmental health, so the nocive impacts are minimized.

4.11P.14

Non-steroidal anti-inflammatory drugs acting as endocrine disruptors in Astyanax altiparanae (Teleostei: Characidae) tissue explants

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Many pharmaceutical compounds act as endocrine disruptors, interfering in various physiological processes, such as reproduction. In fish, the integration of abiotic environmental factors and favorable physiological conditions result in hypothalamic secretion of the gonadotropin releasing hormone (GnRH), a neurohormone that stimulates adenohypophysis gonadotropic cells to produce two gonadotropins, follicle stimulating hormone (Fsh) and luteinizing hormone (Lh), which in turn act on the gonads stimulating the production of gonadal steroids (estradiol, testosterone and 11ketotestosterone). Several studies have been conducted evaluating the ability of non-steroidal anti-inflammatory (NSAIDs) drugs to modulate endocrine function of reproduction-related tissues and cells, however most of them were carried out in temperate region. Therefore, the aim of this study was to investigate the regulation of gene expression of gonadotropins and steroids levels, *in vitro*, using explant models of pituitary and testis, at different concentrations of the NSAIDs diclofenac (DCF) and ibuprofen (IBU). The explants were maintained for 12 hours in Leibovitz's (L-15) medium in different treatments: a positive control – L-15 with 100 nM GnRH (for pituitary) and L-15 with 10 IU/mL hCG (for testis); the medium with positive control; and different concentrations of DCF and IBU (0.1, 1, 10, 100 and 1000 $ng.L^{-1}$). After this period, each pituitary was collected and the analysis of the gene expression was performed, evaluating the relative level of *fshb* and *lhb* mRNA. Testis medium was collected to measure steroid levels. Both, DCF and IBU were able to decrease the gene expression of *fshb* and *lhb* in the exposed pituitary at all concentrations tested, even at very low concentrations, such as 0.1 ng.L-1 and without a dose-response ratio, evidencing an inhibitory response of gonadotropin synthesis in response to GnRH. In testis, DCF did not alter estradiol, testosterone and 11ketotestosterone levels, while IBU was able to alter the levels of these hormones even at low concentrations, showing an inhibitory response of testis to hCG. These results demonstrate a serious a worrying problem that can interfere with fish reproduction, considering that even the smallest dose can be considered an endocrine disruptor compound (EDC), mainly ibuprofen, capable of interfering in gametogenesis and spawning in this neotropical species.

4.11P.15

Diclofenac and caffeine induce hepatic oxidative stress in Astyanax altiparanae (Teleostei: Characidae) in acute and subchronic exposures

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The number of studies about aquatic contamination by pharmacological compounds and their toxic effects on the aquatic fauna has been increasing. Among them, we highlight diclofenac (DCF) and caffeine (CAF) that induce oxidative stress in fish, but most of the studies have been carried out in temperate regions. Therefore, the aim of this study was to evaluate the effects of DCF and CAF, isolated and combined, on the antioxidant system in the liver of adult males of *Astyanax altiparanae*, a neotropical teleost, in acute (96 h) and subchronic (14 d) exposures. Four groups were established: control (CTR), DCF, CAF and a mixture (DCF+CAF). For the acute bioassay, 10% of the LC50-96 h value for this species was used: 3.08 $mg.L^{-1}$ for DCF, 9.59 $mg.L^{-1}$ for CAF, and the same concentrations were combined in the mixture. For the subchronic bioassay, the following concentrations were used: 0.4 $\mu g.L^{-1}$ for DCF, 27.5 $\mu g.L^{-1}$ for CAF, and the same concentrations when combined. The activity of oxidative stress enzymes as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione S-transferase (GST) were measured, as well as lipid peroxidation. In the acute bioassay, DCF treatment decreased the activity of SOD and GPx

compared to the CTR, whereas CAF treatment decreased CAT activity. Regarding the mixture (DCF+CAF) treatment, SOD and GST activity decreased when compared to CTR fish. In the subchronic bioassay, DCF alone inhibited GPx, CAT and GST activities, and DCF+CAF triggered the inhibition of SOD, CAT and GST activity. Unlike the acute bioassay, in the subchronic exposure CAF did not change the antioxidant enzymes, which may indicate that at low concentration, this compound has no toxicity in this species during 14 d. Although in both, acute and subchronic bioassays, DCF decreased the activity of most antioxidant enzymes analyzed, this profile was not enough to generate lipid peroxidation during subchronic bioassay. In the acute bioassay, fish exposed to DCF showed greater damage compared to CTR. In conclusion, DCF separated or combined with CAF, induced hepatic oxidative stress in *A. altiparanae* exposed to high concentrations for short periods as well as low concentrations for a longer time. However, CAF seems to affect CAT at high concentration even for short periods. Therefore, under subchronic exposure even with lower enzymatic activities there was no hepatic lipoperoxidation, while under acute exposure a hepatic damage was observed.

4.11P.16

Consideration of nectarivorous birds in wildlife risk assessments

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In subtropical and tropical climate zones as well as South Africa, nectarivorous birds are commonly distributed. Well-known passerine families are e.g. hummingbirds (Trochilidae), sunbirds (Nectariniidae) and sugarbirds (Promeropidae). In agricultural regions where crops exhibit a flowering phase before harvest, nectarivorous birds may be attracted to feed on nectar of crop flowers, depending on adjacent non-crop habitats, anatomy of mandibles and tongue of the species and flower characteristics. It is unclear whether nectarivorous birds need special considerations for feeding guilt related risk assessments for registration of plant protection products. Some countries where nectarivorous birds occur, partially follow the principles of the EU risk assessment approach (EFSA 2009, EFSA Journal 2009, 7(12):1438), where a screening step assessment is first conducted which serves to eliminate all active substances with low risk from further evaluation. As it considers over-conservative assumptions for fictive 'indicator species' for each crop assuming worst case diet (i.e. exhibiting highest residues), this step is protective for all species possibly entering and foraging in treated areas. For substances which do not pass the screening step (EFSA 2009), a Tier 1 assessment follows. This considers representative 'generic focal species' (still no real species), depending on crop, growth stage at type of product application, diet guild & foraging strata of the generic focal species. We present points to consider on whether and how nectarivorous bird species might be included in higher tier risk assessments regarding (I) species' distribution and habitat needs, including their relationship to floral abundance and morphology, (II) species' physiology, activity and energetic expenditure, and compared to species already included in the EU risk assessment guidance (EFSA 2009), (III) species' diet, i.e. being partially or exclusive nectarivorous, and year-around or restricted to specific time periods, and (IV) crop flower characteristics being avian nectar feeder compatible or not. The poster shall highlight diversity of nectarivorous birds, difficulties in trying to compile a robust risk assessment for specific representative species, and at the same time recommend a pragmatic way forward how one may cover this diet guild following the EFSA (2009) risk assessment approach.

Multidisciplinary Efforts to Advance Knowledge on Environmental Risk Assessment of Chemicals for Amphibians and Reptiles (P)

4.12P.1

Guidance for developing amphibian population models for ecological risk assessment

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Despite widespread acceptance of the utility of population modeling and advocacy of this approach for a more ecologically relevant perspective, it is not routinely incorporated in ecological risk assessments (ERA). A systematic framework for situation-specific model development is one of the major challenges to broadly adopting population models in ERA. As risk assessors confront the multitude of species and chemicals requiring evaluation, an adaptable stepwise guide for model parameterization would facilitate this process. Additional guidance on interpretation of model output and evaluating uncertainty would further contribute to establishing consensus on good modeling practices. We build on previous work that created a framework and decision guide for developing population models for ERA by focusing on data types, model structure, and extrinsic stressors relevant to anuran amphibians. Anurans have a unique life cycle with varying habitat requirements and high phenotypic plasticity. These species belong to the amphibian class, which is facing global population decline in large part due to anthropogenic stressors, including chemicals. We synthesize information from databases and literature relevant to amphibian risks to identify traits that influence exposure likelihood, inherent sensitivity, population vulnerability, and environmental constraints. We link these concerns with relevant population modeling methods and structure in order to evaluate pesticide effects with appropriate scale and parameterization. A standardized population modeling approach, with additional guidance for anuran ERA, offers an example method for quantifying population risks and evaluating long-term impacts of chemical stressors to populations.

4.12P.2

Risk mitigation measures for terrestrial life stages of amphibians in agricultural areas

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At present, there are no management measures to protect amphibians under the approval of plant protection products (PPP). Amphibians need to be considered in the risk assessment, but no guidance is available for how to proceed. As a development of a guidance document for amphibians requires an international effort and most likely several years, Switzerland is seeking options to decrease the exposure of amphibians through PPP within the framework of its national action plan. Management measures to protect amphibians from PPP have already been put together based on a literature review in the scientific opinion by EFSA (2018) and were further supplemented for the small-scale structures in Switzerland. Furthermore, already implemented measures to improve biodiversity were assessed with regards to their benefits for amphibians in agricultural areas. The potential measures were evaluated with regards to their practicability and costs for the farmers, their influence on the production and their verifiability by the cantons. Suitable measures for protection of terrestrial life stages of amphibians were identified that can be implemented easily and without substantial impact on agricultural production. As these management options are outside of the remit of the PPP authorization and rely therefore on the voluntary implementation by the farmers, the raising of awareness of the farmers is considered to be an important aspect for success. The pressure on farmers through constraints for environmental conservation has increased considerably in the last years, therefore the information regarding possible risks and potential management options must be processed in a comprehensible and sensible way.

4.12P.3

Initial juvenile activity of common frogs (Rana temporaria) around natal ponds in an agricultural landscape

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Amphibians currently represent the most endangered vertebrate group in Europe and exposure to plant protection products (PPPs) has been highlighted as a potential threat. The initial juvenile movement phase, when individuals leave their natal ponds, is the first terrestrial life-stage at risk of direct PPP exposure, especially when juveniles enter cultivated fields. Therefore, we investigated the activity via captures of newly-metamorphosed amphibians around their natal ponds, to define the spatial scale and field attractiveness. In June-July 2019, juveniles were trapped using drift fences around four ponds located within the intensively used agricultural area of Münsterland in north-west Germany. We analysed trapping effort at distances of 1-5 m, 10 m, 20 m and 30 m from the ponds in different habitats. Overall, we had 1406 captures of metamorphosed common frogs (*Rana temporaria*). With increasing distances to ponds captures per meter of fence decreased. The first results showed the lowest measured activity was in cereal fields, when compared to copses and grassland. This indicates a lower risk because lacking of attractiveness of cereal fields for newly-metamorphosed frogs. Enhancing habitat quality and attractiveness around ponds with structures such as copses could mitigate potential risk from direct PPP exposure through diverting juvenile amphibians from cultivated fields. The study was carried out within the project AmphiMove founded by the IVA.

4.12P.4

Reproductive Health of Common Toads (Bufo bufo) in Viticultural Landscapes

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Amphibian populations are declining worldwide at alarming rates. Among the large variety of contributing stressors, chemical pollutants like pesticides have been identified as a major factor for this decline. Pesticides enter amphibian breeding ponds in the agricultural landscape through numerous exposure pathways such as runoff, drainage and spray drift. In addition, juvenile and adult amphibians can be exposed during their migration from winter habitats to breeding ponds and vice versa. This biphasic exposure can have a negative impact on reproduction of amphibian populations. Therefore, we investigated the reproduction of common toads (*Bufo bufo*) in the viticultural landscape of Palatinate in Southwest Germany compared to less or unpolluted habitats. In a semi-field study we captured reproductively active common toads during their migration to five breeding ponds of different contamination level. One toad pair at a time was placed in a 40 x 65 x 60 cm net cage equipped with a coat hanger as spawning substrate. The cages were placed on the bank of the study ponds and the toad pair was kept until the female spawned. To determine the degree of fertility, the females were weighed before and after spawning and the spawning lines were photographed to evaluate the number of eggs using image analysis software. In order to investigate the fertilisation rate and the survival of the offspring, a total of 90 randomly selected eggs were taken from each spawning line. They were reared in a climate chamber at 21°C and a 16:8 h day:night rhythm in aquariums filled with FETAX medium until Gosner stage (GS) 25 was reached. In addition, the fertilization rate was identified by the incipient development of the eggs. After embryo hatching, the proportion of animals that developed from embryo to tadpole was recorded. Moreover, the developmental rate was determined by recording the time until GS25 was reached. After reaching GS25, the tadpoles were photographed to measure the length of the larvae. Tadpoles originating from breeding ponds with higher contamination levels showed reduced developmental rates and body length compared to larvae from less contaminated ponds. Moreover, females of contaminated ponds laid more eggs than females of less contaminated ponds. This increased quantity of eggs could suggest an evolutionary adaption of toads to pesticide exposure to balance the reduced body conditions of the offspring.

4.12P.5

Can polymethylmethacrylate (PMMA) nanoplastics affect Xenopus laevis eggs and tadpole's development?

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Polymethylmethacrylate (PMMA) is amongst the least studied plastic polymers within the research field of ecotoxicology. Yet, its production increased almost 20% in the last years, which will certainly result in its increased release into freshwater environments and consequent degradation until reaching nanodimensions (nanoplastics -NPLs). The majority of amphibian species depend on freshwater habitats to carry out reproduction, which makes them a potential target of PMMA toxic effects. Young life stages (eggs and tadpoles) may become in contact with PMMA-NPLs present in the water column but also with NPLs that have deposited in upper layers of the sediments. Thus, NPLs have presented themselves in recent years as another threat to the long list that already existed responsible for the decline of this particular group, but about which little is known. Accordingly, this work aimed at assessing the effects of PMMA-NPLs (1, 100 and 1000 µg/L) to aquatic early life stages of the anuran species Xenopus laevis. For that two types of assay were carried out: i) a 96-h assay to assess the toxicity of increased [PMMA-NPLs] on the survival and developmental stage of the eggs; and, ii) a 48-h assay to assess the toxicity of increased [PMMA-NPLs] in the feeding rate (FR) and morphometry (body weight and length) of the tadpoles. At the end of the 96-h assay with embryos, the occurrence of mortality and malformations (%) was below 10% for the control and all tested PMMA-NPLs concentrations. However, exposure to PMMA-NPLs significantly delayed the developmental stage of the larvae in all tested concentrations ($p< 0.05$; Dunn's). Control larvae were on average in Nieuwkoop and Faber (NF) stage 37, while at PMMA-NPLs exposure larvae were on average at NF stage 36. In the 48-h feeding assay with the tadpoles, no differences were found in the FR of the tadpoles exposed to any of the PMMA-NPLs concentrations comparatively to those exposed in the control. Regarding the morphometric endpoints measured at the end of the feeding assay, results showed no significant effects of PMMA-NPLs in the weight of tadpoles. However, at the concentration 1000 µg PMMA/L, more than half of the organisms presented severe injuries in the abdomen zone (e.g. externalization of the bowel). Amphibians are good indicators of environmental quality on both land and water. Other NP polymers are known to induce injury and dead on young life stages of amphibians; however, no data has been generated so far relatively to PMMA. These results provide important baseline information to further understand the toxic potential of PMMA to amphibian's, and to contribute to more adequate risk assessment frameworks aiming their protection. Despite the lack of knowledge in terms of environmental levels of NPLs, it is expected that sediments will be a final destination and thus, more available to be incorporated by organisms that feed on this compartment.

4.12P.6

Amphibian's eggs jelly coat : structure, functions and implication for amphibians ecotoxicology.

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According to the International Union for Conservation of Nature (IUCN), amphibians are suffering a massive decline. With 40% of endangered species, this taxon represents one of the most threatened within vertebrates. Among the factors that affect the worldwide amphibian population, habitat loss/degradation is the most important. Within this degradation the anthropogenic pollution is listed as an important factor. More specifically, the use of pesticides and antibiotics in farming is suspected to degrade aquatic conditions. Indeed, these molecules are carried by surface and sub-surface runoff as well as drainage into aquatic systems. They were detected in high concentrations in surface waters including ponds. Freshwater ecosystems are of crucial importance for the amphibians' life cycle. Indeed, embryonic/larval development and reproduction, two major phases of the population development, take place in these specific environments. Exposure to chemicals during these critical phases is thus likely to have strong adverse impacts on amphibians' population dynamic. During the last decades, due to a general awareness within the ecological risk assessment community, researchers paid more intention to amphibians' ecotoxicology and several standard protocols have been developed such as LAGDA (OECD, 2015), AMA (OECD, 2009). In these three protocols, the exposure to the tested molecules starts during the early embryonic stages and it is suggested to dejelly the eggs before the exposition. The jelly coat is a glycoproteic envelope deposited around the eggs throughout their movement along the female oviduct. On this poster, we review the knowledge gathered by the scientific community regarding the jelly coat protecting amphibian's eggs. This envelope has a structure that differs between amphibian's orders and that can be different even at an interspecific level. This structure gives the envelope different functions involved, for example, in the reproduction and the protection of the eggs. Interestingly, this envelope was shown to influence the ecotoxicity of the different molecules. This review suggests that more attention should be paid to the implication of this jelly coat to amphibians' ecotoxicology and that dejellying should be considered for more ecological relevance.

Real Risks in Real Soils: Linking Exposure and Effects in a Multifaceted World (P)

4.13P.1

Consequences of the test design on the Minimum Detectable Difference in earthworm field tests

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Minimum Detectable Differences (MDDs) are currently used to assess whether an assessment of direct effects is possible for a sufficient number of potentially sensitive populations in aquatic mesocosm studies. A similar approach for terrestrial field tests, e.g. earthworm field tests, is under discussion. The aim of this project was to explore by means of real data sets, how the MDDs are affected by the study design and which MDDs could be achieved in practice. Abundance data sets of six earthworm field tests, all conducted by the same contract laboratory in the same geographical region in Germany, were analysed by means of the multiple t-tests by Williams and Dunnett. NOECs and related MDDs were calculated for each combination of taxon and sampling date. The preliminary results are as follows: In total, eight earthworm species were found in the studies. Only for A. calliginosa and L. terrestris (and the total number of earthworms) the MDD was per average below 50 %. To expect MDDs < 50 % usually more than 10 animals should be present in the pooled samples per control plot. By means of a regression of the coefficient of variation from control abundances, the total standard deviation was estimated for different classes of abundance in the control and the MDD was calculated for different experimental designs. Using four samples per plot and four plots for the control and each of three test concentrations was considered as the standard scenario for comparison. Changing the number of test concentrations had only minor effect on the %MDD while increasing the number of samples per plot or the number of plots reduced the expected MDD. Approximately 100 animals on average per control plot are necessary to reach an MDD below 35 % in the standard design. For a possible design of 8 control plots and 6 plots per test concentration, it is expected that 15 worms per control plot sample allow to reach per average an MDD <= 35%. By using six instead of four samples per plot, the number of worms in the pooled sample per plot is increased per average by 50 % which would further increase the chance to get low MDDs. However, this design would require approximately doubling the number of samples to be conducted compared to the standard design and thus, would significantly increase the costs. Reducing the MDD down to 10 % seems not to be practical considering the natural variability found in studies.

4.13P.2

The Selection and Co-Selection of Antimicrobial Resistance by Non-

Antibiotic Drugs and Plant Protection Products

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Various studies have shown that non-antibiotic compounds including metals, biocides and detergents co-select for clinically relevant antimicrobial resistance (AMR) genes. Agricultural activities require the direct application of plant protection products (PPPs) such as herbicides and insecticides to soil. PPPs are applied alongside antibiotics and manure which may contain resistant bacteria, antibiotic residues and other co-selective agents. This cocktail of chemicals may result in the development and spread of resistance genes between environmental bacteria and clinically relevant bacteria. The selection pressure exerted by the mixture of these chemicals and how they impact AMR and microbial diversity in terrestrial environments is a novel research area and will be addressed in this presentation. The effects of PPPs on microbial growth were observed using the SELECT method developed by Murray *et al.* (2019; under review). In this method, complex bacterial communities are exposed to ten PPPs (including herbicides, pesticides etc.) and selective concentrations identified by a significant reduction in community growth. Results from these experiments determined the concentration at which long term evolution experiments were carried out. Metagenomic analyses on exposed communities have been conducted to determine if PPPs co-select for known antibiotic resistance genes.

4.13P.3

Carbon content and soil organisms - Trick or Peat?

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Soil organisms are one of the most important but so far also one of the most overlooked groups within Terrestrial Systems and need to come into the focus of research on possible adverse effects of synthetic compounds in the environment. This aspect is also currently supported and under intense discussion with the recently published “Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms” (EFSA Journal 2017;15(2):4690) by EFSA. The current standard for Laboratory testing foresees a set of species to be tested within artificial created soil consisting of certain amounts of components such as e.g. sand and peat. Especially on the latter one, the amount of available carbon being present in the respective soil can make a difference. This aspect is also well known in other soil relevant test designs, such as e.g. non-target arthropods (Heise et al, 2005)[1]. The herewith presented results shall give a detailed view on impact of different soils and their respective compartments chosen for testing, and how this may influence results from specific compounds. Further on it may be matter of discussion if the, as per guideline stated, artificially created soil represent a robust framework for detecting possible effects on soil organisms, or if a rather natural and / or regional relevant soil should be used for more relevant and realistic exposure setups and effect determinations. Lastly, the use of natural / naturally more relevant soils as extended approach over lower stage artificial soil setups may be a good and realistic add-on to the current tiered approach testing, for which especially the soil organism group lacks of intermediate test designs as binding level between lower stage laboratory studies and high-end field setups. 1: Heise, Julia; Heimbach, Udo; Schrader, Stefan: Influence of Soil Organic Carbon on Acute and Chronic Toxicity of Plant Protection Products to *Poecilus cupreus* (Coleoptera, Carabidae) Larvae; Journal of Soils and Sediments; 10.1065/jss2004.10.118; 2005

4.13P.4

PROSOIL - Protection of soil organisms: Development of environmental toxicity criteria for soil organisms in the context of classification & labeling of substances and PBT assessment

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Currently, the identification of chemical substances as hazardous for the environment is clearly focused on aquatic toxicity criteria. Neither the globally harmonized classification and labelling system (GHS) and the European classification, labelling and packaging regulation (CLP), nor toxicity criteria for the PBT assessment consider soil toxicity data. This shortcoming in terms of protecting terrestrial organisms leads to the main scientific question of this project: Are soil organisms adequately protected by current hazard-based, provisions solely based on aquatic data? Indeed, it is highly questionable if there is a scientific rationale to assume that aquatic hazard criteria could protect all compartments of an ecosystem.The recent past has shown, that initiatives to strengthen the policies in the area of soil protection had little success. In 2006, a strategy for soil protection was communicated by the European Commission, an effort that should finally lead to the establishment of a European soil framework directive. This proposal has been withdrawn in 2014. Besides, the idea to integrate soil-specific classification criteria for CLP/GHS or PBT assessment have already been discussed at a European and UN level. However, so far without regulatory success.The present project will focus on a data-driven approach to transparently and reproducibly deduce protective trigger values for soil hazard criteria. In order to develop such criteria, the project aims to create a unified database, based upon input data from a large variety of regulatory data inventories, preferably making use of long-term (chronic) indicators of toxicity. Furthermore, substances and their test concentrations shall be identified that are currently not classified as potentially hazardous for the environment according to GHS/CLP, and those that are not identified as PBT but possibly should be, based on the deduced terrestrial toxicity criteria. We assume that substances which demonstrate a need for soil toxicity criteria might be difficult to test in aquatic test systems or were previously classified as P and/or B under the provisions for an PBT assessment, with the (aquatic) T-criterion not fulfilled. Finally, on this basis, the discussion on the regulatory implementation of soil toxicity criteria at EU level shall be initiated again.

4.13P.5

Filling the lack of effect data of Novaluron-based pesticides to non-target in-soil organisms

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Insecticide formulations with novaluron as active ingredient (a.i.) are important products to control pest damages and widely used in soybean crops. Novaluron is an insect growth regulator that belongs to the benzoylureas group and inhibits the enzyme that catalyses the polymerization of chitin, especially on Lepidoptera. To improve the effectiveness of insecticides, novaluron is often mixed with the a.i. methomyl in the same product. Methomyl is a carbamate insecticide with broad-spectrum in anticholinesterase activity. Both pesticides with one or two a.i. may be applied in the field alone or within a tank mixture. Therefore, the effect of these products to non-target organisms is crucial not only to allow prediction of their impact on terrestrial systems when these products are applied alone, but also to allow the estimation of joint toxicity of mixtures (through mixture toxicity models like concentration addition) when these products are applied in tank mixtures. Given the lack of data in the literature on effect data of the commercial products Rimon® 100 EC (10 g.L^{-1} novaluron) and Voraz® (440 g.L^{-1} methomyl + 35 g.L^{-1} novaluron) to the collembolans *Folsomia candida* and to the predatory mite *Hypoaspis aculeifer*, the present study aimed to investigate the toxicity of these two products to these two non-target species. Laboratory reproduction tests with *F. candida* and *H. aculeifer* were performed following the guidelines ISO 11267 and OECD 226, respectively, and using soils with increasing concentrations of the commercial products taking into consideration the recommended doses used in soybean crops. Standard artificial soil with 5% of organic matter was used as test substrate. Results showed that *H. aculeifer* was the least sensitive species with an EC_{50} of 17.56 mg a.i. kg^{-1} to Rimon® and 1.54 mg a.i. kg^{-1} to Voraz®, while *F. candida* presented an EC_{50} of 1.28 mg a.i. kg^{-1} to Rimon® and 0.03 mg a.i. kg^{-1} to Voraz (almost 10 times lower than the recommended dose). Moreover, taking into consideration the effect data from literature for methomyl acting alone, the present results showed that the resulting toxicity of the commercial products with more than one a.i. can be underestimated when using the concentration addition model. The data obtained reinforce the need of assessing the joint effect of both a.i. to take into consideration possible interactions between substances.

Soils as Sinks for Plastics: Analysis, Transport and Effects of Nano- and Microplastics in Terrestrial Environments (P)

4.14P.1

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 most cases, the focus is on plastic pollution of aquatic ecosystems, whereas the pollution of soil ecosystems (including drainage water) is often neglected. In the "iMulch" project we will analyse the effect of agricultural mulch films on organisms and soil ecosystems. Soil samples from fields with different field crops and known frequent prior use of mulch films will be analysed in order to determine the proportions of mulch film fragments in the real soil. The tests include both petroleum-based and bio-based, biodegradable plastic foils. The consortium develops a methodology that will enable the sampling and detection of micro- and macroplastics in soils and drainage waters, using Raman spectroscopy, FTIR spectroscopy and TED-GC-MS. Further, the aging of the mulch films will be analysed in laboratory and mesocosm tests. The degradation of the mulch films will be additionally monitored in a laboratory scale sewage treatment plant. Experiments will cover the adsorption and desorption of heavy metals and pesticides on microplastic fragments of the mulch film. The transport and fate of the mulch-polymers in soils using ^{14}C marked polymers in outdoor lysimeter experiments will be investigated. Experiments examining effects of the mulch fragments on organisms and the degradation of the film fragments in soils via microorganism will be conducted. Additionally, another approach deals with the "upcycling" of mulch films by bacteria. During this process plastic fragments are degraded in the laboratory by microorganisms and converted into new plastic molecules. Such molecules will then be returned to the value chain and thus increase the recyclable portion of the mulch films. Furthermore, a life cycle assessment will be carried out to measure the environmental compatibility of conventional and biodegradable films. Finally, avoidance and substitution strategies are derived from the results with the aim of reducing plastic film fragments in the environment. First results of the field soil sample measurements and the ad- and desorption experiments will be presented.

4.14P.2

Transport of nano- and microplastic through unsaturated porous media from sewage sludge application

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Wastewater treatment plants have been identified as important hubs of plastic from urban areas to the environment. During wastewater treatment, small particulate plastic, down to the nanometer scale, is removed from the effluent but accumulated in the excess sludge. The reuse of sludge as fertilizer in agricultural practices can lead to accumulation of plastic in the soil. The extent of such accumulation and subsequent particle mobility is largely unknown, in part due to difficulties in detecting plastic in complex matrices. To this end, in this study, nanoplastic particles and microplastic fibers were synthesized with a passive inorganic tracer, palladium and indium respectively, to aid in faster and more quantitative analysis using ICP-MS. The metal-doped plastic was spiked into a pilot scale wastewater treatment plant, where the excess sludge was treated by anaerobic digestion and dewatered, ensuring realistic associations between sludge and plastic. The resulting sludge cake was affixed on top of an unsaturated porous medium column consisting of glass beads to assess: i) the release of particulate plastic (nanoplastic, microplastic fibers) from the sludge, and ii) the accumulation and mobility of plastic and organic matter through the column (analogous to a soil). Pristine nanoplastic particles and a conservative tracer were used as benchmarks for transport through the column. A low detachment from the sludge and reduced mobility through the column was found for microplastic fibers (> 95 % retention) due to size limited transport. However, a co-transport behavior between the mobile organic fraction and nanoplastic particles was observed, with 50% of both retained in the column. These results contribute to the understanding of the fate of particulate plastics after being released to the environment through sludge application, helping to assess the associated environmental risks of particle mobility and percolation, particularly for nanoplastics. Accordingly, accumulation and mobility of various forms of particulate plastic should be more explicitly considered in future sludge reuse policy.

4.14P.3

Using metal-doped plastics to understand soil organism interactions with nanoplastics and microfibers and their potential to bioaccumulate

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Terrestrial environments are subject to extensive pollution by plastics, and are likely to act as long-term sinks for microplastic debris, based on the slow degradation of plastics. However, the fate and behaviour of plastics in terrestrial ecosystems, including their fate in soil organisms, has yet to be fully investigated. Soil organisms will interact with plastics in terrestrial systems, and it has been demonstrated that micro- and nano-scale plastics may adversely affect terrestrial organisms like earthworms, but until recently quantifying micro- and nano-plastic fate in organisms, and their potential for bioaccumulation, has been analytically challenging. The advent of metal-doped micro- and nano-plastics for use in environmental fate studies has opened up the potential for tracing these small-scale plastics in complex matrices, thus allowing their fate to be more accurately quantified. The aim of this study was to investigate the use of palladium (Pd)-doped nanoplastics (NPs) and indium (In)-doped microfibers (MFs) for quantifying the uptake and elimination of these plastics by the earthworm, *Lumbricus terrestris*. In the first experiment, earthworms were exposed for seven days to a range of concentrations of NPs and MFs spiked in soil (nominally 50, 500 and 5000 mg plastic/kg). Accumulation was assessed by measuring the Pd or In concentrations, respectively, in the earthworms. A second experiment was conducted to assess the uptake and elimination of NPs by earthworms over a longer exposure period. Earthworms were exposed to a single concentration of Pd-doped NPs in soil for 21 days, with earthworms sampled at 3, 9, 15 and 21 days of exposure. After the 21-day uptake period, remaining earthworms were transferred to uncontaminated soil for a 21-day elimination period, where earthworms were sampled after 1, 3, and 21 days of elimination. All earthworms (both in uptake and elimination phases) were allowed to void their gut contents for 24 hours after sampling and were analysed for whole body Pd concentrations. The accumulation profiles, particularly over the longer bioaccumulation study, will uncover the potential for earthworms to accumulate NPs in their tissues, as well as the residence time of plastic in their guts following exposure to NPs. This will also give insights into the potential for trophic transfer of NPs in terrestrial food chains.

4.14P.4

Detecting microplastic ingestion by terrestrial arthropods and other invertebrates via Calcofluor White counterstaining

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The potential threats microplastic pollution pose to soil biota necessitate novel methods to detect microplastic ingestion by soil animals. Fluorescent staining with Nile Red dye has shown promise for distinguishing microplastics from inorganic and some biological material, but may be insufficient for separating them from invertebrate remains, even after chemical digestion. Here we report on the development and validation of a novel fluorescent counterstaining technique for detection of microplastics within terrestrial invertebrate biomass and fecal material. We used both laser scanning confocal microscopy and fluorescence stereomicroscopy to image filters containing ground arthropod, earthworm, or gastropod biomass mixed with plastics and stained with Calcofluor White, a dye specific to chitin and cellulose, in addition to Nile Red. Under laser scanning confocal microscopy, arthropod biomass appears blue and purple in contrast to the greens, yellows, and reds of many common plastic polymers. Earthworm and gastropod biomass was stained only by Nile Red, but could often still be distinguished from known plastic particles under the confocal. Using a much cheaper stereomicroscope fitted with a fluorescence adapter, it is still possible to distinguished between arthropod biomass stained by both Calcofluor White and Nile Red and plastics stained by Nile Red alone by alternating excitation lights. Autofluorescence of digested earthworm and gastropod biomass under UV excitation light made it distinguishable from plastic under fluorescence stereomicroscopy via the same technique. Additional laboratory tests found the method able to detect polyethylene and polyvinyl chloride microplastics ingested by living arthropods (*Zophobas morio*), and in field validation, we observed microplastics in earthworm and millipede fecal material and millipede biomass. This method may be valuable for assessing microplastic contamination in soil biota.

4.14P.5

Ecotoxicity of mulch films

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Mulch films are regularly applied in agricultural farming all over the world due to their positive effects on humidity, temperature and limitation of weed growth. They are intentionally brought into the environment and prone of rapidly forming plastic particles upon degradation, facilitated by their low thickness. Most films consist of polyethylene or similar petroleum-based polymers, whose fate and effects on the soil ecosystem have not been assessed thoroughly. Furthermore, new mulch film products consisting of bio-based materials, such as polylactic acid, are introduced to the market without sufficient ecotoxicological assessment. Main goal of the iMulch project is to compare conventional petroleum-based and bio-based mulch films regarding their potential impact on the soil environment and derive recommendations for a sustainable and ecological use of mulch films. The first part of this project focusses on the question whether the mulch films themselves release toxic substances and exhibit relevant modes of actions. Targeted *in vitro* tests are applied but also *in vivo* acute and reproductive toxicity tests and biomarker for soil fauna. Earthworms and collembola have been used as representative soil biota in various studies and will thus be investigated also in this study. These experiments are targeted in the near future of the iMulch project and first results will be presented at the SETAC Europe annual meeting 2020. Physical degradation products of mulch films, namely microparticles, might influence various soil parameters as well as affect soil fauna by internal damage, movement restraint or release of adsorbed chemicals, described as Trojan-Horse effect. Typical candidates for such chemicals are pesticides, which are regularly applied on agricultural fields and to some extent remain in the soil. Sorption of pesticides on microparticles of both bio- and petroleum-based and their joint effects are investigated later in the iMulch project.

4.14P.6

Are polystyrene nanoplastics harmful to soil organisms? - Evaluating behaviour, survival, reproduction and biochemical markers on Enchytraeus crypticus

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There is an increasing concern about the effects of nanoplastics (NPls) from the degradation of plastic in the environment, to non-target organisms. However, until now, there is a limited and controversial information about the toxicity of NPls, especially to terrestrial organisms. The aim of this study was to identify and understand the effects of polystyrene NPls (mean diameter of 42 nm) to the model organism Enchytraeus crypticus, using survival, reproduction, avoidance behaviour and biochemical markers as endpoints. The tested concentrations were: 0.015, 1.5, 15, 150, 300, 450 and 600 mg NPls/kg LUFA 2.2 soil. Survival and reproduction were assessed after 21d of exposure; avoidance behaviour after 2d, and biochemical markers â_ activities of catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST) and acetylcholinesterase (AChE), as well as, the levels of lipid peroxidation (LPO) – were assessed after 3, 7 and 14d of exposure. NPls did not have significant effect in terms of survival and reproduction of E. crypticus. The organisms significantly avoided the NPls contaminated soil at 0.015 mg/kg. The avoidance behaviour decreased with the NPls concentration increase. NPls caused no significant effect in CAT and GR activities, and LPO levels. However, 1.5 mg/kg NPls increased the GST activity at 3d. Despite the lack of observed effect of NPls on E. crypticus survival and reproduction, an activation of stress/biotransformation responses occurred. This activation appears to be enough to avoid oxidative damage, since no effect on the LPO levels was found. Concerning AChE, 300 mg/kg NPls decreased its activity at 3d but increased at 7d. The AChE activity decrease may explain the observed lack of avoidance. This study shows the importance of performing a multi-endpoint approach: the phenotypic endpoints were not altered, although oxidative stress and neurotransmission impairment have been found after short-term exposure to NPls and could have longer term impacts. Keywords: plastics; terrestrial invertebrates; toxicity; multi-biomarker approach.

4.14P.7

Exposure to micronized polyethylene terephthalate microplastics (PET-µPs) affected the growth of the Giant snail Achatina reticulata

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Several monitoring studies have demonstrated that microplastics (µPs) contamination critically affects both aquatic and terrestrial ecosystems worldwide. Despite the ubiquitaian presence of µPs, their toxicity has been mainly investigted on aquatic organisms, while the there is a dearth of information concerning the effects caused on terrestrial species. Thus, the aim of this work was to investigate the adverse effects induced by the exposure to polyethylene terephthalate (PET), a plastic polymer commonly used in food packaging and found in the environment, on the Giant snail *Achatina reticulata*. Snails were exposed via diet for 45 days to two doses (1% and 10 % of µPs on the weight of the administered food) of micronized PET microplastics (PET-µPs). Effects of PET-µPs administration were assessed at biochemical, tissue and individual level. Every other day, snails were weighted, and the length of the shell was measured in order to calculate their grow rate. At the end of the exposure, snails were sacrificed in order to perform histological analyses to check for ingestion/egestion of PET-µPs, as well as tissue damage of the digestive system. Moreover, digestive gland was isolated to assess the modulation of the oxidative status, in terms of amount of reactive oxygen species (ROS), the activity of antioxidant (SOD, CAT and GPx) and detoxifying (GST) enzyme, as well as the onset of oxidative damage in terms of lipid peroxidation (LPO) and DNA fragmentation. The exposure to PET-µPs did not affect the oxidative status and did not induce oxidative damage in the digestive gland isolated from the giant snails. However, snails exposed to both the PET-µPs doses grew more in weight compared to controls, while only snails exposed to the higher dose grew more in length than controls. Our results show that PET-µPs can affect the health status of terrestrial species, suggesting the necessity to investigate their potential adverse effects at different levels of the ecological hierarchy in order to shed light on their real ecological risk.

Wastewater and Sludge Reuse in a Circular Economy: Benefits and Risks (P)

4.15P.1

Impact of organic fertilizers reuse in groundwater quality: dissemination of antibiotics and antibiotic resistance genes

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Pollution of groundwater by antibiotic residues is mainly caused by the usage of organic fertilizers in agriculture, such as livestock waste, or the impact of human sources. Livestock waste can be an important reservoir of antibiotics and bacteria carrying antibiotic resistance genes (ARGs) due to the large amounts of pharmaceuticals used in animal husbandry. In this study, eleven natural springs, located in an area with intensive livestock activity and representing four distinct hydrogeological settings, were sampled in three different campaigns. Natural springs are of interest because they are undisturbed locations where flow paths outcrop and, consequently, they can provide information about the transport of emerging contaminants according to the nearby land use. Therefore, they are surrogate sampling points that characterize the pollution levels that will reach the whole groundwater system. Natural springs were selected based on their nitrate concentrations, attributed to livestock waste fertilization. Samples were collected and analysed for hydro-chemical, isotopic, and antibiotic concentrations. ARGs conferring resistance to the detected antibiotic classes were also monitored as a response of the groundwater microbiome to antibiotics. All sampled natural springs presented agricultural influence as pointed out by nitrate concentrations, usually larger than 100 mg/L. Identified antibiotics were tetracycline, doxycycline, chlortetracycline, oxytetracycline, sulfamethoxazole and sulfamethazine, at concentrations ranging from ng/L to low µg/L, while other analysed antibiotics (up to 25) remained non-detected. For ARGs, sul1 and sul2 (conferring resistance to sulfonamides), tetW (resistance to tetracyclines) and intI1, a proxy for anthropogenic pollution, were detected in most of the samples. Sul1 was the most abundant ARG, with absolute copy numbers ranging from 4x10^2 to 5.6x10^6 gene copies/L water, while tetW was only present in some locations, with copy numbers up to 1.2x10^5 gene copies/L water. Seasonal sampling pointed out a large variability of occurrences and concentrations of antibiotics. This variability is associated to both hydrological factors and reactive transport processes. Our results reveal the difficulties to assess spring water quality for public uses, as well as the implementation of efficient monitoring strategies. [Funded by project H2020-MSCA-IF RESOURCE (ID: 750104) and project PACE-IMPACT, CGL2017-87216-C4-4-R].

4.15P.2

Occurrence of antibiotics and resistance selection risk assessment in selected Kenyan wastewaters, surface waters and sediments.

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Antibiotics are micro pollutants whose continuous flow into the hydrological cycles, especially through wastewater treatment plants, mediate antibiotic resistance in environmental bacteria. Environmental antibiotic concentrations may exceed minimum inhibitory concentrations (MIC), but also lower levels can affect the ecosystem. Here, we investigated the occurrence levels of selected antibiotics in four wastewater treatment plants (WWTP's) and the corresponding rivers to which they discharge. The concentrations were compared to the proposed compound-specific predicted no effect concentration (PNEC) for resistance selection values, above of which antimicrobial resistance evolution is likely to occur. The concentrations of doxycycline (DOX), amoxicillin (AMO), sulfamethoxazole (SMX), trimethoprim (TMP), ciprofloxacin (CIP) and norfloxacin (NOR) within the influents, effluents, surface waters and river sediments ranged between 0.2 to 49.3 μgL^{-1}, 0.1 to 21.4 μgL^{-1}, < 0.1 to 56.6 μgL^{-1} and 1.8 to 47.4 μgkg^{-1} respectively. Surface waters around WWTP1 showed two to five times higher measured concentrations for CIP, NOR and SMX as compared to the effluent concentrations an indicator for indirect loading into water bodies from decentralized sanitation. The risk quotient (RQ) for bacterial resistance selection in effluent and surface water ranged between <0.1 to 53. The risk could be higher in river sediments based on the higher measured concentrations. Generally, there exists medium to high risk of resistance selection for multiple genera of pathogenic microorganism within the respective environmental compartments.Key words: Antibiotics, Antimicrobial resistance, risk assessment

4.15P.3
Host range of antibiotic resistance genes in influent, effluent and dried sludge of a wastewater treatment plant
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Antibiotic resistance is an emerging concern at the global scale. According to the World Health Organization, in the absence of concrete actions to tackle this problem, a post-antibiotic era will occur. Wastewater treatment plants collect wastewaters from households, hospitals and industries, containing both antibiotics and antibiotic resistant bacteria. During the treatment, bacteria with different origins are mixed and can interact. For these reasons, wastewater treatment plants have been indicated as possible reservoirs of antibiotic resistance genes and as putative hotspots for their horizontal gene transfer, as well as a source of dissemination of antibiotic resistance to the environment. Based on previous studies, the abundance of antibiotic resistance genes is reduced during the wastewater treatment process, mainly because the overall quantity of bacteria is lowered. However, some genes seem to be enriched in effluent waters and dried sludge compared to the influent. Also, the taxonomy of the hosts carrying the antibiotic resistance genes might change as a result of horizontal gene transfer events. Our aim was to analyse and compare the host range of six antibiotic resistance genes in influent water, effluent water and dried sludge collected from the Viikinmäki wastewater treatment plant in Helsinki, Finland. Emulsion, Paired Isolation and Concatenation PCR (epicPCR) was used to investigate the host range of the antibiotic resistance genes *blaIMP*, *blaNDM*, *ermB*, *ermF*, *sul1* and *strB*. EpicPCR is a method that can link a gene of interest to the 16S rRNA gene from the genome of the host bacterium, without any cultivation step. All the examined genes were detected both in abundant and in rare taxa. By applying epicPCR, we could identify discrepancies in the host range of the target antibiotic resistance genes in influent water, effluent water and dried sludge. In several cases, in effluent water and dried sludge antibiotic resistance genes were carried by taxa that were not hosting them in the influent. This opens the possibility of horizontal gene transfer events in the wastewater treatment plant. In effluent water and dried sludge, the antibiotic resistance genes were also detected in genera that include pathogenic species, such as *Arcobacter* and *Acinetobacter*, implying that wastewater treatment plants can potentially disseminate antibiotic resistant pathogens to the environment.

4.15P.4
Recovering phosphate in waste water treatment plants while dealing with pathogens and pharmaceutical residues, including antibiotics
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The phosphate mineral struvite can be reclaimed from sludge produced in sewage treatment plants (WWTP).. Phosphate is an important nutrient for crops but supply from mining is finite. Alternative sources are therefore being investigated, for example recovering phosphate in the form of struvite from sewage sludge, which always contains a substantial amount of phosphate. The extraction of struvite also lowers maintenance costs in the sewage treatment plant, as it reduces the formation of crystals in the installation. Several Dutch WWTP are now producing struvite routinely. Because of the possible presence of pathogens and pharmaceutical residues in the sludge, it cannot be stated in advance that the recovered struviet is clean and safe for all uses. Currently, there are few indications that the utilization of struvite would lead to increased risks for public health or the environment. However, this conclusion is based on just a few measurements while some hazards heave been identified in the open literature. For each specific concern, an inventory was made of existing knowledge on occurrence in waste water and possible risks. For pharmaceuticals, a prioritization approach wil be presented, based on prescription and use volumes, combined with measurements on residues in WWTP influent and previous work from the literature. For antibiotics the same approach was followed. This resulted in a list of substances as proxies for the possible presence of e.g. painkillers, hormones and anti-epileptics in struvite. These lists were used by the water treatment boards to define a list of pharmaceuticals for a survey program.For pathogens, an inventory was made of occurrence of four main groups: bacteria, viruses, protozoans and worm eggs. Based on measurements in municipal waste water and some in struvite itself, an assessment was made. Concerns on pathogens focus on spore-forming bacteria (e.g. Clostridia) and it was suggested to perform a challenge test (spiking the incoming sludge) to demonstrate effective reduction in the struvite process.

4.15P.5
Use of biochar from municipal sewage sludge (biocoke) for recultivation of disturbed and contaminated soils
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Despite of many valuable properties of municipal sewage sludge (MSS), such as high content biogenic elements (carbon, phosphorus, potassium etc), no adequate recycling technique was yet found for the second biggest urban waste stream. Present days the MSS management in Europe expands from incineration to composting, landfilling and field spreading. The main goal of this presentation is to discuss our results on the use of a biocoke (BC – produced from MSS) for recultivation of disturbed and contaminated soils. A company "Aktivil" developed an approach to produce the BC from the MSS through a slow pyrolysis process in a proprietary multi-hearth reactor. In the first series of experiments, pot trials of the biochar as a soil conditioner were performed with two types of poor soils collected from tinned spots: loamy grey forest soil (GF) and sandy alluvial meadow soil (AM). The experimental data indicated that the soil amendment with the BC resulted in the increase of water holding capacity on pH of the soils and content of available phosphorus and potassium, and increased the total harvested green mass of ryegrass increased by 30-32%, and root mass – by 54-120% compared to unamended control. Meantime, concentrations of mobile forms of Zn and Pb only slightly increased in the amended soils compared to control, but their concentrations in the green mass and roots practically remained the same in all pots. In the second series, the BC was used instead activated carbon for the adsorptive bioremediation of contaminated soils. First, it was shown that the BC amendment may significantly accelerate bioremediation of an Arctic soil contaminated with crude oil (about 5 mass%) due to reduction of the soil toxicity and increase of the number of petroleum degrading bacteria. Then similar experiments were carried out with a chernozem collected from a territory near a burial site of prohibited pesticides (DDT and HCCH). It was shown that soil amendment with the BC accelerated degradation rate of some pesticide metabolites and simultaneously sharply reduced bioavailability of the pesticides to plants and soil biota. Summing up, our results confirmed a good perspective of application of "locally produced-locally used" sludge-based biochar as soil conditioner in the urban landscape projects, as well as a sorbent for accelerated bioremediation of soil contaminated with persistent chemicals or crude oil. This work was supported by RFBR-mk 19-29-05265.

4.15P.6
AWAREGIO - Modular Wastewater Treatment Approaches to Achieve a Sustainable Reuse of Freshwater, Nutrients and Energy in the Anticipation of Future Water Stress
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Access to freshwater resources is essential for human well-being and survival of human civilization. Global trends such as climate change and growth of human population etc. increase the potential for conflicts about fresh water resources. Droughts as experienced during the last three summers can also lead to future shortages in freshwater even here in central Europe, but more importantly in arid regions.The presented AWAREGIO project, a pilot-study supported by the BMBF, aims to develop innovative, modular wastewater treatment facilities that flexibly allow for the reuse of freshwater, wastewater nutrients, and energy within agriculture, aquaculture and drinkable freshwater substitution. The technologies are also aimed at showcasing and providing new market opportunities for the small companies involved.The modular wastewater treatment approach includes an innovative primary treatment system followed by four different secondary treatment technologies (soil filter, UV treatment, ultrafiltration and reverse osmosis for a part of UF-stream) which were investigated in parallel. The efficiency of the different treatment steps was assessed by various state-of-the-art bioanalytical methods. The usability of the treated wastewater was assessed in an aquaponic facility used for culturing different crops and African catfish (Clarias gariepinus). For monitoring purposes, 2 L water samples were extracted via SPE for later use in bio-assays. Toxicity of these extracts was assessed via the acute algae-growth inhibition assay (OECD 201), acute daphnia immobility assay (OECD 202), fish embryo toxicity (OECD 236), MTT-, ER- CALUX®-, Ames Fluctuation-, Micronucleus- and µ-EROD assays. Chemical analysis was conducted using LC-MS/MS targeted screening covering common indicator anthropogenic micropollutants (e. g. pesticides and pharmaceuticals) as target analytes.Teratogenity and estrogenic activity has been observed for different secondary treatments. Results of the chemical analysis indicated a degradation of some analytes through the soil filter. Only the combination of UF and RO resulted also in a well-working elimination of persistent pollutants comparable to tertiary treatment steps in conventional WWTPs. The best water quality was obtained

using reverse-osmosis followed by ultrafiltration, whereas UV-treatment was identified as not suitable to reach the required water quality. The soil filter produced also a very good quality with very low input of energy.

Developments in the Ecological and Human Health Risk Assessment of Biopesticides: Microorganisms, Botanicals and Semiochemicals (P)

4.16P.1

Challenges associated with registration of botanical active substances in the EU

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Botanical active substances, or plant extracts/oils, together with other biological pesticides ("biopesticides") 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/altered by chemical and/or microbial processes." The qualitative and quantitative composition of botanical active substances depends on the origin of the biological material which is influenced by many factors including geography, climate and soil conditions, as well as processing methods, and therefore their complex and variable nature can make registration challenging. This paper discusses two key challenges of botanical active substance registration in the EU: (1) the lead component concept; and (2) evaluation of technical equivalence. Identification of lead component(s) can benefit risk assessments as long as this approach is fully justified. It is feasible that botanical active substances of the same biological origin will have different compositions. The use of (Q)SAR, literature and comparison of other exposures with pesticide exposure can help to alleviate any toxicological or ecotoxicological concerns and demonstrate that difference source(s) of technical material can have a comparable level of hazard for human health and the environment to that of the reference specification, such that technical equivalence can be concluded. Real-life examples and strategies are discussed; however, the substance identities have been sanitised to ensure confidentiality.

4.16P.2

Botanical (plant extract) active substances: a review of ecotoxicological risk assessments under EU Regulation (EC) No. 1107/2009

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Unlike conventionally synthesised chemicals, botanical plant protection products (also known as 'plant extracts' or 'biochemicals') are produced by the processing of plant material, often using water or ethanol extraction methods and/or physical processes such as pressing, milling and crushing. Nevertheless, botanical active substances are subject to the same regulations and data requirements as chemical pesticides for their approval in Europe (EU Regulations (EC) 1107/2009 and 283/2013). Whilst specific guidance for botanical active substances is available (SANCO/11470/2012– rev. 8, 2014; ENV/JM/MONO(2017)6), this guidance does not provide a general testing strategy for the ecotoxicological assessment of the diverse and complex range of botanical active substances. Additional guidance is provided by some member states (e.g. the Netherlands Ctgb has published an Evaluation Manual for the Authorisation of Biopesticides, v.1.1; July 2018), nevertheless applicants are advised to propose a relevant testing strategy for their specific substance in line with its mode of action, proposed use and relevant exposure to non-target organisms. With several botanical active substances currently approved at the EU level, what can be learnt from previous evaluations to assist future applicants with approval or renewal of their botanical active substances? Here, we will present a review of the ecotoxicology sections of recent EFSA conclusions for botanical active substances, with the aim to identify common areas of concern and trends in the type of data gaps identified during the peer review. Based on these findings and our specialist expertise, we aim to assist future applicants to better focus their submissions to avoid some of the common difficulties and pitfalls.

4.16P.3

Toxicological assessment of biopesticides from agricultural waste

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Chemical-origin pesticides are known to produce different kinds of environmental effects in non-target organisms and human beings. It is necessary to develop alternative plant protection approaches that assure the safety of aquatic and terrestrial organisms from the toxicology point of view. Here we present the study WASTE4GREEN "Sustainable and green agri-waste based biopesticides", as an innovation and development initiative of two biopesticides, whose active substances will be obtained from agroindustrial waste. This will improve food safety by producing waste free fruit and minimizing the environmental risk. To achieve this goal a toxicological assessment model was designed to determine health and environmental effects of bioactive extracts obtained from plant residues. Subsequently, the two acquired natural formulations will be evaluated. In the first phase the toxicological profile of bioactive extracts will be determined based on a bibliographic review taking into account Toxnet, ChemAgora (EC), SEARCH-TOX, ECOTOX (EPA) databases; EFSA phytosanitary products evaluations and food products and additives registration; EMEA medicines and medicinal plants registration; chemical products registration (REACH); and a review of toxicological assessment reports from other international institutions and organizations (EU, EPA, IARC). We will also evaluate bioactive extracts ecotoxicity, including exposure and effect markers, based on aquatic (*Daphnia magna*) and terrestrial (earthworm *Eisenia foetida*) bioindicators. In the second phase, the resulting formulations' health and environmental effects will be evaluated using the QSAR ToolBox and ecotoxicological tests with above mentioned organsism plus the algae *Scenedesmus subspicatus*, the fish *Brachydanio rerio* and the plant *Lactuca sativa*. The methods used follow OECD guidelines for chemical product control and are carried out under a quality assurance system according to EN ISO/IEC 17025, accredited by the National Accreditation Entity (ENAC) for testing in aquatic environments. With this toxicological assessment model we expect to mitigate adverse effects on the environment and human health that originate from chemical products currently used for plant protection. This project will allow the reduction of commonly used chemical-origin pesticides by an estimated 56%, with the use of two safe and sustainable natural origin formulations, whose active ingredients will be obtained from agroindustrial waste.

4.16P.4

QSARs models as a toxicological assessment tool for novel biopesticides regulation

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Pesticides are compounds used worldwide for plant protection and disease vector control. However, most of the conventional chemical-origin pesticides manufactured few decades ago have ended up producing different kinds of side effects in non-target organisms, or even in human beings. For this reason, it is necessary to develop alternative plant protection approaches, such the so-called "biopesticides", which are products made up of natural active substances, with high effectiveness and specificity against pests and vectors, being environmental friendly. In order to include these biological products in the plant protection products market, they must be regulated under the classic steps for risk assessment. However, experimental toxicity tests are costly, time-consuming, and require a high volume of laboratory animals. For this reason, *in silico* approaches like the quantitative structure-activity relationship (QSAR) models, can be used to predict chemical toxicity with no experimental data, following the 3Rs principle (Replace, Reduce, Refine of laboratory animals). In addition, the use QSARs models has been already accepted by the European Union in 2006 under the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH), and nowadays have become a computational approach to toxicity assessment and regulatory decision support. The aim of this study was to evaluate the current regulatory state-of-art of biopesticides in order to identify the main gaps in the registration and evaluation process, and include the use of different QSARs models already considered in other important European regulations for biopesticides regulatory processes. After thorough review of the regulation, we noticed that there is a lack of biopesticide specific legislation in Europe, and natural active substances registration is taking place as conventional chemical-origin pesticides (e.g. Plan Protection Produts Regulation (EC) No 1107/2009 and their modification (EC) No 1432/2017). In this sense, the working methodology used in other regulations, such as that described in REACH, could be transferred for future evaluation and registration guidance of novel biopesticides. Likewise, since the level of risk resulting from the use of natural active substances is lower than the risk associated with chemical-origin pesticides, QSARs models described above could be considered during biopesticide toxicological assessment and registration processes, thus reducing animal testing.

4.16P.5

A mesocosm approach for evaluating freshwater macroinvertebrate community responses and ecosystem functioning to field-relevant levels of a Bacillus thuringiensis var. israelensis bioinsecticide

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Bioinsecticides based on the bacterium *Bacillus thuringiensis* var. *israelensis* (*Bti*) are increasingly being used in pest control as environmentally friendly alternatives to synthetic insecticides. *Bti*-based insecticides are highly selective, being specific to Diptera, and decompose rapidly in the environment. Nevertheless, their safety to non-target species and freshwater ecosystems is still under debate. This work aimed to evaluate the potential effects of a *Bti*-based insecticide on structural and functional responses of a macroinvertebrate community using indoor mesocosms. For that, artificial streams colonized with a macroinvertebrate community plus periphyton collected in a reference stream together with alder leaf packs were exposed to a control and to three *Bti* concentrations (12 ug/L, 120 ug/L and 1.2 mg/L) for 7 days. These concentrations are within the recommended dosages of application in the aquatic compartments. Besides community structure and invertebrate abundance, effects were also evaluated regarding leaf decomposition and primary production as measures of ecosystem functioning. Community structure was significantly altered in all *Bti* treatments when compared to control, mostly owing to reduction of chironomids, which were, as expected, the most affected in *Bti*-treated streams, followed by oligochaetes. Accordingly, fine sediment eaters were the only functional group with reduced abundance. Effects were also observed in terms of Ephemeroptera-Plecoptera-Trichoptera (EPT), which gradually decreased in abundance along the concentration gradient. Moreover, leaf litter decomposition was greatly reduced under *Bti* exposure, also reflecting sublethal effects (toxic anorexia) of detritivores and indicating impairment of ecosystem function. On the other hand, no alterations in primary production were observed, which is in accordance with the absence of effects on grazers. Our results raise important concerns as they indicate deleterious ecosystem-level effects of field-relevant concentrations of a *Bti*-based insecticide. Results also show that other non-target species besides chironomids are affected by *Bti* exposure with consequent effects on natural macroinvertebrate communities and ecosystem functioning. Furthermore, the present study provides valuable information that could be used to revise the thresholds used concerning the application of these bioinsecticides for an improved environmentally friendly pest control management.

Dyes as Environmental Contaminants (P)

4.17P.1

Aquatic toxicity assessment of auramine dyes

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Around 10000 ton of dyes are produced worldwide every year and used for several ends. Dyes are considered as emerging contaminants, because they have been detected in the environment in concentrations that may induce risk to the aquatic organisms. The dyes C.I. Basic Yellow 2 and C.I. Solvent Yellow 34 (often referred as Auramine and Auramine O) are synthetic colorants, still widely used in clothes and food products, despite their potential carcinogenicity to humans. Since they are still manufactured and utilized, those dyes are constantly discarded to the environment, especially in the aquatic ecosystems. Thus, the aim of the present study was to assess the toxicity of both dyes to aquatic organisms from different trophic levels (*Raphidocelis subcapitata*, representing autotrophs, *Dapnhia similis* representing primary consumers and *Hydra attenuata* and *Danio rerio* representing secondary consumers). Moreover, the predicted non-effect concentrations (PNEC) for both substances were calculated. Both dyes induced an inhibition in growth rate in exposed algae; negatively affect the reproduction of *D. similis* and induced deformities in H. attenuate and *D. rerio*. PNEC values of 0.92 ug/L and 4.0 ug/L were obtained for C.I. Solvent Yellow 34 and for C. I. Basic Yellow 2, respectively, based on results obtained with algae, the most sensitive test system in the present investigation. Experiments testing the possibility of an inhibition of photosynthesis caused by the color of the dyes blocking the light were conducted, and results demonstrated that the toxicity recorded was only related to the chemical exposure and not to some physic influence. The obtained results were submitted to a Criteria of Reporting and Evaluating Ecotoxicity Data (CRED) analysis, confirming their reliability and relevance. Thus, PNEC values were derived for both dyes and may be an useful information in future risk assessments of those substances in freshwater systems.

4.17P.2

Does wash-off from façade paintings pose a risk for the aquatic environment?

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Façades are frequently coated with paints containing biocides to prevent algal and fungal fouling. Rain fall events can wash-off these substances and biocides can enter surface and groundwater bodies, with potential negative effects on the local biota and the ecosystem services they provide. At present, only little is known about the effects of façade paints in aquatic environments. Therefore, we systematically assessed the acute and chronic effects of selected façade paint products on aquatic organisms. A first set of acute bioassays with *Desmodesmus subspicatus* and *Daphnia magna* established dose repose relationships of individual biocidal ingredients, which are frequently part(s) of façade paints, namely: Tertbutryn, Octhilinone and Diuron. In a further step we assessed acute and chronic effects of six commercially available paints or additives either containing no, one or two of the same biocide(s). First immobilization data with daphnia revealed 48 h-EC_{50} values of Tertbutryn, Octhilinone and Diuron as high as 11.73, 0.68 and >10.00 mg/L, and are therefore in good agreement with scientific literature. In contrast, results for paints containing biocides showed significantly higher 48-h EC_{50} values when compared to the single biocide application, indicating a reduced toxicity for entire paints by a factor between 2 and 90. Further, paints without any biocides were up to 165-fold less toxic if compared to those containing biocides. These insights point towards a broad range of ecotoxicological effect thresholds of façade paints warranting further study. However, a direct comparison for the ecotoxicity of façade paints based on their biocide content remains difficult. This is because each product represents a complex mixture of (secret) ingredients, additives and widely unknown concentrations, complicating ecotoxicity testing and ultimately risk analyses. Consequently, further acute and chronic *Daphnia* studies as well as algae tests are planned to further our understanding of façade paints' aquatic ecotoxicity.

4.17P.4

Purification of the natural dye (willow bark hot water extracts) for toxicological evaluation

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Willow (Salicaceae) bark contain a variety of antioxidant phytochemicals depending mainly on the hybrids. In this study, the hot water extraction can be coupled with the selected resin adsorption to produce the concentrated phytochemical extracts from the bark of Salix hybrids based on its adsorption and desorption capacities. Toxicological activity studies focusing on the evalutations of the hot water extracts before and after purification (i.e. sugar elimination), considered as one of the key criteria for the classfication as an environmental friendly dye, will also be discussed.

Environmental Risk Assessment of UV filters (P)

4.18P.1

Bioaccumulation of phenolic benzotriazoles in the benthic freshwater amphipod Hyalella azteca

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The identification and scientific assessment of compounds that bioaccumulate in organisms and biomagnify in food webs play a key role within the PBT-assessment. The bioaccumulation potential of compounds is commonly expressed as bioconcentration factors (BCF), determined in flow-through studies with fish according to OECD 305. The performance of aqueous exposure bioconcentration fish tests requires the possibility of preparing stable aqueous concentrations of the test substances. For highly hydrophobic organic chemicals (HOCs; octanol–water partition coefficient [log KOW]>5), testing via aqueous exposure may become increasingly difficult. For these substances the dietary test is recommended which yields dietary approach a biomagnification factor (BMF) rather than a BCF. An alternative test system for BCF tests using the freshwater amphipod *H. azteca* (HYBIT) was recently suggested. Hyalella BCFs show a strong correlation with fish BCF values. About 20 organic compounds with different hydrophobic characteristics (log K_{ow} 2.4 – 7.8) have been tested so far. However, only a few studies have been carried out with HOCs. In this study two phenolic benzotriazoles (UV 234 and UV 329) which are highly hydrophobic (log Kow >6) were tested following the HYBIT flow-through protocol. The UVs which are commonly used as ultraviolet filters, e.g. in sun protectors or in polymer based products to extend their lifespan, are difficult to apply due to their very low solubility in water. Therefore, only very low exposure concentrations (≤ 1µg/L) could be applied in this study. Nevertheless, the measurement of substance concentrations in test water and *H. azteca* samples collected during the uptake and depuration phase was still possible and allowed the determination of BCF_{SS} and BCF_{kin} values for both compounds. The results reveal the high bioaccumulation potential of the two test compounds. The BCF studies showed that the HYBIT is also suitable for the testing of HOCs such as phenolic benzotriazoles and may thus help to tackle the challenges involved in the testing of HOCs as observed in the flow-through fish test, also due to the shorter duration needed to gain steady state.

4.18P.2

Down-the-drain exposure modelling of UV filters in the U.S.

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UV filters are used in a variety of cosmetic and non-cosmetic applications, such as sunscreens, apparel and plastic goods due to their ability to absorb certain wavelengths of ultraviolet radiation. The presence and effects of UV filters in the marine environment have received scrutiny within scientific, regulatory and public domains in recent years. Most of these concerns are related to the potential effects of UV filters on coral resulting from their presence in coastal marine areas, especially those receiving high numbers of tourists. In contrast, the freshwater environment has received less attention in terms of the risk organic UV filters may pose. UV filters can enter the environment through domestic wash-off and wastewater treatment plant (WWTP) effluent release. In the U.S., the majority of these effluent releases are to the freshwater environment. A recent literature analysis identified that there is currently no North American freshwater monitoring data for UV filters. Therefore, we ran the iSTREEM® down-the-drain exposure model to provide a screening level assessment of UV filter concentrations in US freshwater. A variety of input scenarios were applied representative of worst-case exposure through to more refined estimates. A probabilistic risk assessment was performed to identify the 95th percentile risk quotient for UV filters in the US freshwater environment. Worst-case modelled exposure estimates were below concentrations measured elsewhere throughout the world. Collection of high-quality freshwater and WWTP monitoring data are needed to determine whether this trend is also observed in North America. A source apportionment study may be required to further investigate trends in UV filter exposure in the freshwater environment.

4.18P.3

Histopathological assessment of UV-filters (TiO2-NPs and BP-3) in Scophthalmus maximus liver at environmentally-relevant concentrations

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The worldwide usage of sunscreens, formulated with organic and inorganic compounds has been increasing year by year, that was also associated with the increase of coastal and maritime tourism and the awareness of the dangers arising from exposure to solar radiation. This increase of sunscreens usage by humans raise the concern about the potential increases of the concentrations of these compounds in aquatic systems. Several studies have revealed the toxicity in aquatic organisms associated with exposure of UV-filter compounds, such as titanium dioxide nanoparticles (TiO2-NPs) and benzophenone-3 (BP-3), however most of them do not consider environmentally-relevant concentrations of these compounds. In the present study, Scophthalmus maximus (turbot fish) were exposed through intraperitoneal injection to environmentally-relevant concentrations of both UV-filters, TiO2-NPs, BP-3 and their mixture. The toxicity assessment of these compounds was performed after 3 and 7 days using histopathological analyses of turbot liver and semi-quantitative histopathological indices. Overall, the histopathological observations of fish liver suggested a slight increase in the immune/inflammatory response after exposure to UV-filter compounds, as well as a slight intensification of the progressive alteration fat vacuolization of hepatocytes. Despite these observed alterations, no significant differences between treatments were observed and no link was found between alterations and exposure time. Through microscopic observations made using Nuclear Red (NR) staining, no nanoparticle clusters were found in the liver, suggesting that there was no accumulation of nanoparticles during the exposure time. Summing up, this work suggested that environmentally-relevant concentrations of this compounds leads to slight alterations in turbot hepatic tissue. Acknowledgements Thanks are due to Fundação para a Ciência e Tecnologia (FCT)/MCTES for the financial support to MARE (UID/MAR/04292/2019) and CESAM (UID/AMB/50017/2019).

4.18P.4

Under the influence of regulations: occurence and temporal trends of the UV filter EHMC in German rivers

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Globally, 2-Ethylhexyl-4-methoxycinnamate (EHMC) is one of the most frequently used UV filters in sunscreen and personal care products. In the EU, it is currently listed under the European Community Rolling Action Plan (CoRAP) as suspected persistent, bioaccumulative and toxic (PBT) and a potential endocrine disruptor. Through wastewater discharges and rinsing from the skin while swimming EHMC is released to surface waters. Due to the widespread usage, the occurrence of EHMC in the aquatic environment is already documented. Against this background, EHMC has been included in the first Watch List under the Water Framework Directive (WFD) referring to a sediment PNEC of 200 µg/kg dry weight (dw). The objective of this study was to obtain spatio-temporal trends for EHMC in freshwater. We analyzed samples of suspended particulate matter (SPM) retrieved from the German environmental specimen bank (ESB). The samples covered 13 sampling sites from the major German rivers including Rhine, Elbe and Danube and have been collected over the past decade. Our results indicate decreasing concentrations of EHMC during the studied period. In the mid-2000s, the levels for EHMC ranged between 3.3-72 ng/g dw. The highest burden could be found in the Rhine tributary Saar. In 2017, we observed a maximum concentration ten times lower (7.9 ng/g dw in samples from the Saar). In 62% of all samples taken in 2017 concentrations were even below the LOQ of 2.7 ng/g dw.

Environmental Risks of Neonicotinoid Insecticides: Are They the Outliers? (P)

4.19P.1

Confirming the SSD and the msPAF approach for the ecotoxicological risk assessment of neonicotinoids under semi-field conditions

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Neonicotinoid insecticides have been identified as contaminants of concern for freshwater ecosystems due to their frequent occurrence at concentrations that may exert toxic effects to aquatic invertebrates. Recently, the European Commission has included five neonicotinoid insecticides (i.e., acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam) in the Watch List of contaminants that need to be monitored in European surface waters. This implies that a water quality standard and mixture toxicity model need to be proposed to calculate risks from cumulative neonicotinoid exposures. In this study, a mesocosm experiment was conducted with imidacloprid and an equimolar mixture of the five neonicotinoids included in the Watch List to derive threshold concentrations for freshwater invertebrates and to test the multi substance Potentially Affected Fraction (msPAF) model for mixture toxicity assessments. Among the tested species, Cyclopoida, *Cloeon dipterum* and *Chironomidae* showed the highest sensitivity, with calculated NOECs below 0.2 µg/L. The tested imidacloprid and neonicotinoid mixture concentration ranges displayed similar short-term effects on the evaluated invertebrate populations and communities, indicating that the concentration addition model can be used as a plausible hypothesis to assess direct short-term responses. Slight differences were observed in the magnitude of the long-term effects and the recovery potential of some taxa, which was mainly attributed to the different persistence of the compounds included in the mixture. Species Sensitivity Distributions (SSD) for imidacloprid and the msPAF for the mixture based on acute toxicity data were not sufficiently protective for semi-field effects. This is partly due to the moderate persistence of these compounds in the water column and due to their delayed effects on freshwater insects and crustaceans. The SSD for imidacloprid based on chronic toxicity data provided an accurate prediction of the observed effects in the mesocosm experiment. Due to the absence of chronic SSDs for each compound in the mixture, the available chronic toxicity data for all compounds was molar-transformed to build a new mixture sensitivity distribution and to calculate an msPAF. This molar-transformed msPAF provided a conservative estimate of neonicotinoid mixture effects on invertebrates and can therefore be recommended for use in the retrospective risk assessment of neonicotinoid mixtures.

4.19P.2

Capitalizing on existing knowledge of pesticide-CYP9 enzyme interactions to understand chemical susceptibility across bee species using the SeqAPASS tool

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As pollinators of numerous crops, bees are critical to global agriculture. Therefore, it is important to protect the health of these insects to ensure chemicals used to maximize and protect crops do not have unintended adverse consequences to bees. Flupyradifurone is a butenolide neonicotinoid used on crops affected by sucking pest insects that are resistant to other neonicotinoids. Recently, it has been shown that the leafcutter bee (*Megachile rotundata*), is 170-fold more sensitive to flupyradifurone than other managed bee pollinators. Further, evidence suggests that the pesticide tolerance of certain bee species is due to metabolism by specific P450 enzymes in the CYP9Q and CYP9BU subfamilies. A new approach method that can be used to computationally understand flupyradifurone susceptibility across insect species, and more specifically bee species, is the US EPA Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool. The SeqAPASS tool allows users to rapidly evaluate available protein target sequence and structural similarity at the primary amino acid sequence, conserved domain(s), and individual amino acid residue levels, to understand conservation and therefore predict chemical susceptibility. Using the individual amino acid residue comparison feature of SeqAPASS allows for the identification of species-specific predictions of chemical susceptibility. A case study was developed comparing bees of differing sensitivities to flupyradifurone to explore possible individual differences in amino acid residues in the CYP9Q3 protein. Critical amino acid residues from the catalytic pocket of CYP9Q3 were compared across species. Although there is limited sequences available for key bee species, a critical lysine

residue (K219) was identified as important for certain pesticide-CYP9Q interactions. An individual amino acid residue SeqAPASS evaluation predicted dissimilarity in susceptibility between the leafcutter bee and other managed bee pollinators due to a difference in the critical amino acid located at position 219. Through a review of available literature, along with computational assessments of susceptibility using the SeqAPASS tool, this study provides lines of evidence for differences in pesticide susceptibility among managed bee pollinators based on conservation of the CYP9 enzymes. In addition, this work demonstrates the ability of the SeqAPASS tool to rapidly identify differences in chemical susceptibility across other species of concern.

4.19P.3

Imidacloprid-based insecticide toxicity to soil fauna in loamy and sandy soils from Brazillian Cerrado

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Imidacloprid is one of the most widely traded pesticides in the world, with a broad spectrum of action, high persistence and low soil sorption, and high ecotoxicity. Brazilian Cerrado is one of the major agricultural areas of the world, with an intensive use of pesticides at one hand and little knowledge of their occurrence and environmental impacts on the other hand, especially on non-target soil organisms. Therefore, the aim of this study was to evaluate the effects of an imidacloprid-based insecticide to non-target soil organisms in two soils with contrasting texture (56% against 9% of clay content) from the Brazilian Cerrado. For this purpose, avoidance behavior tests with earthworms (*Eisenia andrei*) and collembolans (*Folsomia candida*), and reproduction of collembolans (*F. candida*), were carried out. The soils were spiked using the commercial product Evidence® 700 WG (700 g.kg^{-1} imidacloprid), based on the recommended dose for sugarcane (400 g.ha^{-1} of the commercial product, corresponding to 280 g.ha^{-1} a.i. imidacloprid): 0, 0.11; 0.22; 0.45 0.90 and 1.80 mg.a.i.kg^{-1}. The field predicted dose of imidacloprid was calculated as 0.45 mg.a.i.kg^{-1}. Standardized ISO guidelines were followed to avoidance and reproduction tests. For earthworms, a possible habitat function loss was observed, once an avoidance behavior above 80% were found in both soils for all tested concentrations. For collembolans, the avoidance behavior under predicted field dose occurred only in the clay soil. The values of EC_{50} obtained for reproduction of collembolans were 0.255 mg kg^{-1} for the clay soil and 0.015 mg kg^{-1} for the sandy soil.. Imidacloprid sorption in soils is low (K_f of 0.54-1.10 in tropical soils) and mainly driven by hydrophobic interactions. The similar organic matter content of these two soils (36.9 and 35.4 g dm$^{-3)}$ together with the strong differences in EC_{50} values showed that texture played an important role in the ecotoxicity effects. This study showed that imidacloprid is toxic to soil invertebrates, causing avoidance and reducing the reproduction even in concentrations below that expected to occur in the field.

Difficulties in Waste Ecotoxicological Tests for HP14 Classification: Legislation Debate and Testing Challenges (P)

4.20P.1

Critical points of the EU Directive on the HP14 classification: An Italian perspective

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Several people from Academia, industry, private laboratories, Regional Environmental Protection Agencies (ARPAs) and members from Italian National Institute for Environmental Protection and Research (ISPRA) attended a recent meeting entitled "First meeting of applied ecotoxicology" (21th November 2019, Livorno-Italy), in which a section was devoted to the classification of waste with hazard property (HP14). It was pointed out that the current legislation has several points that need to be clarified. No guidelines or recommendations currently exist at EU level for a specific methodology for assessing of the ecotoxicological property of waste HP14. As a result, HP14 classification is performed in different ways throughout EU Member States, including Italy. The result of the meeting was the lack of a harmonized approach for the application of ecotoxicity tests on waste samples. In fact, for example, the recent Council Regulation (EU) 2017/997 defines a "conventional" method for HP14 classification (summation method of hazardous substances) based on determination by chemical analysis of waste as it is. However, the Reg. 2017/997 does not explain how determine the ecotoxicity if the bioassays are used and if their results "prevail" on the chemical one. Furthermore, the most important issues that needs to be clarify are: -the definition of "Representative sample" for different waste typologies; useful indications can be derived from International Standard methods (ISO 5667-13:2011; ISO 5667-16:2017; ISO 18400-206:2018); - which is the most suitable subsampling for specific ecotoxicological testing (on solids or liquid subsamples), although useful indications could be found in some ISO documents (ISO 18772:2008 Soil quality; ISO 21268-1:2019, Part 1-4 and ISO 14735:2005); - the elution ratio, defining which sample has to be treated using UNI EN 14735 or other standard methods; - the selection of the appropriate ecotoxicological test battery could affect the final result; some indication could be found in the ISO 17616:2019 Soil quality. Unfortunately, even the best approach for one of these steps is not enough, unless an equally good approach is devised also for all the other steps. Consequently, there is the need to harmonize the method used at Italian and at EU level, considering that waste can be transferred among different Countries. A forum at Italian level that involve Academia, industry and regulators is now in progress.

4.20P.2

Challenges in development of a leaching test for evaluation of ecotoxicity (HP14) of municipal solid waste bottom ashes

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In the European Union, the regulatory framework for classifying HP14 is based on regulations for classifying chemicals stated in the CLP, *The regulation for classification, labelling and packaging of chemicals* (Regulation (EC) 1272/2008). Thus, it is reasonable to base test methods for HP14 assessment of waste on the same principles as in the CLP. The aim of the project reported here was to develop a test method for classifying the ecotoxicity (HP14) of municipal solid waste incineration bottom ash (MSWI BA) based on an internationally recognized OECD test (the transolution/dissolution protocol, T/Dp) and CLP guidelines. Focus was on three metals that are known to be hazardous, leachable and occur in relatively high concentrations in MSWI BA, copper (Cu), lead (Pb) and zinc (Zn).Three approaches for testing and assessment were investigated:• leaching tests at a liquid solid (L/S) ratio of 1 000 000 and pH 5.5 for 28 days (following the T/Dp)• extraction tests for estimating relevant release of Cu, Pb and Zn faster and easier than by leaching at L/S 1 000 000. • leaching tests combined with geochemical modellingSeveral difficulties were encountered in performing leaching tests at L/S 1 000 000 and pH 5.5 for the required time, the most severe being substantial contamination of samples. Laboratory procedures were adjusted to minimize the effects, but the results still had large uncertainties. The extraction tests, based on ISO 17586 and EN71-3:2 for environmental and oral availability, provided reproducible results similar to the results of the leaching tests but due to the contamination in leaching tests at L/S 1 000 000 it was not possible to determine if these two types of tests provided equivalent results. In many cases, extracted amounts of Cu, Pb and Zn were lower than amounts leached in the L/S 1 000 000 tests. The objective of the geochemical modelling was to predict leaching at L/S 1 000 000 based on results from leaching at L/S 100, but the model used could not simulate solid-solute interactions for ashes at such dilute conditions. Consequently, leaching at L/S 1 000 000 was not considered a viable option for routine testing of MSWI BA and none of the studied approaches yielded a fully ready-to-use test method. The extraction tests were the most promising of the tested approaches but reliable and non-contaminated results at L/S 1 000 000 are needed to verify method performance.

4.20P.3

Mechanized tunnelling and management of spoil material containing foaming agents: currents trends and future perspectives

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Several tons of excavated materials (spoil material) have been produced during the tunnelling for the construction of underground railways, highways etc. The reuse of excavated soil as a by-product is desirable and taken into consideration in most Countries for avoiding the production of huge amounts of waste. The Tunnel Boring Machine- EPB technology (TBM-EBP) requires the use of foaming agents and in some cases other additives such as polymers. Many commercial products used for this purpose contain as the main component, the anionic surfactant sodium lauryl ether sulphate (SLES). SLES is a biodegradable compound; however, a risk for the aquatic environment is not excluded, if the spoil material enters in contact with the aquatic environment. This possibility depends on the amount of SLES residues which pass in the aqueous phase of the soil, which in turn depend on the soil intrinsic characteristics (soil texture, mineralogy) and

residence time of the excavated soil in the temporary deposit of the tunnelling construction site where it can be degraded. Several experimental studies performed for several real case studies found that to establish a SLES concentration threshold in soil is not a suitable approach for evaluating the environmental compatibility of the spoil material. An ecotoxicological approach seems the best solution for facing this issue. In Italy, spoil material from excavation processes can currently be re-used as a by-product if it meets environmental legislative criteria related to the Italian Decree 152/2006. At present, specific limits for substances (e.g. surfactants) contained in foaming agents used for TBM-EBP technology have not been established in EU Directive 98/2008. In this study, several case studies with different type of excavated soils treated with different commercial products will be shown, applying ecotoxicological tests for aquatic and terrestrial compartments. The ecotoxicological results, together with the chemical analyses of SLES were used for elaborating site-specific Protocols to be used during the excavation phase for classifying spoil material as a by-product.

4.20P.4

From HP 14 classification to the environmental criterium of End-of-Waste status: the case of inert waste

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Adopting sustainable End-of-Waste (EoW) criteria is crucial to promote the transition to an efficient Circular Economy, especially within the inert waste treatment sector. In particular, methodologies established by European regulations on the assessment of compliance with the environmental requirement ("Will the use of the material lead to overall adverse environmental or human-health impacts?") can represent an issue possibly hindering the marketability in the civil works sector of the so-called "recovered aggregates". In this context, a scientific working group involving the Department of Civil, Environmental and Architectural Engineering of Padua University and other independent experts is proposing a full protocol (i.e. including standard references and technical instructions for all analytical phases, from sampling to data interpretation), to update current regulations on the field. According to the current proposition, each analytical phase must be performed in accordance with technical guidelines established for the characterization of waste material. The innovative approach underlying the proposed procedure includes the possibility to perform ecotoxicological tests, whether the results from chemical characterization does not comply with the established CL necessary to obtain EoW status. In particular, the analytical procedures laid down for ecotoxicological EoW assessment is based on the so-called French-German approach for HP 14 classification of waste by testing. Results will be presented from the application of the proposed protocol (from sampling to sample preparations, from chemical to ecotoxicological characterization) on 5 different samples of recovered aggregates, characterized by different input waste composition (C&D waste, slags, incinerated bottom ash and natural mineral material) and analysed both in unbound and bound-like forms. Also, critical issues encountered during the performance of bioassays on recovered aggregates will be investigated (pH adjustment, precipitates occurrence during storage, etc.), and the proposed solutions will be discussed during the conference session.

New Developments in UVCB Risk Assessment (P)

4.21P.1

OECD 301 Ready Biodegradability Test Set-up for UVCBs and Difficult-to-test Substances Using Gas-to-Liquids Products as a Case Study

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Biodegradability assessment is an integral part of chemical risk assessment. The outcome of biodegradation testing is controlled by the properties of the test substance, the microbial inoculum, and the test set up. As indicated in the OECD test guidelines, test substance properties should inform test design and set up. This is particularly important for 'difficult-to-test substances', such as substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs), volatiles, and poorly soluble substances. Here we will present, a suite of aquatic biodegradation data generated for various Gas-to-Liquids (GTL) substances. GTL substances are synthetic hydrocarbons manufactured from natural gas via the Fisher-Tropsch process. Their synthetic nature means they have very low aromatic content and relatively simple hydrocarbon structures. However, they are UVCBs and contain volatile and poorly soluble constituents. This presentation will show how the GTL substances perform in several OECD 301 ready biodegradability tests (RBTs), with outcomes varying according to test set up. Based on Quantitative Structure Activity Relationships, it is expected that all of the tested substances would be readily biodegradable. However, the most widely tested substance, GTL Gas Oil, has test outcomes that either pass or fail the criteria for ready biodegradability. This variability is linked to the type of test and test system, with OECD 301B tests showing lower biodegradation than the OECD 301F tests. This was attributed to abiotic losses of the GTL Gas Oil from the test system, due to either volatilization or sorption of the test substance to the components in the test system. Such losses are also believed to account for the lack of CO2 production in the biodegradation tests for GTL kerosene and naphtha, both of which have volatile constituents. This work demonstrates the importance of proper test system selection for biodegradation testing of difficult-to-test substances. For tests with UVCBs containing volatile or poorly soluble constituents, test equipment with fewer plastic components to limit sorptive losses and limited headspace to reduce losses from volatilization are recommended. Recommendations for such systems that maximize bioavailability and provide a more accurate reflection of aquatic biodegradability will be discussed. Using multiple lines of evidence of biodegradation, it was possible to account for abiotic losses in the test systems and reach robust conclusions for these complex substances.

4.21P.2

Sniffing out cedar wood oil: further characterising an UVCB and evaluating its hazard potential

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Cedarwood oil is a chemical substance of unknown or variable composition, complex reaction products and biological materials (UVCB), which makes the assessment of its (bio)degradation, bioaccumulative and toxic potential very challenging. The hydrophobic nature of most constituents present in cedarwood oil further complexifies such assessments. Recently, a new partitioning-based experimental platform was developed by Birch et al. (Chemosphere 2017 184 400-407) to determine biodegradation kinetics of hydrophobic organic hydrocarbons at environmentally relevant low concentrations by use of GC-MS. This study will present the results of developing and/or setup analytical methods to further characterize cedarwood oil as well as the application of the new partitioning-based approach to cedarwood oil to investigate the potential of generating biodegradation kinetics data as well as linking the fate data to toxicity data, a so called fate directed toxicity approach.

4.21P.3

How to develop combined toxicological assessment for complex inorganic UVCBs: "Slags, Doré furnace" assessment

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Metals production requires several refinement steps to remove so-called non-target constituents and finally produce (single) enriched metal substances. During the refinement steps, inorganic UVCB (iUVCB) enriched intermediate substances, e.g. blister/anode copper, doré, lead bullion are produced and further processed or transported. Side products are also removed to collect both non-target constituents and "left-over" target constituents, e.g. slags, flue dusts, drosses. Assessing iUVCBs requires to understand their unique specificities: known but variable elemental composition and often unknown mineralogical speciation. Risk assessment aims at identifying a substance hazard and at describing its safe conditions of use for human populations (worker and if relevant general population) and for the different exposed environmental compartments (water, sediment, soil and secondary poisoning). When assessing iUVCBs, the main assumption is that their overall fate and hazard properties are driven by the fate and hazard properties of their individual constituents (i.e. constituents/assessment entity (AEs) based approach). For most of the iUVCB substances, the ecotoxicological profiles of the individual (inorganic) AEs have already been assessed in Europe (REACH Registrations). Once all the iUVCB AEs are identified, the relevant AEs will be further selected for combined assessment: however, at industrial sites, simultaneous metallurgical processes typically occur, increasing the complexity of the combined exposure assessment, often based on monitoring/emission that are hardly assignable to the different specific sources. The AEs based approach better addressed the 'multidimensional' scale of iUVCBs, resulting in a robust risk assessment methodology composed of several parallel Risk Assessments (RAs). Assessing several mixture toxicity assessment studies and metal specificities (e.g. impact of natural background concentrations, metals essentiality, metal solubility and bioavailability, "added risk approach" versus "total risk approach", etc), the combined AEs based approach was refined in a Tiered approach to improve realistic assumptions and assess the iUVCB AEs for all environmental compartments, considering potential additional effects due to combined toxicity/exposure mechanisms. The Slags, Doré furnace was assessed following this approach and the uncertainty analysis supported that it was more robust and less uncertain compared to a standard substance approach.

4.21P.4

Predicting mixture properties using COSMOtherm for human exposure risk assessment

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Mechanistic mass balance exposure models are designed to estimate human exposure to chemicals, often in the context of regulatory assessment. Chemical mixtures present a particular challenge in exposure modelling within any existing mass-balance model, due to the need to account for changes in mass transport properties of the individual constituents of these complex mixtures (e.g. diffusion, volatilization). There are few estimation methods for predicting mixture properties. The most commonly applied tools include UNIFAC[1] and COSMOtherm,[2] Both of which perform calculations based on equilibrium criteria. COSMOtherm has outperformed UNIFAC in several comparative studies, especially with increasing molecular complexity.[3-6]COSMOtherm has also been applied to model the partitioning of chemicals involving complex phases or those with poorly characterized composition, including binary and ternary mixtures,[4,7] polymers,[7-9] and UVCBs.[10] The objective of this study is to evaluate COSMOtherm's capability to predict mixture properties such as vapour pressures, solubilities, partition ratios, and others. Predicted values will be compared to UNIFAC-predicted values and evaluated against experimental values. Ultimately it is hoped that we improve the current understanding of key mechanisms impacting inhalation and dermal exposure to multiple chemicals originating from common, and sometimes complex, sources (mixtures) in order to derive realistic and representative human exposure estimates for chemical mixtures.

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4.21P.5

The Advantage of Using Benchmarking in Dietary Exposure Bioconcentration Measurements in Fish

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The bioconcentration factor (BCF) is an established criterion for the (regulatory) assessment of chemicals. While BCF testing is standardised for single component chemicals, there is a lack of standard methods for the assessment of complex mixtures. An example are essential oils - fragrance materials that are registered as natural complex substances (NCS) under the European REACH legislation. NCS are widely used, but their potential environmental hazard is not fully understood. REACH screening criteria indicate that many NCS could meet the B criterion due to their lipophilicity and relatively low molecular weight. A challenge for determining the bioconcentration factor (BCF) of essential oils is that it cannot be readily measured using a standard flow-through experiment. The OECD 305 guideline provides the option to determine the BCF using a measured depuration constant (kT) from dietary exposure and an estimated uptake rate (k1). However, this approach has been criticised for the variable quality of k1 estimates based on the used models - and hence potential limited reliability of the resulting BCFs. To assure the quality of BCFs from dietary exposure, we used two benchmarking methods (conservative and threshold benchmarking) to evaluate the bioconcentration of cedarwood oil constituents, lavender oil constituents, and diesel oil constituents from a single dietary exposure experiment on rainbow trout. Using benchmarking, we established a robust method for the simultaneous measurement of kTs of hydrophobic UVCB constituents in a single dietary exposure experiment on rainbow trout. The presented method and quality control criteria provide a possible solution for the quality assurance issues related to k1 estimates in dietary exposure OECD305 studies. While the proposed threshold benchmarking provides the opportunity to quality control the derived BCFs and even assess regulatory threshold transgression of chemicals based on depuration rate constants alone.

Challenges, New Approaches in Life Cycle Inventory Data Collection and Modelling (P)

5.01P.1

How to treat odorous emissions in Life cycle Assessment?

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Unpleasant odour emissions from waste treatment and industrial activities are a serious problem affecting many people living in area sorrounding the plant. For decades their presence has been considered as a simple "nuisance", to the point of not receiving any specific standardization. Only in 2003 the European regulation UE 13725 has defined the odorimetric units per cubic meter (U.O./m3) as the unit of measurement of odour, that shall be assessed using the dynamic olfactometry. This methodology aims to evaluate odour concentration according to the perception of odour of the sample under examination by panelists, that by doing so will identify the threshold of perceptibility of the substance (O.T.) when it is perceived in 50% of cases. The quantification of odour emissions represents a challenge within a LCA study due to the scarcity of primary data. The aim of this study is to present the preliminary findings of an ongoing study whose aim is to identify and quantify odour emissions from a Mechanical-Biological Treatment (MBT) plant, the Tre Monti plant, located in Imola (Bologna, Italy), where Municipal Solid Waste (MSW) are treated, also with aerobic processes, leading to significant odour emissions. In particular, the proposed methodology faces and proposes solution to two main issues: what is the composition of the emitted gaseous mixture and what is the concentration of each analyte contained in? The environmental analysis is carried out on the basis of the amount of emitted substance, calculated on the basis of the resulting concentration. The damage evaluation method chosen for the analysis is IMPACT 2002+, modified in order to include characterization factors of indoor emissions with a preliminary method. Odour emissions damage is assessed according to the categories already included in the selected method. The contribution to total damage of odour emissions is limited to 0.24%, where the most affecting substance on human health is represented by ammonia. In addition to the environmental analysis, a conformity test is also carried out on the results obtained with those measured and analysed by laboratory services on the biofilters of a composting plant, in order to identify any critical aspects corrective actions of the developed model. In conclusion, this preliminary approach allows to identify and quantify the substances responsible for the odour impact, in order to attribute them the relative damage on human health as well as on ecosystem quality.

5.01P.2

High-throughput Exposure and Risks Assessment of Chemicals in plastic Toys

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A wide range of chemical additives is used during the manufacturing of plastic toys to obtain or increase specific properties in the final product. Hence, public concerns continue to rise about the possibility of plastic toys containing toxic chemicals, which can pose risks to children's health. Chemical substitution in combination with exposure and risk screening are useful tools to identify harmful chemicals in plastic toys and to evaluate alternatives. However, existing methods lack efficient and flexible approaches to quantify exposure for the thousands of marketed chemical-plastic toy combinations. To address this gap, we propose a high-throughput framework for evaluating exposure to chemicals in plastic toys, and test our framework in a case study on a wide range of organic chemical additives.We first quantify the chemical mass for hundreds of substances found in plastic toys. For each chemical-toy combination, we then calculate the fractions of chemicals transferred to air and humans in a matrix approach. By matrix inversion, we estimate the Product Intake Fractions (PiF), relating the chemical mass taken in by children per unit mass of chemicals in toys. We finally determine exposure doses expressed in mg/kgBW/d, Hazard Quotients (HQ) and Hazard Index (HI) for each chemical-toy combination. Based on these steps, we generate high-throughput exposure results for chemicals found in plastic toys.Considering an average total mass of 18.3 kg plastic toys purchased yearly per child in western countries, the estimated exposure doses become substantial. Across 456 assessed chemical-toy combinations, the PiF ranges widely from a median of 0.2% to a maximum of 11% children intake per unit mass in toy. Resulting exposure doses for children vary from 0.015 to 10.5 mg/kgBW/d, dominated by dust ingestion and dermal gaseous uptake. Combining exposure estimates with toxicity data, we derive HQs and sum them to obtain HIs, which range widely and reach up to 1450 across chemical-toy combinations. Our results demonstrate that we are able to systematically identify main chemicals of concern in plastic toys and rank possible alternatives based on common chemical function. In addition, we are able to propose for each chemical-toy combination a hypothetical 'safe' threshold concentration. Our mass balance-based framework can be readily used in chemical substitution to screen a wide range of chemical-toy combinations, and can be extended to also assess other products.

5.01P.3

Improving LCI data collection methodologies: A case study on microalgae cultivation for phycocyanin extraction

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Life Cycle Assessment (LCA) methodologies are an efficient tool to implement sustainable development in the bioeconomy. They have been used for decades to assess environmental impacts of various production systems and to support decision-making for policy and businesses. More recently, LCA studies have been applied to emerging technologies at an early stage of development such as microalgae cultivation systems. Microalgae are a sustainable and promising feedstock for biorefineries and have received considerable research attention for bioenergy production. Numerous LCA studies focused on microalgae cultivation and biomass processing for biofuel and biogas production. However, only a few analysed the production processes of high-value biomolecules such as phycocyanin, a blue pigment with applications in the agro-food and cosmetic industries. Arthrospira platensis, commonly known as Spirulina, naturally synthesises phycocyanin and is widely cultivated in outdoor open raceway ponds. The SpiralG (H2020-BBI) project aims to develop a new biorefinery for phycocyanin production and co-products valorisation. The LCA analysis focuses on assessing the environmental impacts of Spirulina cultivation and biomass processing as well as identifying the process steps contributing to the greatest impacts for improvement. The data collection highlighted the importance of developing a Life Cycle Inventory (LCI) data collection methodology adapted to microalgae cultivation systems. Most of the pilot and industrial scale processing plants are poorly equipped with sensors. The data collection stage is time-consuming, the quality of the data limited, and datasets present gaps. Therefore, most of the LCA studies of microalgae cultivation systems are based on extrapolated laboratory scale experiments or models rather than primary data. The data collected in the newly built biorefinery were analysed to identify the weaknesses of the LCI data collection approach used. A method for microalgae cultivation systems was developed from these results to improve data quality and temporal resolution while ensuring the collection of relevant and well-characterised data to make them reusable.

5.01P.4

Methodological issues associated to GHG avoided emissions accounting in waste management and recycling

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Since COP21 meeting in Paris in 2015, companies and organizations are invited to commit actions and solutions to reduce GHG emissions and contribute to the struggle against climate change. Then, in March 2019, the European Commission adopted a comprehensive report on the implementation of the Circular Economy Action Plan. The waste management and recycling sector is a key actor and enabler in the circular economy ecosystem, allowing reduction pressure on resources and ecosystems. Thus, companies need some guidance to measure and report their contribution to GHG savings thanks to their solutions they propose on the market. The literature review revealed that several dedicated guidance were produced these last years, (EPE, 2013 & 2018, ICCA & WBCSD, 2013, 2019 etc.), however there is not yet a unique dedicated standard on which to refer. Thus, existing standards such as GHG Protocol, ISO 14040 and ISO 14060 series, are used as a basis for avoided emissions accounting, leading to different interpretations and possible applications, without precise requirements and recommendations. Consequently, additional precise recommendations and requirements are welcome to be sure that all actors use the principles with the same approach for waste management and recycling activities. In 2019, a consortium including companies covering a large variety of waste management and recycling activities worldwide (Veolia, Suez, Paprec, Séché), non profit organization (WBCSD and Gold Standard Foundation) and experts of LCA and GHG accounting (Quantis), decided to collaborate in a project supported by EIT Climate KIC. The common objective of the consortium was to identify key challenges associated to avoided emissions accounting, and produce preliminary recommendations for waste management and recycling sector. Work is ongoing and partners wish to produce a future methodological framework for avoided emissions accounting for several defined goals, in waste management and recycling sector. This poster focuses on the presentation of the results of the literature review of almost 30 documents expressly developed for avoided emissions or that are applicable for accounting avoided emissions, and also provides an overview of the methodological challenges associated to waste management and recycling "avoided emissions". It reveals that the definition of the goal of the study is the first key question to solve, that allows afterwards to define the most adapted methodological approach.

5.01P.5

Improving Local Life Cycle Inventory approaches: challenges, proposals and future perspectives to support cities towards sustainability transitions

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To date, the concept of "sustainable city" is becoming more and more popular, and cities aim at becoming smarter, greener, low-carbon and/or circular. In order to implement efficient environmental friendly urban policies, a local Life Cycle Inventory (L-LCI) that can be representative of the local community in terms of materials and energy flows is crucial for an effective application of LCA at the urban scale, and i.e. a thorough assessment of environmental performances and impacts. Despite the big efforts and ambitious intentions, many cities lack knowledge and expertise to get access and properly manage local data. Ongoing research about urban footprinting shows that collected information by local authorities may be considered sufficient for policy support, but they are insufficient to perform thorough LCA studies. Identified limitations mainly include: i) time consuming data mining, as the information is not centrally available and data are collected from a wide range of stakeholders; ii) dishomogeneous structure, as data are collected applying different protocols; ii) heterogeneous data quality, especially regarding geographical representativeness, highly dependent by accessibility, direct control of local authorities, and complexity of the information provided. Very similar constraints appear when cities are asked about the main challenges they see in relation to the "smart city" concept. During the Smart Flanders programme funded by the government of Flanders, Belgium, representatives from thirteen cities were interviewed and they arrived at four significant challenges related to local data: i) sufficient data hygiene in the organization, ii) how data coming from Internet of Things applications can be better gathered, combined, used and turned into actionable information, iii) how to make local data centrally accessible, and iv) how to optimize interaction with vendors.From these two examples, it is clear that having access to key local datasets is vital towards improving local policy making and performing more complete L-LCIs.Based on the outcomes of these studies, the present contribution provides guidance and food for thought for future research needs in order to solve current limitations and preparing enhanced and efficacious L-LCIs able to support cities to become smarter, sustainable and/or circular.

5.01P.6

Model-based technology integration to support life cycle engineering of all-solid state batteries

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Lithium-ion batteries are essentials for consumer electronics, stationary storage systems and especially electromobility. There is a wide variety of lithium-ion batteries (LIB) and an even greater diversity of subsequent all-solid state batteries (ASSB) under development. Life cycle assessment (LCA) of LIB reveal that most of the environmental impacts come from the manufacturing phase, consisting of complex process chains, while great uncertainties are often involved. These results show the demand for an optimization of processing steps as well as life cycle inventory (LCI) data collection in lithium battery production. In addition, the current dynamics in ASSB development cannot be matched by conventional retrospective LCA approaches. Furthermore, the expected advantages of ASSB compared to LIB in the technical, economic and environmental dimension cannot be guaranteed with the current state of knowledge. In an interdisciplinary project the development of emerging ASSB production techniques is accompanied by an integrated computational life cycle engineering approach. LCI data collection is realized by massive integration of measurement technology on lab scale and demo scale of unit processes in material, cell, module and system development. In a first step technologies are identified that are especially suitable for LCI data collection, e.g. optical, acoustic or temperature sensor technology. Appropriate metrology enables a systematic and continuous analysis of the effects of new material and any dynamics in production technologies. Measurement data is subsequently prepared and fed into a digital model of ASSB production lines enabling LCA of complete possible future ASSB production. This innovative approach in LCI data collection applied to an early development stage of an emerging technology serves as a basis for prospective LCA.

Integrated Life Cycle Approaches for Decision Support towards Sustainable Development of Existing and Emerging Technologies, Products and Services (P)

5.02P.1

Digitalisation of the supply chain to assess chemicals life cycle - First experiences in using blockchain technology for mass flow analysis

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While promoting sustainable and circular economic models, regulators, industry and civil society in general, are all striving to identify and control potential risks posed by chemicals. To achieve this, developing solid knowledge on how chemicals are used is considered essential.While robust scientific methods exist to assess hazardous properties, generating representative data on chemical uses and applications remains a major challenge. Despite governments are imposing increasing regulatory requirements to force industry to pass information on certain hazardous chemicals along the supply chain (e.g. REACH in Europe, registries of nanomaterials in various Member States, Proposition 65 in California, etc.), we are still far from reaching a robust, comprehensive and constantly updated picture of how, where and in which quantities chemicals are used.Most of the information on chemicals is currently lost during their life cycle, mainly due to the complexity of the supply chains spanning across sectors and regions. The absence of a unique, integrated and trustworthy communication system allowing data owners to control data access, while guaranteeing confidentiality where needed, represents a limitation to the modern vision of sustainable and circular economy.Among emerging technologies that could overcome this barrier, blockchain is gaining momentum in various supply chain related applications: decentralization, immutability and transparency are the key characteristics that allow blockchain to be a valid candidate.The project Chemchain aims at assessing the feasibility of blockchain technologies to create a trusted, globally accepted and open blockchain infrastructure. While allowing industry to exchange and track chemical-related information along the supply chain, the blockchain will accumulate an unprecedented set of data on chemical uses and applications. This poster will present an overview of ongoing pilots, including the potential use of blockchain to support mass flow analysis of metals, looking at how - and in what quantities - they are used across sectors and products, from manufacturing to final disposal and recycling.

5.02P.2

Future environmental impacts of key metals and consequences for low-carbon technologies

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The environmental benefits of low-carbon technologies such as photovoltaic (PV) modules or electric vehicles (EV) have been under debate since their large-scale deployment requires a drastic increase in global metal production. This is of concern since higher metal demand is known to accelerate ore grade decline and can thereby further intensify the environmental footprint of metal supply. To account for this interlinkage known as the "energy-resource nexus", energy and metal supply scenarios need to be assessed in conjunction. We investigate the trends and drivers of future impacts of metal supplies and low-carbon technologies, such as electricity from PV or production of lithium-ion batteries, considering both metal and electricity supply scenarios. We develop metal supply scenarios for copper, nickel, zinc, and lead, for a use in Life Cycle Assessment (LCA) extending previous work. Our models consider future developments such as ore grade decline, energy-efficiency improvements, and secondary production shares. We also include two future electricity supply scenarios from the integrated assessment model of IMAGE using a recently published methodology. Both scenarios are incorporated into the background database of ecoinvent to realize an integrated modelling approach, i.e., future metal supply chains make use of future electricity and vice versa. Thereby, more consistent databases are created representing future systems which can be used as background for prospective LCAs. The scenarios are incorporated with the Brightway2 packages of Presamples and Wurst. We find that impacts of the modelled metal supplies and low-carbon technologies will decrease in the future. Key drivers for impact reductions are the energy transition, increasing secondary production shares, and decreasing hydrometallurgical copper production shares. Among the modelled metals, changes in copper supply revealed the highest influence on reducing impacts of low-carbon technologies caused by metal supply. Considering both metal and electricity scenarios has proven to be essential since they drive impact reductions in different categories, namely human toxicity and climate change, respectively. Thus, an energy transition can only partly compensate for ore grade decline, i.e., only for climate change impacts, but not generally for other impact categories. To complete the picture of future impacts, more integrated and in-depth scenarios are needed as well as scenarios for more metals.

5.02P.3

Joint use of life-cycle approaches and multi-criteria decision analysis tools for evaluation of renewable natural gas production systems

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According to global projections, natural gas will continue to be widely used for the production of energy. However, despite being considered as a transition option, more favourable systems are required for sustainable energy production. Thus, in order to reduce the dependence of fossil fuels, systems based on biomass processing have gained increasing attention. These systems allows obtaining energy products with the quality of natural gas, but based on renewable sources. The main aim of this study is to support decision-making through the evaluation and prioritisation of three representative systems for the production of renewable natural gas (RNG), jointly using life-cycle approaches and Multi-criteria Decision Analysis (MCDA) tools. The following systems were evaluated: (i) manure fermentation and subsequent upgraded of biogas by CO_2 removal, (ii) manure fermentation and biogas methanation using renewable hydrogen from electrolysis, and (iii) biomass gasification in the atmosphere of oxygen and methanation of the resulted gas. Eight criteria were used for the assessment of the selected RNG cases, covering environmental, economic and technical dimensions (the first two analysed under a life cycle perspective): global warming, cumulative non-renewable and total energy demand, photochemical oxidation, acidification, eutrophication, levelised cost of RNG and, efficiency of RNG production system. Regarding the multi-criteria analysis, three MCDA techniques for the prioritisation of alternatives were applied: Weighted Sum Method, TOPSIS, and PROMETHEE. The calculation of the performance indicators leads to different interpretations depending on the dimension evaluated. Case 3 presents the best result of efficiency, while case 1 shows the lowest levelised cost of RNG. Regarding environmental performance, case 2 emerges as a suitable trade-off solution. The application of the MCDA tools allows identifying case 2 as the best alternative when equal-weighting approach is assumed. In contrast, case 3 is selected as the most appropriate alternative when the MCDA tools follow a hierarchy-weighting approach (same weights applied to each dimension evaluated). Thus, this work shows the potential of the emerging RNG systems for production of gas based on renewable resources and how their comprehensive evaluation and prioritisation could support decision-makers towards elaboration of energy plans based on sustainable production patterns and less dependence on fossil fuels.

5.02P.4

Life cycle analysis (LCA) of biojet fuel obtained from Pinus pinaster resin

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The reduction of greenhouse gases (GHG) emissions caused by the aviation industry is a difficult challenge. Alternative fuels can significantly contribute to lower emissions but there are important technical and economical obstacles. Raw materials to produce alternative jet fuels must not interfere with food cultivation; the total environmental impacts, not just CO_2 emissions, need to be considered; and the production process has to yield a product with a cost-competitive final selling price compared to the traditional jet fuel. Hence the importance of performing an accurate Life Cycle Analysis (LCA) and Life Cycle Cost Analysis (LCCA) to assess the feasibility of alternative jet fuels. In this project, LCA and LCCA are used to evaluate the production of biojet fuel obtained from *Pinus pinaster* resin in Spain during 2018. ASPEN PLUS v.9 software was used to simulate (1) the resin distillation to produce rosin and turpentine, (2) the decantation to remove the water from the turpentine and (3) the hydrogenation to remove double bonds present in turpentine composition (α- and β-pinene). Data from this simulation and other collected from companies, papers and different reports were introduced in SimaPro v.9.0. The life cycle to produce biojet fuel considers the whole process, from the pine cultivation, the extraction of the resin, the treatment of the resin for the production of turpentine, its hydrogenation for the production of biojet fuel, the final combustion in a jet engine and the associated transports between processes. In order to quantify the environmental impact, the Product Environmental Footprint (PEF) method was used, considering a functional unit of 1 MJ of biojet fuel. The LCCA took into account not only the actual costs of the production, but also the external costs due to environmental impacts calculated by the environmental prices methodology. The results show that, when considered a yield of 4 kg/year of resin per tree, CO_2eq emissions are 0.0158 kg/MJ, which means a reduction of 83.21 % compared to the fossil kerosene. In addition, considering externalities or increasing the resin yield to 6 kg/year per tree makes the biofuel cost-competitive compared to the price of fossil kerosene. In conclusion, the analysis probes that biofuel obtained from pine resin can be blended with traditional jet fuel and help to reduce the environmental impact of the aviation industry.

5.02P.5

Integration of Product Environmental Footprint with RothC model for the valorisation of organic agriculture environmental performance: the case of organic olive oil production in Italy

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European Product Environmental Footprint (PEF) method allows the practitioner to calculate the environmental life cycle performance of products for several mandatory impact categories, which can be also combined with the use of additional environmental indicators included in the Product Environmental Footprint Category Rules (PEFCR). When applied to agricultural production, these additional criteria can highlight specific aspects of the investigated production process and therefore help to valorise organic agricultural supply chains, to which LCA studies may allocate higher environmental impacts if compared to conventional productions, because the yield strongly affects the results. In this study, after performing the PEF study of an Italian organic olive oil in the framework of the LIFE EFFIGE project (www.lifeeffige.eu), the RothC model was applied, as suggested by the current draft version of the PEFCR for olive oil production, for the calculation of organic carbon storage in the soil of olive groves, with the aim to both valorise the environmental performance of the organic olive oil and highlight the model strengths and weaknesses. RothC evaluates the turnover of organic carbon in topsoil according to the soil type, the temperature and rainfall, the soil moisture and the soil cover of the studied area. A combination of site-specific data provided by the company and literature data were used for the application of the model. Climate data consist of the monthly average air temperature and total rainfall, obtained from regional specific databases and the monthly open pan evaporation, which was calculated by literature formulas and reference data for the studied area. The clay content of the soil, its organic carbon content and the monthly input of organic fertiliser were derived from primary data provided by the company. The input of plant residues was calculated by literature average data, whereas the depth of the soil layer was estimated according to the agricultural work processes in the olive grove in the reference years (2016 and 2017). The results of the annual return of organic carbon to the soil, calculated by the model, will be presented at the conference, as well as the results of different scenarios obtained by the variation of some main parameters, such as the quantity of organic fertilisers and the amount of plant residues. Finally, both the limits of the model and its integration with the PEF results will be discussed.

5.02P.6

Environmental Life Cycle Assessment of Innovative Dry Paper Recycling Technology

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A new 'dry paper recycling technology' that creates new paper from used paper at office have been developed. The developed technologies consist three technologies ; 'defibration technology" for decomposing used paper into pulp fiber, 'sheet forming technology' for forming fibers again into a uniform sheet, 'pressing and binding technology' for increasing fiber density and bonding pulp fibers each other to create new papers. By doing these in dry processes, a small sized and low power consumption paper making machine (named PaperLab) to be able to create on-demand paper recycling system have been realized. This recycled paper can be used to print as a PPC paper and so on. This study aimed at evaluation of environmental aspects of this technology using the framework of product life cycle perspectives. The scope of our assessment includes raw material extraction, transportation, manufacturing, usage and recycling. The machine is assumed to be made at Japan, to be used and recycled at European countries. The result of environmental assessment of this system was compared with that of conventional paper produced in Europe. The assessed inventory items are carbon dioxide and water consumption. According to the result, this new system is revealed to contribute the reduction of 90 percentages of water consumption, because it enables us to avoid the water usage in making pulp for recycling paper.

5.02P.7

Integrated environmental and economic life cycle assessment of vanadium redox flow batteries

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The rising production of electric energy from renewable sources (wind and photovoltaics) requires electrical energy storage systems to compensate for fluctuations in energy generation. Especially the vanadium redox flow battery (VFB) is a promising battery system with unique properties, which can make a valuable contribution to the reliability of electric energy supply. The power and the capacity of VFB can be individually scaled. Thus, a stationary battery can be customized for nearly every requirement in terms of power and energy capacity [1]. VFB can achieve low energy specific costs for high capacities and the battery also offers the opportunity for a sustainable production [2]. However, an integrated economic and environmental assessment of VFB has not yet been considered in detail in the literature. In order to close this gap, we present the results of an integrated environmental and economic life cycle assessment of megawatt-scale VFB storage systems. Since the change in energy supply require large scale approaches our study focusses on batteries with 1 MW nominal power and reasonable nominal capacities of 4 MWh and 8 MWh. Based on primary data, a detailed life cycle inventory as basis for integrated LCA and LCC has been developed. By evaluating the data, potential weaknesses and optimization possibilities of this battery system were determined in the life cycle phases of manufacturing, use and end-of-life. To reduce costs and emissions a recycling of components is necessary. Thus, a focus of our work is to analyze different recycling paths and the consequences on the costs and potential environmental impacts. Some critical components result in very distinct energy consumption or comprise severe environmental risk due to their toxicity. Recycling or reusing these components plays an important role to reduce the specific costs of the energy storage system and to lower the environmental impact. A detailed assessment of the end-of-life enables a path to life cycle engineering and thus, a sustainable production of key components of VFB systems. [1] C. Minke, U. Kunz und T. Turek, *Journal of Power Sources* **2017**, *361,* 105–114, DOI: 10.1016/j.jpowsour.2017.06.066. [2] S. Weber, J. F. Peters, M. Baumann und M. Weil, *Environmental science & technology* **2018**, *52,* 10864–10873, DOI: 10.1021/acs.est.8b02073.

5.02P.8

Life cycle assessment of rubber tires: Evaluation of the environmental benefits of a new activator in the vulcanization process

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Tire manufacturing is a large and growing industry with its corresponding impacts on the environment. Zinc oxide (ZnO) is an activator used in the vulcanization process during tire production. ZnO is added to the rubber matrix to increase vulcanization efficiency and the mechanical properties of resulting tires. It is in turn released in different forms in the environment through tire wear and tear. It can also leach from crumb rubber which is used in several civil engineering applications. High zinc concentrations have been shown to have negative effects on ecosystems. Consequently, scientists and the tire industry have been pushing for a reduction in the use of ZnO in tire production. To achieve that goal, recent research describes an alternative activator composed of ZnO nanoparticles (NPs) anchored to Silica (SiO2) NPs, namely, ZnO-NP@SiO2-NP. Silica is also employed as a reinforcing filler in rubber compounding. The method of production has been validated on the lab scale and is currently being upscaled within the EIT-RawMaterials project SAFE-VULCA. The resulting rubber compounds meet the mechanical characteristics required of commercial tires, allowing a reduction of ZnO amount by increasing its efficiency. To see whether the alternative technology is environmentally beneficial, Life Cycle Assessment (LCA) is used to assess the impact of a passenger tire made using ZnO-NP@SiO2-NP. The results are compared to the impact of a traditional tire. Emphasis is put on (i) the impact of zinc released via multiple pathways and (ii) different end-of-life scenarios. The former is addressed by specifically including the characterised impacts of zinc in the impact assessment phase. The latter is addressed by including results of recycling tests that are conducted within SAFE-VULCA. Because the production of the new activator is currently being upscaled, the environmental impacts of the future industrial-scale process need to be prospected for a fair comparison. To do so, a systematic approach is followed, including expected process changes, size scaling, process line synergies, and industrial learning. This work intends to provide new insights into the environmental impact of a tire's life-cycle which are scarce in current literature. It shows the practical assessment of the environmental impacts of an upscaled technology, assisting with decision making. Finally, it attempts to draw attention to tires as a source of zinc contamination in the environment.

5.02P.9

Life Cycle Assessment of Flame Retardants: LIFE-FLAREX project

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Flame retardant (FR) textiles are extremely important since they offer human and environment protection against fire. In recent years, increasing concerns about the toxicological and environmental consequences of using halogenated FR chemicals on textile substrates have been raised. Many of these chemicals are now recognized as global contaminants and are associated with adverse health effects in animals and humans, including endocrine and thyroid disruption, immunotoxicity, reproductive toxicity, cancer, and adverse effects on fetal and child development and neurologic function. LIFE-FLAREX project aims to contribute to the mitigation of the environmental and health impacts on European ecosystems caused by toxic compounds of FR textiles additives. There is limited information available about the environmental behaviour and toxicological properties of FR, and their potential impact on the environment is still difficult to be assessed in an accurate way. In order to evaluate the FR, a Life Cycle Assessment (LCA) has been performed to compare halogenated FR with more environmental friendly alternatives. The LCA has been developed based on ISO 14.040 and ISO 14.044; following the four interrelated steps: goal and scope definition, life cycle inventory analysis, life cycle impact assessment and interpretation of the results. During the goal and scope task, the following aspects have been defined: The objective of this LCA is to compare the environmental impact of environmental friendly alternatives with conventional FR in different types of textileFunctional Unit: 100m^2 of textiles finished with FR.System boundaries: life cycle stages included in the system boundaries are raw material extraction, production stage, use and end-of-life. Also the transport is included in the assessment. For LIFE-FLAREX project, the LCA methodology selected is the ILCD impact assessment method, released by the European Commission, Join Research Centre in 2012. The impact categories selected to express the environmental performance are: Climate change, ozone depletion, human toxicity (cancer and non-cancer effects), acidification, freshwater eutrophication, freshwater ecotoxicity and mineral, fossil & renewable resource depletion. LIFE-FLAREX will provide more data to know the environmental performance of specific FR. Despite at this stage of the project there are not final results to show, it is expected to present preliminary results during the coming months.

5.02P.10
Ecorkwaste: environmental and socio-economic assessment of cork waste gasification
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As one of the leaders in cork production, Catalonia faces the problem of sustainably disposing the industry related residues, approximately 15% of the extracted raw cork ending up as forestry waste, while considerable shares of the processed cork are also considered cork residues. Indeed, cork residues are usually sent to landfill with no recovery or second use, as there are few specific "collect and recycle" programs. The present work suggests gasification as a thermal strategy to convert cork wastes into energy, so that this debris is energetically valorised. This contributes to the implementation of the Waste Framework Directive (2008/98/CE) following the objectives and goals of the Roadmap for a Resource-Efficient Europe. Technological, R&D and industrial partners were gathered under the framework of the LIFE+ Ecorkwaste Project and thus, a gasification plant with capacity to treat 15 kg of cork residues per day was designed. Life cycle assessment (LCA) and life cycle cost (LCC) were performed to assess the environmental and techno-economic impacts, as well as a socio-economic matrix integrating various aspects of the technique was developed to evaluate the social impacts. The functional unit was "the production of 1 MWh by means of cork valorisation through gasification technology" and a comparison with the conventional scenario of using the national electricity grid was performed. The LCA scenarios of possible management strategies for the produced wastes were modelled using Simapro Developer and the CAPEX-OPEX concept was applied for the LCC. Major remarks are the fact that the gasification strategy depicted a sustainable profile with lower environmental impacts than the conventional scheme of energy production while also entailing lower costs. Cork gasification proved to be a key basis to the economic and social development at a regional scale. Keywords: waste-to-energy, LCA, LCC, social assessment

5.02P.11
Simultaneous Heat and Cooling from District Heat - Environmental Impacts of the Innovative Thermal Energy Conversion Process TeTra
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District heating (DH) systems supply heat efficiently to urban customers. However, there is a growing need for a simultaneous supply of not only heat, but also of cooling services. The innovative device "TeTra" is based on an absorption chiller process, but it can provide not only cooling services, but also heat.The objective of this work was a prospective screening LCA that compares two ways of providing heat and cooling simultaneously from DH: a) a two-device reference system with a DH-transforming heat exchanger that provides DH to customers and a DH-driven absorption chiller that provides cooling, and b) the novel, one-device TeTra system that provides both heat and cooling from a DH input.The LCA followed a system expansion approach to ensure functional equivalence. An additional source of off-heat from a large natural-gas fueled combined-heat-and-power (CHP) plant was added to the reference system to compensate for its lower efficiency in using DH. Both systems are sized to provide 5 kWth of cooling power at 10 °C and 10.6 kWth of heat at 65 °C to a customer, plus 6.3 kWel electricity. The electricity output is a co-product from the additional CHP heat source in the reference system that is matched by a generic Austrian electricity mix in the TeTra system. Primary data were obtained from manufacturers of heat exchangers and absorption chillers, from the DH network operator, and from thermodynamic system simulations using the IpsePro software. For secondary data and upstream processes, the ecoinvent database version 3.5 was used. LCA modeling involved the open LCA v. 1.9 software and ReCiPe (H) Midpoint and CED as impact assessment methods.Results show that the TeTra system is favorable in combustion-related impacts such as global warming potential, acidification potential, and non-renewable cumulative energy demand. This is almost exclusively due to the system expansion processes, that is, the production of additional CHP heat in the reference system, and the provision of additional electricity in the TeTra system. The production of the absorption chiller, heat exchanger, and the TeTra device, is only relevant when resource use impacts are considered, e.g. metal depletion. Overall, the LCA demonstrates the usefulness of the system expansion approach for comparing energy systems and its results suggest that the TeTra system could be an environmentally favorable way of providing heat and cooling simultaneously through DH systems.

5.02P.12
Life Cycle Assessment (LCA) as a decision support tool to achieve more sustainable ecosystem for port operations: the case of PortForward Project.
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Future Ports will be able to enhance sustainable development, managing resources efficiently. Moreover, future ports will be oriented to port community. In this context, port of the fu¬ture will be smart, through the application of ICT solutions, to improve exchange of information flows between port and port community. Also, port of the future will be interconnected by using different modes of transport and the integration of different technologies. Finally, port of the future will be green through the adoption of green technologies, with the purpose of reducing environmental impacts of port operations, saving resources and promote economic and social benefits. PortForward Project is in line of all these premises. Main objectives of PortForward are: - To introduce and Internet of Things (IoT) concept for port assets (infrastructure, vehicles, cargo, people). - To analyse the environmental and the socio-economic influence of the port interface with the surrounding are and the port-city, as well as the rest of the logistic value chain. To achieve the second objective, a Sustainability Assessment will be performed, based on the Environmental Life Cycle Assessment (LCA), Social LCA and Life Cycle Costing (LCC) methodologies. Sustainability Assessment will be focused on the case study of the Port of Vigo, and specifically, for the Container Terminal operations. Three main group of activities will be included in this analysis: 1) The Yard Crane Operations, including the loading and off-loading of vessels and the cranes use; 2) The Storage Yard, considering the movement of containers by using as machinery the reachstackers or the transtainers, and also considering the electricity consumed in the refrigerated containers and cleaning operations of containers when needed; 3) The movement of vehicles, both internal port trucks and external port trucks. A comparative analysis is being prepared between baseline scenario and the scenario with the improved operational performance of the port, after the implementation the of PortForward solutions. Preliminary results of environmental assessment reveals: • Main environmental impacts of container terminal are related to the vessels loading/off-loading operations. • The second main environmental impact contributor is the movement of vehicles. • The use of transtainers contributes with the third main environmental impacts, followed by reachstackers. • Cranes cause the lowest environmental impacts in the container terminal.

5.02P.13
Should We Replace Plastic In The Milk Supply Chain?
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Plastic is found throughout the milk production life cycle, from the wrap used to preserve silage to the bottle used to contain the final product. However, the durability and low cost of plastic that have led to its ubiquity also make it potentially environmentally damaging, and its impact is a concern to many consumers. Industries including agriculture and food production are feeling significant consumer pressure to reduce plastic use. However, the replacement of plastic products with alternative materials without full consideration of the impacts risks simply shifting, rather than reducing, the environmental risks.The "Plastic: Redefining Single Use" project at the Grantham Centre for Sustainable Futures aims to consider the entire life cycle of milk in the UK. By studying the full product life cycle and a range of alternative scenarios at key stages, the total impact of reducing plastic use can be considered. Two interlinked studies will be described: A cradle-to-gate study of the production of milk on a UK dairy farm, and a gate-to-grave assessment of the impact of milk packaging, considering the following interventions:Reducing plastic use by reducing silage use and increasing the use of supplementary feedsReducing plastic use by reducing silage use and increasing the duration of summer grazingIncreasing the recycling of agricultural plastics such as silage wrap and cover materialsReducing plastic by supplying milk in glass bottles for consumer purchase and steel churns for café useReducing plastic use by supplying milk in bio-based plastic bottles instead of fossil-based plasticAn initial delivery comparison suggested that the use of plastic bottles produced CO2 emissions over seventeen times greater than the delivery of milk in churns. Energy use per litre was around seven times lower in the churn case, and water use sixteen times lower.Initial results on the impact of alternative feeds and the potential to increase their use and reduce winter silage requirements highlighted the key role of imported ingredients. If a feed contains ingredients such as Soy or Palm kernels, global warming impact is significantly greater than feeds which do not contain these ingredients. However, the manufacture of a single use Polypropylene bag was found to make up over 75% of the total CO2 emissions of the combined product. Efforts to reduce plastic use must be carefully considered to avoid simply transferring impacts.

5.02P.14
Detecting Inter-Industrial Clusters in the 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 footprint of their products to contribute the climate change mitigation, thus the inter-industry (or inter-firm) collaboration is essential in order to reduce the supply chain emissions effectively. To detect the critical supply chains for emissions, the environmentally extended input-output analysis has been used to trace the embodied emissions in whole supply chains. Some previous studies regarded the set of industries and the inter-industrial transactions as the "network", and applied the network analyses to the input-output tables to identify environmentally important industries which should be collaborated, that is, the clusters. However, the clustering methods used in these studies have some problems specific to the input-output analysis. These problems can occur by integration of the multiple hierarchical supply chain network into single hierarchy for ease of handling. This study develops a new approach to detect industrial clusters for input-output analysis that takes into account the hierarchical supply chain network structures. Here, we apply the cluster coefficient to the input-output framework and introduce the new concept "I-O clusterness". The degree of I-O clusterness in this study can be interpreted as the degree of backward and forward linkage effect of three industries and three transactions that construct the cluster in the global supply chains. We used the EXIOBASE 3 and calculated the I-O clusterness scores for GHG emissions with a focus of the industries in Japan, United States and China as a case study. This new approach can help firms to find collaborators and mitigate GHG emissions in their supply chain networks.

5.02P.15
A two-fold perspective on End of Life of photovoltaic panels not properly confined
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Nowadays, the photovoltaic (PV) technology is widely applied and the PV panels are powerful sources of renewable energy. Hence, since the early 1990s, significant PV installations were carried out and, considering that PV panels have a lifespan up to 25-30 years, an increasing number of modules will reach the end of life (EoL) in the coming years. If not properly handled, EoL PV panels can release dangerous substances, as trace metals, with adverse effects on the ecosystems. Therefore, the environmental loads and the potential ecological risks deriving from PV panels not properly confined have to be also carefully assessed. Starting from a case study on the metals released from two different types of PV panels, namely crystalline silicon (c-Si) and thin film (TF) panels, the consequent environmental impacts are evaluated. by means of a two-fold approach based on Life Cycle Assessment (LCA) and Ecological Risk Assessment (ERA). SimaPro 9.0 software, coupled with the ReCiPe H v.1.03 Midpoint method, is used in compliance with the LCA ISO-standards. The focus is set on the freshwater and marine ecotoxicity impact categories, since the investigated baseline scenario envisages an EoL PV panel not properly confined and exposed to rainfall, with a consequent leaching of metals in the environment. The propagation of the uncertainty linked to foreground data input (concentration of released metals) and output (generated impacts) is tested by means of Monte Carlo function. The use of local emissions experimentally measured allows, in this study, to overcome the limit of LCA methodology providing an overview of potentially generated impacts far away from the streamlined procedure. However, the preliminary results show that the impacts produced on the investigated impact categories are highly variable and negligible at a certain extent, calling for a further validation in terms of real effects on the investigated ecosystems. Indeed, the screening evaluation of potential ERA posed by metal release, conducted applying a combination of Hazard Quotient and the Ecotoxicological Index, indicates that the detected metals represent a risk for aquatic organisms for both c-Si and TF PV panels. The application of both methodologies (ERA and LCA) in the present case study confirms the need for a tailored LCA including a deeper understanding of the environmental hazards that ERA is helpfully capable to turn into quantitative risks.

5.02P.16
A conceptual framework for Integrating Life Cycle Assessment and Environmental Risk Assessment
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Integrating life cycle assessment (LCA) and Environmental risk assessment (ERA) has been driven by the need to achieve a more complete environmental impact assessment that genuinely address both local and global impacts, to appropriately inform decisions and policies related to specific product or process systems. Previous studies integrating LCA and ERA vary in terms of 1) methodological choices (e.g., components employed, and stages considered in the assessment) and 2) the type of indicators used in reporting outcomes of integrated assessment. The omission of important components of either LCA or ERA, and significant variations in the outcomes of integrated assessment, leads to difficulty comparing studies, and lack of clarity in available or required knowledge to progress in this field. A comprehensive approach representing a set of procedure that capture the theories and principles of both LCA and ERA, is needed. Such tool is important in establishing an understanding of various needs, concept and constraints in integrating LCA and ERA, to help obtain more harmonized, objective, and robust environmental assessments across various studies. A conceptual framework was developed to serve as a guide for integrating LCA and ERA, in choosing the appropriate components towards achieving a balance, and consistency in assessments, and provide a basis for comparison of outcomes of integrated assessments. The framework incorporated an interactive feature between LCA and ERA, to enable identification and management of problem shifting. An interface between LCA and ERA consisting of elements associated with the individual tools was created, to develop an understanding of the potential exchange of elements between LCA and ERA, and was used as a basis for selecting the relevant components of the framework. A tiered approach was employed to organise and prioritised assessment stages. The main features of the framework include 1) Definition stage (Problem formulation), 2) Hazard identification and risk prioritisation 3) Exposure Assessment and 4) Impact assessment. The interactive feature of the framework provides the opportunity to identify certain pitfalls early in an assessment, improve exposure assessment and understanding of the underlying trade-offs. Keywords: LCA; ERA; Framework; Impact Assessment

5.02P.17
How to conduct prospective life cycle assessments for emerging technologies A systematic review and methodological guidance
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Emerging technologies have the potential to support sustainable development. However, the environmental assessment of emerging technologies is challenging, especially at an early stage of development. A review of studies concerning prospective life cycle assessments (LCAs) of emerging technologies was systematically conducted to answer the question: how can LCA be applied prospectively to emerging technologies? We found 33 case studies, two review papers, and 13 papers on methodological guidance on how to perform a prospective LCA of emerging technologies. Prospective LCA is recommended to, for example, comparatively assess emerging with mature technologies. Three main challenges for conducting prospective LCAs were identified by the application of the content analysis: Namely, comparability, data, and uncertainty challenges. Issues on defining the aim, functionality, and system boundaries of the prospective LCA as well as the specification of LCIA methodologies comprise the comparability challenge. Data availability, quality, and scaling are issues within the data challenge for the application of prospective LCA. Finally, uncertainty challenges exist as an overarching issue when applying prospective LCA. These challenges are especially crucial for the prospective assessment of emerging technologies. However, this review shows that within the methodological papers and case studies several approaches exist to tackle these challenges. These approaches were systematically summarized within a framework to overcome the issues when conducting prospective LCAs of emerging technologies. Therefore, this framework is useful for LCA practitioners who are analyzing early-stage technologies. Finally, most authors conclude that performing prospective LCAs lead to new insights and could guide policymakers. Nevertheless, further research should address scale-up schemes and include uncertainty analyses for a more in-depth interpretation of results.

5.02P.18
LCA as decision support to understand and reduce potential environmental impacts of advanced materials and processes developed or applied in the EU Horizon 2020 PRESTIGE Project
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PRESTIGE is a Horizon 2020 project with the central aim of developing advanced materials and processes (printing and overmoulding) that offer better performing functionalities and enable high-end aesthetics. To achieve this, PRESTIGE splits into several business case studies, each focusing on a particular technological application, including a haptic effect in a steering wheel to assist safer and future autonomous driving (electroactive polymers), decorative energy harvesting integrated into a wristband (photoactive and energy storage materials), and smart labels for new packaging solutions (conductive inks, coatings and nano-coatings). LCA, based on the ISO 14040/44 methodology, has been chosen as the primary tool to evaluate and support the development process and results from an environmental perspective. For this purpose, a goal and scope has been defined for each of the project's three main business cases, and the collection of complex inventory data has been initiated. Cumulative energy demand will be analysed in addition to a set of relevant environmental impact categories. In regard to the novel photovoltaic and energy storage technology applied within the project, additional indicators such as Energy Payback Time and Embodied Energy serve to

evaluate the performance of the project outcomes. As most of the advanced materials and processes are not covered by current LCA databases, it has been found necessary to create new processes based on secondary resources, estimations and approximations that pose additional challenges for the completeness and reliability of results. Nonetheless, initial partial screening results regarding potential environmental impacts have been obtained and applied to influence the project trajectory towards more sustainable solutions.

5.02P.19

Scaling approach to assess the potential of the use of wood in an emerging market through material flow analysis

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Wood-based structural products are gaining interest in the building sector, due to their potential in sequestering greenhouse gas emissions. Their increasing uses are allowed by developments in fire and structural performance, design expertise, and regulations. However, increasing wood materials use in the built market can have unintended consequences in the wood supply chains and modify the balance between supply and demand. Consequential Life Cycle Assessment (CLCA) allows the assessment of changes in the material supply chain. To quantify and link those consequences, the affected physical flows need to be estimated. However, the emerging market assessment requires data that may not exist because of the use of technologies at the early development scale. Material Flow Analysis (MFA) can build a framework of flow for CLCA modelling with representative and quantitative information by filling some data gaps. Indeed, MFA considers physical constraints (technology performances and material availability), in addition to account for mass balance. Wood flows are tracked to identify their end-use markets and trends in consumption. To bring insights into the sector's dynamics, such as stock variations and potential discarded flows that supply dependent markets, MFA and residence time model are used. To overcome the lack of data on wood consumption, extrapolations and correlations between physical and economic parameters are also used. The estimation of the increasing wood consumption for NR buildings employes parameters, that are challenged through sensitivity analysis. According to assumptions on parameters, results show how flows can increase in the market before reaching their physical constraints, such as the available wood stock in the forest. Main results present wood flows for NR building structures and for the harvested roundwood. The insight into the parameters and the modelling strengths and weaknesses show how results can be sensitive. This sensitivity is relative to the scale of the NR market and the harvest. Even if the role of the approach is to help life cycle inventory on the potential of the use of new product, more research on parameters desegregating can improve it.

5.02P.20

Use of a simplified LCA tool in the naval sector: advantages and limits of the methodology.

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Although life cycle assessment has become a well-established tool to support eco-design within a wide range of industrial sectors, the naval sector is still lacking behind and not many LCA studies that investigate the effect on the environmental footprint of different designing choices can be found in the literature. To drive a change of pace, and spread the adoption of the LCA approach within the naval industry,a new simplified LCA-based tool dedicated to the naval industry has been developed. The aim of this parer is to investigate the advantages and the limits of this new software, as well as its investigating then its suitability for the naval sector, and to do so the tool has been implemented in a case study concerning the desingning and production of hull for a sailing boat realized by the University of Padova. The methodology and results have been analysed in parallel to those of a full LCA performed using a traditional software (SimaPro). Although the study is still a work in progress, its already possible to notice some differences with SimaPro: the model of the product system is already incorporated within the software and for a specific unit process the data required are readily available at the even at the designing stage. This simplifies a lot the inventory phase. The impact categories considered in the LCIA are only seven and, opposite to SimaPro, there is no possibility of adding or removing one or more. The elimination of the modelling phase makes the LCA study accessible by a non LCA expert. The choice to limit the database to the most common materials and practices might stop companies from investigating innovative solutions. In the same way, limiting the number of impact category makes it easier to communicate the results to the stakeholders, but not giving the option of selecting different ones will prevent companies from assessing the impact in categories they consider important. At this stage of the study it is not possible to say if this new software is the right tool for the naval sector. For now, the main advantage of this software is to spread to a new sector the LCA approach.

5.02P.22

Integrated approaches to analysis of the influence of feedstocks and processing technologies on valorisation of bio-waste streams

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Within the context of circular economy (CE), different bio-waste materials are attractive potential sources of functionalised molecules, that can enter chemical supply chain as higher value intermediates, rather than be degraded to syngas, or used for energy generation. Specifically, for the case of the paper industry waste, terpenes contained in the crude sulphate turpentine (CST) or 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-waste, however, is not a clean feedstock, and is characterised by significant variability and, often, heterogeneity. Frequently, there may be more than one possible choice for the bio-waste 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 feedstock and purification technology options as a more holistic analysis. Thus, integration of process modelling for technological scenarios generation with life cycle assessment (LCA) for subsequent evaluation of environmental impacts and costing is the most appropriate method of evaluating the holistic problem. The aim of this work is to develop LCA-based tools for assessment of technologies at the early stage of development. For this, case studies that target (i) identification of the most environmentally and economically viable routes towards platform molecules based on terpenes sourced from the bio-waste streams and (ii) demonstration of the generic methodology of assessment of chemical processes within CE, are developed. We demonstrate that mutiobjective decision making based on rigorous models and life cycle impacts evaluation can be achieved through an integrated approach. We further have shown that using life cycle scale of analysis and information on life cycle impacts is critical in decision making on the choice of a suitable upstream processing steps for bio-waste conversion.

LCA for Urban and Territorial Footprinting: Advancements, Trends and Applications to Promote Sustainable Consumption Patterns and Territorial Management (P)

5.03P.1

A methodology to identify sustainability indicators in small, medium and large cities in Spain

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The growth of the urban population in recent decades has concentrated most of greenhouse gases emissions, resource consumption and waste generation in the cities. In addition, this situation also affects socio-economic parameters such as unemployment rate or poverty. Besides, the concern for assessing sustainability in urban systems is reflected in the Sustainable Development Goals of the United Nations. Sustainability assessment of cities with indicators is a methodology that considers aspects of the three traditional pillars of sustainability: social, economic and environmental. In the present study, the sustainability of 32 Spanish cities is evaluated using three indicators (one per sustainability pillar): woman unemployed rate (social), city unemployment rate (economic) and MSW collected (environment). These three indicators have been identified as key indicators by means of the combination of two analysis models: CART and Random Forest. In addition, the models of analysis also determine the corresponding thresholds for each indicator. Furthermore, according to the number of thresholds that are exceeded in a city, it can be classified following a colourful ranking. Cities that are considered sustainable are marked in green; cities highlighted in yellow exceed only one of the thresholds; cities that overpass two thresholds are displayed in orange; and cities that surpass all thresholds are shown in red. Bearing in mind the results, most of the cities in red are located in the south of the country, especially in the region of Andalusia. Thus, the differences between the cities located in the north and the south of the country in terms of sustainability are remarkable, so that the sustainability of cities can only be evaluated with the three key indicators without requiring a large amount of data, providing useful information and support to policy makers.

5.03P.2

Exploring complementarity of Life Cycle Thinking & Landscape and Urban Planning towards sustainable urban co-design Case study: a last kilometer electric tricycle-based logistic technology

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Nowadays, sustainability is gaining importance in territorial policies. Yet, no substantial efforts have been deployed for systematically integrating environmental, social and economic aspects in early stages of urban service co-design. While Life Cycle Assessment (LCA) is a suitable method for impact assessment of products and services, Landscape-Urban Planning (LUP) provides information on how these products and services interact at the territorial scale. Our understanding of urban systems' needs could go a step further towards better consumption patterns and resources optimization by coupling both approaches.This work explores the synergy between Life Cycle Thinking and Landscape-Urban Planning by their application to an essential urban service: logistics. The challenging metabolism of high-density urban systems is taken as an opportunity to consider emergent logistic solutions, specifically electric tricycle technologies for last kilometer delivery.Firstly, we start by applying each approach individually; following LUP perspective, we define a territorial reading grid, based on a set of criteria describing physical, socio-cultural and economic resources of a landscape. Then, using LCA, we quantify potential impacts or benefits of electric tricycles technology compared to the existing delivery technologies. Electricity for tricycle batteries' charging is provided by photovoltaic systems.In a second step, results from both approaches are coupled to determine local urban hotspots that describe the main aspects to meet stakeholders' needs and improve their living conditions. Sustainability design parameters are, thus, defined to optimize the tricycle technology.Finally, the design and optimization of the electric tricycle-based delivery system is carried out. The Geographical Information System (i.e .Quantum GIS) is used to explore local data on solar energy potential spots, cycling infrastructures, potential urban logistic platforms, etc. Energy required has been computed to determine adequate charging infrastructures, taking topography and climate conditions into account. Intelligent logistic planning is performed to improve efficiency, optimize the streamline to reduce environmental impacts and simulate interaction with other urban transportation flows.Based on the current case study, the urban service co-design framework has been fully established in order to gain sustainability in a territorial management scale through LCA and LUP perspectives.

5.03P.3

Handprint approach turns the focus on consumer's impact

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Over the recent years more and more countries and organizations have unveiled their net-zero targets. The net balance calculations are based on greenhouse gas (GHG) emission accounting and reporting standard created by Greenhouse Gas Protocol. However, there is a significant deficiency in the instructions as they do not demand the inclusion of GHG emissions derived from a consumption. Therefore, a remarkable share of GHG emissions arisen in a decision making of the inhabitants are excluded. Anyway, in order to curb climate change it is essential to pay attention to the purchase decisions either individuals or firms are making daily. GHG emissions of a product chain are very much dependent of the provenance of individual products. Carbon handprint is an approach by which a manufacturer aims to show to its customer how much their product can reduce customer's carbon footprint of a product. The approach applies carbon footprint ISO-standards and reflects the positive climate impact on customer's product. The approach suggest the use of primary data from the use and end-of-life phase and calculation of avoided emissions is acceptable. The carbon handprint approach presents new definitions and consistent guidelines for comparing the possible handprint product against the baseline product. The handprint results from the reduction in greenhouse gas emission when a handprint product is utilized instead of a baseline product. The developed approach is demonstrated with one case study about renewable diesel. Results of the case study indicate the carbon handprint that can be achieved when a customer chooses renewable diesel instead of baseline diesel fuel. The results show that the carbon handprint exist for renewable diesel in case it replaces baseline diesel fuel. The customer in this case was a typical consumer of diesel fuel, but many other possible customers can also be recognized like logistics service providers. The handprint approach brings a missing part to carbon balance calculations. From cities and organizations perspective, it will hopefully also increase the supply of such climate friendly products in which the actual climate impacts in an operating environment are taken into account. Hopefully, it will also turn the direction of climate discussions more to purchase decisions what either business customers or individual consumers are doing.

5.03P.4

Comprehensive Analysis of Carbon Footprint Based on the Relative Location in the Global Supply Chain Networks

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For equitable and effective policy making of mitigating climate change, environmental emission accounting has been developed in the Input-Output analysis. Production-based accounting tells us larger emitters (e.g., energy sector), and consumption-based accounting enable us to understand larger final consumer inducing larger emissions (e.g., construction sector). Liang *et al*. (2016) suggested betweenness-based accounting for effective emission reduction through the approach focusing on the critical transmitters (i.e., the sectors emerging in a lot of supply chain paths with large emissions such as metal sector). Comprehensive analysis combining above three emission accountings gives us useful information for environmental policy making. Large production-based accounted sectors are often upstream sectors in production process with high emission intensity and needs technological improvements. On the other hand, most sectors with large consumption-based emissions are downstream sectors in production process and consumption policy such as eco-labeling is effective. High betweenness-based accounted sectors are located in middle-stream of production process and need green supply chain management. Hanaka *et al.* (2019) developed these accounting and allocated the emissions associated with a production in a country to "production-oriented emissions," "consumption-oriented emissions" and "betweenness-oriented emissions." Using this method, they clarified what approaches (technical improvement, eco-labeling or supply chain engagement) sectors should take, and concluded which countries with large emitting sector should follow. In this study, I expand the methodology to transaction between sectors, and suggest a new visualization that provides the information which countries and transactions are responsible for large emissions in global supply chains and what policy approach reduces the emissions associated with the countries and transactions effectively. I used Eora26 database (Lenzen *et al*., 2013) and visualized the emission responsibility map in 2015. The new methodology can give us the information for mitigating policy making more understandable than conventional visualization.

5.03P.5

Carbon footprint reduction potential of waste management strategies in tourist cities

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Tourism is one of the major economic factors contributing to growth and jobs worldwide. An average value of 1.67 kg waste is generated per tourist and overnight stay. Waste prevention and recycling should therefore be major objectives in tourist waste management by municipal authorities. Within the EU H2020-funded project "URBANWASTE – Urban Strategies for Waste Management in Tourist Cities", eco-innovative waste prevention and management strategies were implemented in 11 pilot cities with high levels of tourism, in order to reduce urban waste production and improve municipal waste management. Based on case studies the impact tourism has on waste generation and associated greenhouse gas emissions has been assessed and the possibilities to mitigate greenhouse gas emissions by eco-innovative waste prevention and management strategies has been evaluated. The assessment of GHG savings followed the Life Cycle Assessment approach (ISO 14044). The goal of the assessment was to provide qualitative data that show how waste prevention and improved waste management practices employed in the selected pilot cities have performed in comparison to the (status quo) activities. All pilot measures achieved a saving in greenhouse gas emissions compared to the situation before the implementation of the measure. The time frame for which changes were measured and reported differed between the pilot measures. For comparability reasons reported data was normalised to one year. Especially cities still disposing waste on landfills achieved in particular for pilot measures dealing with the prevention of food waste comparable high relative and absolute greenhouse gas emission savings. Drawing conclusions based on overall and absolute savings achieved per pilot measure has some limitations. Some of the measure had a very wide reach and reported data was based on several hotels while some where only implemented on a small scale. All pilot measures had the functional unit of 1 kg of waste treated or prevented.

5.03P.6

Carbon footprint for sustainable tourism and mice in japan

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More than 3 million people travel around the world a day, and about 1.2 billion people travel abroad every year. It has been pointed out that tourism that has brought about a huge amount of movement has a particularly high impact on climate change. In existing papers, macro-level evaluation using the Input-Output Table (I / O) was conducted. The world's tourism industry's CO2 emissions (CO2eq) were reported to be four times the existing calculations. As for international trends, it is said that "supply chain evaluation", "multi-criteria evaluation" and "materiality identification" are important. The SDGs recommend a life cycle approach. However, Japan does not quantitatively evaluate the environmental impact of tourism and MICE. In this study, Japan's tourism and MICE will be quantitatively evaluated using the carbon footprint (CFP) to understand the current situation. In addition, the results are broken down for each inventory item to confirm the high impact. This study has achieved the following: -Quantitative evaluation of GHG emissions , -Breakdown to high impact inventory items , -Establish procedures for quantitative evaluationHowever, the following issues need to be cleared in the future. Individual product services cannot be evaluated in detail. And load changes with money. It is known that environmental responses (resource efficiency, etc.) are difficult to reflect in the results.

Life Cycle Impact Assessment and Ecosystem Impact Assessment: Strengthening the Link Between LCA and Ecology (P)

5.04P.1

Integration of ProScale toxicity potential assessment in LCA applied to Utility Poles

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So far, methods to assess human toxicity impacts are focusing on the indirect impact of chemicals emitted into the environment. ProScale was therefore developed as a method for a hazard and exposure-based quantitative scoring system for comparing direct chemical risks (corresponding to near-field human toxicity) to workers, professionals and consumers associated with products in a life cycle perspective. This work presents the integration of ProScale assessments in LCA, applied to a case study on utility grid poles.This LCA study is carried out to assess the environmental impacts, of production, use and end-of-life of utility poles, i.e. poles used to carry air cables used for electric power transmission. Toxicity potential assessments using ProScale are an intergrated part of the assessment. Preliminary and only partial results are provided here for the production phase for one of the poles under study for illustrating the integration of ProScale in LCA – a polymer composite pole, made up of structure of glass-fibre reinforced polyester, covered with a layer of polyethylene.The results achieved are “obvious” in the sense that the ProScale methods points at other components or processes as being “Hot spots” in a dominance analysis, compared to other impact categories, here illustrated by global warming potential. This is in line with expectations. Comparison with USEtox for he pole case is in still in preparation, but previous theoretical comparisons predicts large difference also in this case due to the different scope and model parameters for ProScale compared to USEtox. This illustrates well that ProScale adds value to LCA studies. A webtool and a default ProScale database has been created and is gradually populated to facilitate the convenient use of ProScale. Discussions are ongoing with the EU Commission regarding how the ProScale method, tools and data bases can be offered to support and supplement the PEF method package regarding direct chemical risks to workers, professionals and consumers associated with products in a life cycle perspective.

5.04P.2

Development of a parsimonious characterisation model to address space debris emission-related damages for the LCA of space systems

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Satellites orbiting Earth are used in many areas and disciplines, including space science, Earth observation, meteorology, climate research. A rising sustainability concern is occurring in the space sector: 29,000 human-made objects, larger than 10cm, are orbiting the Earth but only 6% are operational spacecraft. The others, considered as space debris, are today a significant and constant danger for all space missions. Life Cycle Assessment (LCA) has been identified as the most appropriate methodology to measure the environmental impact of space activities. However, there is still a need to integrate debris related impacts within the LCIA step to broaden the scope of LCA for space systems. The objective of this presentation is to propose a set of characterisation factors to compute the damage caused by the generation of debris on the economic space activities. Following the methodology of emission-related characterisation models in LCIA, the CF of a given substance addressing environmental damages at the endpoint level is expressed as the product of the fate factor (FF), exposure factor (XF), effect factor (EF). We propose to adapt each factor to the orbital environment. Fate factor represents the survivability of the debris over time depending on its altitude. Exposure factor expresses the number of probable collision of the debris with active satellites. Both FF and XF are assessed with models developed in the space community. The effect factor corresponds to the monetary value loss for society due to the inactivity of a satellite, expressed in dollars. EF will be assessed by the time of the conference. We computed interim CFs (FF*XF) for the low earth orbit (LEO) region from 400 to 2000km and 0° to 190° with 2°×50km interval bins. These CFs represent the probability of collision between a debris generated in a specific orbit and any active orbiting space object during the residence time of the debris in the orbital environmental. For a specific inclination, CFs are increasing with the altitude because debris generated in high altitude cross all orbital cells before their elimination (below 400km) in the atmosphere and therefore are exposed to a higher number of space objects. The final set of CFs comprising FF, XF and EF will enable to link the emission of debris and final economic damages to space activities.

5.04P.3

Product Biodiversity Footprint - A case study on salmon

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Terrestrial and marine life biodiversity have become major concerns in recent years. In order to limit this crisis, human beings must take measures. Among them is the shift of dietary habits. Current food transition scenarios show the relative share of fish in human diet to be stable if not increasing; in a world with an increasing population, fish resources will therefore be under pressure. One of the most consumed fishes is salmon (FAO: 2 381 579 t production), which is produced for its bigger part in Norway (FAO Fishery Statistics, 2006). There are two ways of producing salmon: aquaculture and fishery. The goal of this study is to compare the impact on biodiversity of both wild-caught salmon and aquaculture. The method used is Life Cycle Assessment (LCA) applied on the life of wild-caught salmon and aquaculture from ‘cradle to the harbor’. This study excludes the life of the fished salmon after its arrival at the harbor. Indeed, it presumes that both wild-caught and farmed salmon evolve similarly beyond the harbor stage, during transport, processing and consumption, and have the same nutritional functions. Functional unit is “1 kg fresh Atlantic salmon at harbour gate” How to compare impacts of both practices on biodiversity? In 2005, biodiversity has been defined by five drivers in the Millenium Ecosystem Assessment (MEA): habitat change, pollution, climate change, overexploitation, and invasive alien species. We will hence explore in this case study: - How the most recent LCA method cover those drivers? - Beyond LCA how can we fill the gaps? We see an important trade off between overexploitation for wild caught fish and invasive species for farmed fish that we address in priority: o Literature seems to highlight i) damage on the ’invasive alien species’ driver for farmed salmon through the escape of farmed fish and the spread of parasites (sea lice) and ii) overexploitation for wild-caught salmon. Those two aspects will be explored in priority and we aim at defining indicator with the ambition of being quantitative and comparable. - According to the results, we will list and hierarchize the other gaps in order to steer future research. We expect LCA to cover aspects of pollution and climate change. Due to the pelagic attribute of salmon, we do not expect much impact in terms of habitat change. Characterizing overexploitation and invasive species seems to be instrumental in relevant comparison between the two.

5.04P.4

Developping a life cycle impact assessment model based on functionnality to assess competition between users

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Key words : Sand depletion / life cycle impact assessment / functionality Massive sand extraction is already having an impact in the construction and industrial sectors. Sand is a partly renewable resource as there is a continuous sand formation rate, but the use yearly use rate has been estimated by as being twice the quantity of sand all the rivers in the world can carry (estimated at 25 Gt/yr). The objective of this project is to develop a life cycle impact assessment model that takes into account sand functionality and is consistent with IMPACT World+'s approach to resource scarcity The first step is to determine the different sand categories that have to be characterized (and the distribution of existing users within those categories). Based on a literature review and on economic databases, the different technical uses of sand at the global scale have been identified and categories have been established based on the technical parameters that make the sand functional or not for each of these uses. It has to be noted that the vast majority of the global stock is not functional for any of the current sand uses and has been therefore excluded from this available stock assessment : sand from the Sahara desert and Rub-Al-Khali (estimated based on USDA, 2014 values) was excluded because subtropical desert is composed of easily extractible sand, but too thin for construction and not pure enough for chemical uses. Based on the above data, we were able to calculate the time before depletion if sand is used at the same rate without any substitution. This has been detemined globally and for each sand category. The substituability by other types of ressources for each sand category has been determined based on a literature review and discussions with experts in each of the sand use areas. Based on this, it will be possible to calculate the fraction of users of each sand (sub)category that will not able to adapt to the lack of sand before the exhaustion of easily accessible sand resources, accounting for the adaptation. This corresponds to the SaCSI. We propose here an operational model allowing to account for sand functionality, which is key to be able to highlight the criticity of some of the sand categories. One of the biggest challenge of this project is the data availability, hence proxies have been developed to infer the missing data, which are hence preliminary and have to be considered with care. However those preliminary interim results highligth that the sand related issue is probably highly underestimated in current practice in LCA.

5.04P.5

Domestic freshwater use in Life Cycle Assessment: enhancing the

characterization of Human Health impacts

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Several research works have addressed the consequences on human health of water use in LCA but no consensus has been built to recommend a specific methodology to assess the impact of *domestic* water use. Building on Boulay et al. [1], this project aims to enhance the regionalized effect factor of the characterization of the impact pathway linking water use or degradation to the potential impact on human health through insufficient drinking water, sanitation and hygiene. Method: The updated effect factor is the ratio of the water related burden of disease WRBD building on the national amount of DALYs generated by inadequate *"drinking-water, sanitation and hygiene"*, and the overall national domestic water deficit. We revisited the quantification of this domestic water deficit specific to each country, recognizing that only a fraction of the population within a country has access to water supply on premises and their domestic water use (DWU) could greatly vary compared to the population without access. We therefore introduced a factor p being the multiplier of DWU of households with vs. without access to water supply on premises. Results: The updated value of WRBD demonstrates that Boulay et al. [1] results overestimate the national water-related burden of diseases expressed in DALYs by a mean deviation of 34% (s=18%). We demonstrate great correlation between the access to domestic water supply on premises and the water related burden of disease (R2=0,68, P=4,57E-47). The revisited approach to quantify domestic water deficit accounting for inequalities in domestic water consumption provides more consistent results than Boulay et al. [1]. Revisited results now highlight some of the regions known for being prone to suffer from water shortages, such as South Africa or Burkina Faso. With a value of p equal to 6,2, we suggest to determine the new effect factor described as the slope of the linear regression between the domestic water deficit and the water related burden of diseases specific to each country (EF=2,88e-3, R2=0,69, P=1,1e-45). A subsequent work will focus on the identification of national patterns, relevant to cluster countries with geographical or socio economical similarities, to explore regionalization patterns of the effect factor. [1] A. M. Boulay, et al., "Regional characterization of freshwater use in LCA: Modeling direct impacts on human health,"2011.

5.04P.6

Importance of region definition for air pollution model: example in Africa

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In 2018, the WHO estimated that Air Pollution is responsible globally for 7 million deaths (1 million in Africa) every year especially due to stroke, heart disease or lung cancer. In recent years, in the field of Life-Cycle Assessment (LCA), several global models have been developed evaluating the impact of air pollution at a global scale. Following the model, the definition of the regions differs: for example 56 in (Van Zelm et al., 2016) or 10 in (Tang et al., 2015). Both previous models are using a Chemical Transport Model with similar characteristics (e.g.: Background concentration, transboundary effect) however the two methods showed different results up to 200, 300 DALY per kton of pollutant following the region. Additionally, even though the African population is important, the damage factors for the continent are shown much lower than in Europe. Africa being only divided respectively in 2 and 4 parts in the previous models, this definition might have an impact on the results, it is then necessary to redefine the regions and redo a calculation to confirm our hypothesis. Therefore, this study aims at three points: developing for the first time damage factors for African countries, showing the importance of the region definition in models and finally updating the list of diseases caused by air pollution. In this study, four pollutants were considered: Primary PM2.5 (BCOC), Nitrogen Oxide (NOx), Sulfur dioxide (SO2) and ammonia (NH3) and five types of diseases: Acute Respiratory Lung Infection (ALRI), Chronic Obstructive Pulmonary Disease (COPD), Ischemic heart disease (IHD), Lung Cancer and Stroke, As highlighted in the previous studies, the impact of primary PM2.5 is directly linked to the concentrated number of the population living in the area. Therefore: Burundi, Egypt, Nigeria, Rwanda and Uganda show the highest results, on the opposite the results are lower in Namibia, Botswana as the total population of the region is lower than 5 million. Secondary PM2.5 have a much higher dispersion, the African population in many regions being mainly and unevenly spread all around the territories, concentration increases in area where just a few people are living, causing a lower impact than primary PM 2.5. To improve the reliability of LCA studies, it is important to develop damage factors, at country-level or even prefecture(county)-level. Also, it is important to consider childhood diseases especially when evaluating regions with a young population.

5.04P.7

Regionalization of the AWARE Characterization Factors for a Colombian watershed

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Keywords: Impact characterization , life cycle assessment (LCA), Eater Scarcity, ENSO. The existing characterization models for the evaluation of water scarcity in the frame of Life Cycle Assessment tools, traditionally consider data on a global scale, based on average information of each of the variables that are considered in the characterization factors (CF). In some cases, this broad perspective may be no representative of the specific hydrological conditions of watersheds, where specific geoclimate characteristics, as well as the continuously changes of human activities, modify the availability of water resources. The objective of this study was to develop site specific CFs for the Alto Suarez River basin, which is located in the Colombian Andes. Primary information regarding water supply consumption, and historical flow measurements at 66 spatially distributed recording stations, for a period of 38 years (1980 to 2018) were collected. Furthermore, the specific water consumption profiles (demand) was quantified by user sector (agricultural, livestock, industrial and human consumption), for the entire region and for each crop, economic activity and water supply. Using this data the water scarcity index of the studied basin was determined. Based on the integration of the life cycle approach to assess the potential impact of water consumption, the relevance of regionalization was apparent. Furthermore, the studied basin presents a clear hydrological seasonality, with three hydrological profiles (typically dry year, typically wet, and average year), based on the El Nino Southern Oscilation (ENSO) recurring climate pattern. From the regionalization of the three profiles analysed, CFs were obtained for the 10 sub-basins that compose the study area. The results from the comparison between the CFs of the original AWARE method and the adapted local AWARE CFs shown that the hydroclimatic seasonality of the basin has a significant effect on the estimated factors.

5.04P.8

Assessing biodiversity impacts of ecological intensification activities - a case study in southwest Germany

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The ecological intensification of agricultural production systems aims at the mitigation of adverse environmental impacts by reinforcing regulating and supporting ecosystem services. Conservation and enhancement of agro-biodiversity is central to this approach, as this promotes a range of associated ecosystem services beneficial to the entire system. Common ecological intensification practices include, amongst others, the cultivation of perennial crops such as miscanthus and wild plant mixtures on a small proportion of a farm's arable land. It has been suggested that this provides both economic and environmental benefits. Farmers diversify their revenue streams and local ecosystems profit from enhanced habitat provision and connection, resulting in overall increased biodiversity potentials. However, it is imperative to ensure that environmental benefits on a local scale are not offset by negative substitution effects on a global scale. In this respect, it is essential to discuss how well current LCA biodiversity impact methods account for potential implications of ecological intensification. This study compares the results of two assessment methods with regard to their applicability and meaningfulness. First, the impact model by Chaudhary et al. (2015; Chaudhary & Brooks 2018), which is recommended in the UNEP-SETAC Global Guidance for LCIA Indicators is applied to a case study of a typical cereal farm in southwest Germany. A business-as-usual scenario of conventional farming practices and an ecological intensification scenario are considered. In the latter, the least productive ten percent of the farm's arable land is dedicated to miscanthus cultivation and the foregone production is taken from the global market. The results are then critically discussed with respect to a range of biodiversity parameters. The discussion draws on literature data and expert opinions, which are quantified in accordance with the methodology suggested by Lindner et al. (2019). This methodology regards biodiversity as a fuzzy object and combines it with the hemeroby concept, thus providing a more holistic depiction of biodiversity within the LCA framework. The comparison of results based on the two methodologies contributes to the current debate on the representation of the multidimensional nature of biodiversity. Moreover, it indicates that global biodiversity can only be enhanced by ecological intensification that does not result in transformation of pristine land.

5.04P.9

Renewable resources in LCA: proposal of a new characterization approach based on criticality

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So-called renewable resources are increasingly being used and promoted as a way to go beyond fossil fuel and abiotic resources based economy. Their characteristics remain however hard to define and so far no consensus has been reached, neither about the notion of renewability nor about the linked concept of sustainability. Life Cycle Assessment is an effective method to evaluate the environmental impacts of systems exploiting renewable resources. Still, it is limited when specifically assessing effects on renewability. Life Cycle Inventories include few renewable flows, and Life Cycle Impact Assessment methods developed to evaluate the potential depletion of renewable resources are generally specific and limited in scope. The work presented here proposes a method for the characterization of impacts on resources renewability. It relies on a state of the art of both generalist and LCA literature, and the elaboration of a definition and a typology of renewable resources according to their origin, nature, dynamic aspects, and conditions for their renewal. The method derives from the "availability minus demand" criticality approach used in the AWARE water scarcity index. It distinguishes between resources produced by either natural or anthropic processes; and one of the natural resources support this production. It evaluates the renewability of the resource produced by considering the criticality of a relevant function of the selected supporting resource. The approach is applied to the case of agricultural crops. It proposes an indicator based on soil quality data, and more precisely on its biotic production potential. The availability and demand of Soil Organic Matter (SOM, evaluated by the quantity of Soil Organic Carbon, SOC) are used to characterize the sustainability of crop production in a given area: the calculated characterization factor expresses the Soil Organic Carbon scarcity and is applied to a SOC flow calculated for the assessed system. This development is illustrated by a case study on miscanthus production for biofuels. It takes into account the localization of the production and comparison with different crop rotation alternatives.

5.04P.11

The development of life cycle assessment method in desertification considering land use and climate change.

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In August, IPCC published special report about climate change and land. It was reported that drylands currently cover about 46.2% (±0.8%) of the global land area and are home to 3 billion people. Desertification is land degradation in arid, semi-arid, and dry sub-humid areas, collectively known as drylands, resulting from many factors, including human activities and climatic variations. The climate change and the unsustainable land management exacerbate the desertification rate cross the both temporal and spatial scale. Desertification has reduced agricultural productivity and incomes and contributed to the loss of biodiversity in some dryland regions are commonly acknowledged. It was considered to reduce the provision of dryland ecosystem services and lower ecosystem health. Even forming to be a threat higher the risk of harm to human health. Considering the desertification is a kind of sophisticated dynamic process, the quantitative assessment of desertification is hardly ever conducted. What's more, the variations inducing desertification is quite variously. Due to the complexity of desertification, quantitative desertification is hard to conduct in each processing step, thus, assessments of desertification are almost conducted as qualitative analyses. Life cycle assessment (LCA) can be a quantitative environmental assessing method. However, the framework assessing desertification is waiting to be completed. In this study, by applying the life cycle assessment (LCA) method, we aim to unroll the life cycle of desertification and visualize the whole process, considering the land use and climate change to assessing the environmental impact quantitively. Using the observational land cover data set integrated for presenting land use changed situation on the spatial-temporal basis. The dryland expansion has a high possibility to reduce the carbon sequestration of land, also constrain the gross primary production. Based on the land cover change situation estimating the greenhouse gas dynamic change would give a relative accurate preliminary in predicting the climate change. The desertification expansion can be projected under different representative concentration pathways. In each scenario, the desertification will have different extents on endpoints (ecosystem service, biodiversity loss, human health, social economy loss). We aim to give a damage amounts of desertification in conclusion, which make the damage more visible to the society so that benefit in adjusting the land use mechanism, combating desertification with a more effective operation.

5.04P.12

Quantitative environmental impact assessment for rice cultivation caused by exposure of artificial light at night

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In recent years, it became clear that the brightness of artificial light at night has been increasing year by year, and its scale is expanding. According to Science Advances, it is reported that from 2012 to 2016, the artificially illuminated outdoor area increases by 2.2% per year and the annual radioactivity total radiance increases by 1.8% (CCM Kyba et al.,2017). Light pollution is described as the situation in which these lights are obstructing or having damages through their leaks, a common example is the outdoor lighting. Light pollution has various effects on human activities, wild animals, plants, agricultural crops and livestock. It is known that the degree of damage by the exposure of light differs depending on the type of light source. As a new indicator of LCA, the assessment of impacts from light pollution on biodiversity and human health has been proposed, and it is pointed out that the development of impact assessment methods for light pollution is important (S.Cucurachi et al., 2013). There is a meta-analysis and systematic review of the effects of nighttime irradiation on human health. The results of this systematic literature review are recommended to be used in developing a human health related impact category of light pollution in the LCA methodology (L.Tähkämö, 2019). Numerous studies have been done on the effects of climate change due to conversion from incandescent lamps to LEDs, but quantitation on the effects of light pollution has not been made in LCA studies. Rice is also one of the crops affected by light. Rice is a short day plant where flower buds begin to form when the light exposure becomes longer than the critical dark period, which is the minimum requirement time needed for flowering. When exposed to light at night, flowering will be delayed, leading to reduced yield and quality. However, today road and sign lightings are increasing with the countryside urbanization. Therefore, the influence of light pollution on rice is expected to increase due to the increase in artificial light at night. In this research, we aim to develop a method for life cycle impact of artificial light pollution. This study focused on the impact on rice production as a part of significant endpoint of social asset. The method can be divided into several steps; fate analysis, exposure analysis and damage analysis. Fate, exposure and damage analysis were developed by conducting experiments which are evaluated the effects on the heading and yield of *Oryza sativa L. cv. Koshihikari* under actual paddy field condition with LED lighting irradiation at night. Characterization factors was expressed by monetary unit per a unit of electric power using the formula obtained in the experiments. It was found that the effect varies considerably depending on the distance and shape from the light source. Furthermore, we carried out comparing the amount of damage from light pollution and climate change. Compared to the impact of road lights on climate change, the difference was about 10 times. The results obtained through these studies are expected to contribute to the design and installation of lighting equipment with a comprehensive viewpoint.

5.04P.13

Characterising land use impacts on pollinators abundance for LCA

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Nature's contributions to people -commonly referred to as ecosystem services- are critical for the life of humans on earth. From the atmosphere to the soil where food is grown, a myriad of ecological processes are constantly shaping the world we live in. In recent years, an ecosystem service that has attracted major attention due to its high relevance and worrisome decline are pollination services. Around three quarters of food crops around the world depend partly on insect pollination. Given the relevance of impacts on pollination and their direct effects on crop yield and food security, we consider that the development of a new impact category in LCA explicitly addressing this topic is in line with current societal and environmental needs.This study proposes two alternatives for how to include and characterise land use impacts on pollinators in LCA. The first alternative is the application of the Lonsdorf model, which provides a process-based understanding on pollinators' abundance. Unfortunately, the data intensive nature of this model does not currently allow to practically characterise impacts on pollinators with LCA. The second alternative is based on a simplified characterisation of land use types relying on expert knowledge. This simplified characterisation is directly applicable in practice and allows for the connection with background data from one of the major LCA databases, Ecoinvent. Based on this simplified model, we characterized land use categories as present in Ecoinvent, using expert knowledge. This set of characterisation factors provides the basis for a contemporary assessment of pollinators' abundance in LCA, while continuing the search for better but practical methods in future. This study is the first to develop a new midpoint impact category and related characterisation model to estimate land use impacts on pollinators abundance for LCA. The application of the presented characterisation factors will provide the opportunity to consider these potential impacts during the assessment of product systems and decision making, helping prevent or remediate impacts on pollinator communities.

5.04P.14

Calculation of global average monthly "AWARE" water scarcity CFs at watershed level and evaluation through application to hydropower plants

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According to ISO 14046, the quantification of the water scarcity footprint a water scarcity assessment of the water consumption at location of the reservoir on a spatially and temporally explicit level needs to be considered. For a reservoir, which has a storing function, e.g., for irrigation in the dry season or constant power production. Therefore, storage in wet seasons and release in dry seasons can counteract water scarcity and lead to a reduction of overall water scarcity in the watershed. Additionally, average water scarcity CFs are required, as many hydropower plants lead to non-marginal changes for monthly water availability. Recently, average AWARE factors have been published by Boulay et al (2019). However, two issues in these CFs need to be considered: (1) the equations used in that publication are erroneous and the average CFs are based on the integral of final AWARE CFs that contain a cut-off of the water scarcity measure. We integrate the water scarcity functions before the cut-off is applied, since this represents the impact function. This is a normative choice, which we analyze in detail. We also provide corrected average AWARE CFs on global level for all watersheds and months. We apply the different average CFs as well as the original, marginal AWARE CFs on important hydropower plants to further assess the effects of these model choices on the water scarcity footprint of hydropower. This study builds on previous research regarding detailed hydropower modeling and extends the water scarcity assessment including the sensitivity to both, LCI and LCIA model choices. We applied the global analysis to 1473 hydropower plants covering >100 countries, and added a detailed assessment for a subset of important power plants to discuss the limitations of global assessments. We thereby provide the most complete WSFP of global hydropower with state-of-the-art methods. The two water scarcity metrics applied lead to larger differences than expected, since the monthly dynamics of dam operations can lead to stronger differences for the water scarcity footprint than the differences in the applied water scarcity factors. These insights help to better understand the water scarcity footprint of hydropower and its uncertainties and allow to assess sensitivities from both, LCI and LCIA model choices. Keywords: water scarcity footprint; hydropower reservoir; LCIA, power production.

5.04P.15
The demonstration of the sustainability of multi-trophic aquaculture systems under a holistic perspective: LCA and Ecosystem Services Assessment
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In recent years, the Integrated Multi-Trophic Aquaculture (IMTA) approach has gained more attention as a promising solution for the sustainable development of aquaculture around the globe. The Horizon 2020 project IMPAQT aims to support IMTA by developing modelling tools, as well as new and emerging technologies, which enable economically, environmentally and socially sustainable aquaculture development throughout the EU. Within this project, the LCA methodology was chosen for the environmental assessment of six aquaculture pilots (four in Europe, one in Turkey and one in China). The corresponding baselines (monoculture) are compared with the situation of these pilot sites once other species from different trophic levels are introduced to the farms. The goal and scope have already been defined considering all the inputs and outputs with a *cradle to gate* perspective. The reference flows have been stabilised and the inventories have been completed in relation to these flows. The environmental assessments of the baselines have revealed that focussing on the on-growing phase, the potential environmental benefits associated with multi-trophic systems would be visible. However, as current LCA methods could not fully address the environmental impacts on biodiversity, ecosystems and their goods and services, an additional Ecosystem Services Assessment was carried out. This assessment has been also done to understand and analyse the effects and benefits of Ecosystem Services of an IMTA compared to conventional aquaculture systems. This basic analysis has been carried out using a so called Bayesian Network. These statistical models work on the basis of causal dependencies between system elements of interest. Some of the underlying data for this Bayesian Network analysis were derived from advanced IMTA – ecosystem models that allow quantification of the carbon and nutrient fluxes through the different trophic levels. The developed network included various Ecosystem Services relevant for IMTA systems and compared them to monoculture systems. Results have showed that a Bayesian Network can capture the benefits of IMTA systems, given enough data from models, in-situ measurements or Earth Observations is provided. Finally, the LCA together with the Ecosystem Services Assessment provide an holistic analysis of the benefits associated to the integrated multi-trophic systems, where the environmental impact categories are represented with relevant ecosystem services.

5.04P.16
Biodiversity In Life Cycle Assessment: A Comparison Of Impact Assessment Methods
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Biodiversity loss is one of the greatest environmental challenges of our times; understanding the drivers behind it is essential. Life Cycle Assessment (LCA) is considered a reference method for appraising the environmental performance of products along their supply chains. Recently, the LCA community has been very active in developing life cycle impact assessment (LCIA) methods to better capture impacts on biodiversity. However, there is no consensus on a method for addressing biodiversity and all of the developed approaches are built upon different models and assumptions thereby leading to different results.In this context, the goal of this study is to understand the differences between results and the different biodiversity aspects covered by the different currently available LCIA methods. For this purpose, 12 different LCIA methods were employed to evaluate the biodiversity impacts of three case studies, which are representative food products of biodiversity loss drivers (beef, coffee and chocolate).These results can potential allow establishing the advantages and disadvantages of using certain methods to cover specific aspects of biodiversity. In addition, these results can provide information on where to focus efforts to improve LCIA methods in what respects biodiversity impact assessment.

Quantifying Life Cycle Emissions and Environmental Impacts of Agricultural Practices Related to Pesticides and Fertilisers (P)

5.05P.1
Comparison of operational N direct field emissions models used in LCA - suitability for contrasting agricultural situations
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The use of fertilisers is a key driver of N losses in agriculture, causing a series of impacts. These losses, conditioned by both pedoclimatic conditions and agricultural strategies (e.g. rotations, fertilisation), take the form of NH3 volatilisation, NO3 leaching, nitrification-driven NO emission to air and denitrification-driven N2O emissions to air. Modelling is a common approach for estimating N losses associated with agricultural systems under *ex post* (actual situation) or *ex ante* (scenarios) approaches. Simple or operationnal N models using a restricted numbers of parameters and input variables were chosen based on their suitability for LCA, following a literature review. They differed in their consideration of the biophysical processes determining N losses from agriculture, namely plant uptake, nitrification, denitrification, volatilisation, leaching, erosion and run-off, consideration of the crop rotation, and other N emissions to air. The models were also constrated regarding management drivers such as over-fertilisation and fertilisation technique, as well as mineralisation of soil organic matter (SOM) and organic fertilisers, drainage regimes, and the allocation of fertilisers (and emissions) among crops in a rotation. Selected simple models were ecoinvent v3, World Food LCA database v3, Indigo I-N v2 and AGRIBALYSE v1.3, two recent French research models, and FAO N-balances. For three agricultural datasets, all tested models estimated slightly differing N emissions. From the analysis of the reasons for such differences, we derived recommendations to enhance modeling of N losses in LCA: i) added nutrients and their impacts should be allocated among crops along the rotation, based on crops nutrient uptake dynamics, ii) mineralisation of N in added organic matter should be considered by means of mineralisation kinetic curves, iii) mineralisation of N in SOM should be estimated from soil parameters, iv) volatilisation of NH3 should be computed considering correction factors for agricultural management and climatic conditions (and then substracted when computating the other emissions), v) emissions factors corrected with factors for agricultural management and climatic conditions are sufficient to estimate N2O and NOx emissions, vi) NO3 leaching computation should consider plant N uptake dynamics, drainage regimes, mineralisation of added organic matter and SOM, catch crops, mineral N inputs, and increased N losses due to over-fertilisation.

5.05P.3
Combining LCA and the soil model DNDC to assess the environmental impacts of catch crops
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The cultivation of catch crops has many positive effects, especially concerning the management of nitrogen in agricultural soils. In this study, the effects of the catch crops oil radish, vetchlin, bristle oat and blackseed in combination with soybean as the main crop will be investigated. The focus of this work is on the calculation of field emissions using the simulation model DNDC (DeNitrification DeComposition) and the integration of the results into LCA models. The primary data originate from field trials in Groß-Enzersdorf, Austria. The environmental impacts of the entire cultivation systems were calculated using the LCA software openLCA (v.1.8) and the ecoinvent database (v.3.4). The environmental impacts of six impact categories are studied using 2 functional units: One kg of soybeans (DM) and one ha of land with and without catch crop cultivation. First results show that simulated nitrate leaching in fallow lands is 4-14 times higher than in systems with catch crops. Jet in all impact categories, besides marine eutrophication potential, catch crop systems achieve worse results than fallow lands. This is due to the additional use of machinery and seed production for catch crop cultivation. For example, fallow lands emit 388 kg CO_2-eq/ha and 0,57 kg N-eq/ha (both during a defined crop rotation) when looking at the GWP and marine eutrophication potential. In comparison, vetchling with 1,867 kg CO_2-eq/ha is related to the highest GWP of all studied catch crops, while blackseed shows with emitting only 0,2 kg N-eq/ha that catch crops can have a positive influence on soils (depending on what impact categories are studied). In the near future further studies will be needed in order to find ways to display soil effects and in special the positive effects of catch crops within LCA studies properly and it will be necessary to keep testing the performance of the LCA-DNDC-model combination.

5.05P.4
Life Cycle Assessment of wheat production in France: impact of mineral and organic fertilizers
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Wheat is an important cereal with many applications, not only in the food sector. In the growing context of biobased products, a good understanding of the contribution of the fertilizers to the environmental impact of agricultural products is essential. In this study, the production of wheat in France has been considered. The first part of the presentation will underline the contribution of organic and mineral fertilizers to the environmental impact with traditional agricultural practices combining mineral and organic fertilizers. Especially, when possible the field emissions will be separated between those due to organic and mineral fertilizers, to have a complete picture of their impact. After that, this first case will be compared with 3 case studies, leading to 4 scenarios to analyze: 1) Scenario 1: "Traditional": mix between livestock manure and mineral fertilizer supplement 2) Scenario 2 100% organic fertilizers and conventional agriculture 3) Scenario 3: 100% mineral fertilizers and conventional agriculture 4) Scenario 4: 100% mineral fertilizers with some inhibitors and conventional agriculture To isolate the contribution of the fertilizers, the idea is to conduct the study with no other change that the nature of the fertilizers and/or the use of the inhibitor. This means that the amount of fertilizer used is calculated so that the wheat always receives the same amount of nutriment; the phytosanitary products applied stays equal in all cases, etc. Therefore, the hypothesis is that the yield will be similar for all the scenarios. The results for these four case studies will be analyzed with the ILCD recommended methods, including all the categories that are relevant for biobased products (climate change, eutrophication, acidification, toxicity, etc.).

5.05P.5
Life Cycle Assessment of Belgian organic apple cultivation using different allocation options for organic fertilisers
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When conducting Life Cycle Assessments (LCAs), special care needs to be taken when choosing the allocation procedure. In the case of organic fertilisers originating from waste products of other production systems, this can be difficult, as these fertilisers are generally considered as residual products of which the realised environmental impacts are allocated to the main products of the systems of origin. The aim of this study was to determine the most appropriate method to allocate the impacts of organic fertilisers to the organic production system so that organic farmers carry their fair share of the environmental impact derived from waste products even though they did not produce them.An LCA of the Belgian organic apple cultivation was conducted, to investigate where the difficulties and uncertainties lie and how big the results differ between the different allocation options. Four allocation options for the organic fertilisers – blood meal and cow manure – were explored: waste assumption, mass allocation, economic allocation and system expansion.For mass allocation, the allocation factor was based on the weight of nitrogen available in the fertiliser product relative to the cow's live weight. For economic allocation, the factor was based on the price of the fertiliser product produced by one cow relative to the price of the live animal. For system expansion, an organic plant-based fertiliser was used as a substitute for the organic fertilisers. The amount of active nitrogen supplied to the orchards by the substitute had to be equal to the amount that was actually supplied by the organic fertilisers.The different impact categories can have very different results depending on which of the four allocation options is used. Mass allocation has a a higher impact for all the impact categories compared to economic allocation because it has a higher allocation factor.Since it is possible to use multiple substitution products, system expansion can lead to different results, making it less suitable. Though, finding a true replacement can be quite difficult. Likewise, economic allocation is based on prices, which can be very variable, which do not have a universal value and do not give an indication about the environmental performance of the product. It is recommended to use mass allocation to allocate the impacts caused by the production of organic fertilizers. Though, special care should be taken when selecting the masses used as basis for the allocation factor.

5.05P.6
Evaluations of benefits and impacts of nutrients recovery techniques for dairy manure
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Opportunities exist to improve nutrient use efficiency and nutrient cycling in dairy food supply chains, particularly through by closing the loop between livestock production (animal manure), food waste (losses at production, retail, and consumption) and crop production (fertilizer losses). In response to these different needs and opportunities, this study aims to assess the life cycle environmental performances of four innovative nutrient recovery techniques. We ensure consistency between nutrient mass balance, inventory flows at farm level and we analyse trade-offs between reduction in emissions and increased impacts due to energy consumption during nutrient recovery. The selected promising nutrient recovery techniques include a) Centrifuge Technologies, b) Dissolved Air Flotation, c) Ultrafiltration, and d) Evaporation technologies. We first used a mass-balance based approach to analyse the respective performances of nutrient recovery technique, in term of N,P and K nutrients as well as total and solid masses. In parallel, data in energy and chemical consumption of each system were collected. P content adsorb to solid and the DAF technology enables to recover close to 80% of manure P content and 60% of Organic N in dry solids, while all ammonia and some of the remaining organic N is in the diluted effluents. Evaporation techniques enhances these performances further by recovering more than 90% of manure P, 85% of Organic-N in dry solid, 84% of NH4-N as concentrated Aqua Ammonia, the residual water being rejected as cleaned water. Since these nutrient recovery systems affects other farm emissions (barn and field emissions during manure and fertilizer applications), the data from each nutrient technique are then integrated within the Integrated Farm System Model (IFSM) to derive consistent Life Cycle inventory flows (e.g. Ammonia and Nitrate emission) associated with each system and determine related nitrogen, phophorus and carbon footprint and efficiencies. Finally, trade-offs between energy and chemical consumption related impacts and reduction in nutrient recovery impacts are analysed applying the ImpactWorld+ LCIA method, ensuring a high consistency between LCI and LCIA approaches. This study shows the potential interest of nutrient recovery techniques in term of reduction of nutrient related emissions and N- and P-footprints. It is however crucial to take advantage of non-renewable electricity and heat production systems to ensure that the energy consumption and related impacts is lower than for mineral fertilizer production.

5.05P.7
Quantifying the Environmental Impacts of Trace Elements Used in Animal Nutrition
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The present challenge of livestock production is to meet the growing demand for animal products at low environmental impact. Available life cycle assessment (LCA) studies have shown that feed production significantly contributes to the environmental footprint of edible animal products and therefore an important element to take into account when considering mitigation options. For this reason, the Product Environmental Footprint (PEF) Category Rules of animal feed was approved by the EU commission in 2018, with the feed industry being the first sector to have its PEF. However, for feed additives such as trace minerals, the assessment related to the models of their production process are still being improved. Thus, the objective of this study was to develop a comprehensive dataset for a zinc (Zn) and copper (Cu) sources named HiZox® and CoRouge®, respectively (Animine, France), in compliance with PEFCR requirements. The PEF study was based on the method as described in the PEFCR Feed for food-producing animals, and the experimental unit was 1 kg of zinc or copper used in animal nutrition. The system boundaries were from cradle-to-plant, and the environmental indicators included all PEF impact categories, as well as the toxicity ones. The modelling was performed in the SimaPro version 8.5 and the latest PEF datasets were used. The carbon footprint of HiZox® and CoRouge® was 5.70 and 3.30 kg of CO_2-eq. per kg of mineral supplied in the diet, respectively. The impact on freshwater ecotoxicity (EcotoxF) was 15.8 and 53.6 CTUe for Zn and Cu, respectively. For resource use (ResUse) - mineral and metals, the impact was, respectively, 1.92E-03 and 2.45E-03 kg Sb-eq for Zn and Cu. The LCA of HiZox® and CoRouge® was compared to a reference inorganic sulphate source. For ResUse, HiZox® had a higher impact than the reference sulphate, while CoRouge® had 77% of the impact of a sulphate source. For Carbon Footprint, the relative impact was 44% and 37% of the reference sulphate source, for HiZox® and CoRouge®, respectively. In conclusion, now Animine is meeting its commitment to provide a high-quality PEF related dataset to be used by the feed industry in their own PEF assessments. As perspective, the animal production system, as well as the speciation of zinc and copper in animal wastes, could be accounted in the boundaries of the LCA. This would be relevant because of the high contribution of metal speciation to the toxicity impact.

Quantifying the Environmental Impacts of Marine Litter: Interdisciplinary Knowledge towards Addressing Marine Litter in LCA (P)

5.06P.1
Exposure to selected Micro and Microplastics and Effect on Selected Species in Marine Environments
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Macro and Micro Plastics are reported to harm the ecosystems at various levels. Both macro and microplastics basically harm the organism level through entailments, ingestion and transporting invasive species. Moreover, the habitat level effects could occur due to occupancy in aquatic environments (Woods et al, 2019). These effects could lead to ecosystem level damages. This study conducted a keyword search related to plastic, ecological damages at various levels (such as sub-organism, organism, population, habitat, ecosystem) to filter the prereview research publications from 2010 to 2019. The categorized publications were subjected to systematic reviews to find the relevant information to summarize the effects at various ecological levels. Then a meta-analysis of the data in each category was done to obtain relationships between ecological levels and two basic plastic categories of micro and macro. Microplastic concentration data were

compiled according to particles/m^3 and mg/m^3 (in order to observe the relationship with the effects). Macroplastic concentration data were compiled according to particles/km^2 of surface area and mg/km^2 of surface area. Global small fish distribution maps were obtained from biodiversity mapping organization and the potential exposure and the risk on of small marine fish species to the microplastics were calculated.*Reference:* Woods, J.S., Rødder, G., Verones, F. (2019). An effect factor approach for quantifying the entanglement impact on marine species of macroplastic debris within life cycle impact assessment. Ecological Indicators, 99, pp. 61-66.

5.06P.2

Conceptual framework for assessing marine plastics pollution

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Since marine plastic debris was first recognized as an environmental concern in the 1970s, an increasing number of studies and initiatives has brought the issue to public attention, to the extent that it has become an indicator to monitor our progress towards the United Nations' Sustainable Development Goal no. 14. As a result of human activities, plastics are lost to the environment at different points across the plastics value chain, from where they undergo transport and transformation processes before reaching the marine environment where they cause damages to marine environment and wildlife. Although an increasing number of studies addresses separate issues related to plastics' environmental fate and their effects on different taxa, methodologies for comprehensively quantifying these potential damages are still lacking. This prevents us from gauging the magnitude and severity of marine plastics pollution. We propose a comprehensive and integrated framework for quantifying the potential impacts of plastics, from releases of plastics to the environment, through their environmental fate, up to their eventual effects on marine ecosystems. We have operationalized the first part of the framework by quantifying global plastics losses with an unprecedented granularity and uncertainty characterization. We find that in total 9.2 Million tonnes (Mt) of plastics ended up in the environment in 2015, with a 95% confidence interval of 4.3-23.3 Mt. The majority of macroplastic losses stems from littering and inadequate waste management, while the majority of microplastic losses occurs during the plastics use stage. We have developed overall modelling approaches for addressing fate and effects of plastics. Future tasks include further developing and fully operationalizing the full impact assessment framework to ensure findings made within different research fields are compatible and can be integrated into this life cycle based assessment framework. For instance, findings on ecosystem effects should be compatible with findings related to fate of plastics to facilitate integrated assessments. Indeed, such approaches are necessary to provide stakeholders in industry and authorities with scientifically robust assessments to prioritize measures for curbing plastics pollution.

5.06P.3

Environmental Assessment of Possible Leaching of Additives Present in Plastics

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The occurrence of plastics in the environment concerns society. Microplastics that are emitted to the environment could have harmful effects on marine organisms and human health. While remaining in an environmental compartment, potentially harmful additives can leach to the environment. Additives are substances which are added to plastics in small amounts to achieve or improve certain properties. In terms of the precautionary principle, the emission of plastics especially with harmful additives should be avoided. The goal of this work is to analyze the impacts of possible leaching of additives on the ecosystem and human health. In the first step, plastic types were identified that contribute to a large part to the total amount of microplastic emissions. For the identified plastic types, the typical additives were determined taking into account the information provided by Stenmarck et al. (2017). The minimal and maximal concentration of the additives in plastic were taken from the publication by Hahladakis et al. (2018). The additives identified by this approach were examined regarding their human and ecotoxicological effects using a systematic literature analysis. The metadatabase TOXNET (https://toxnet.nlm.nih.gov/) with a special focus on the databases HSDB, CTD, IRIS, and ITER were searched in order to identify human and ecotoxicological effects. Additionally, we searched the USEtox-database and analyzed the coverage of the identified additives. Using this approach, we found a total of 247 additives. These additives are used in PE, PP, PS, PVC, PA, PET, and PUR plastics. Toxicological data could be determined for two-thirds of the investigated additives. For the toxicological unspecified additives, we used a neural network (CLiCC-Tool, https://clicc.net/) in order to investigate the potential toxicological impacts . References Hahladakis, John N.; Velis, Costas A.; Weber, Roland; Iacovidou, Eleni; Purnell, Phil (2018): An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. In Journal of Hazardous Materials 344, pp. 179–199. DOI: 10.1016/j.jhazmat.2017.10.014. Stenmarck, Å.; Belleza, E. L.; Fråne, A.; Busch, N.; Larsen, Å.; Wahlström, M. (2017): Hazardous substances in plastics. Copenhagen: Nordic Council of Ministers, checked on 6/12/2018.

5.06P.4

Single-use plastic bags and their alternatives - Recommendations from Life Cycle Assessment studies

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In the past few years, much debate has evolved around the environmental impacts of one of the most consumed items globally: single-use plastic bags (SUPBs). However, alternatives to SUPBs also come with an environmental impact, which do not necessarily outperform plastic bags in all environmental categories. Consequently, the environmental impacts of SUPBs compared to their alternatives need to be investigated under a life cycle perspective to identify which solution is more sustainable depending on the context. The aim of this study was to carry out a meta-analysis of eight LCAs comparing single-use plastic bags and their alternatives, including reusable plastic bags, and cotton and paper bag. The findings of the LCA studies were summarised while critically looking of the assumptions and contextual differences between them. The analysis shows that environmental impacts of the bag do not only depend on its material composition (material type and weight) and production processes; they also significantly correlate to the number of use cycles and its end-of-life settings. Therefore, the analysis and interpretation of such impacts shall be put under specific context on the design, sourcing and production of the bag, as well as on user behaviour and treatment infrastructure along the plastic value chain. Single-use plastic bags have lower climate change impact than single-use paper and cotton bags. However, paper bag can perform better if paper mills use renewable energy to produce it and it is reused and recycled, and finally incinerated rather than deposited at landfills. Its impact will be lower when used for multiple times. Cotton bag's impact on climate change will be lower than plastic bag if it is reused more than 50 -100 times with proper end-of-life treatment; it should be noted that cotton reusable bags are generally designed and used in this kind of setting. Single-use plastic bags have lower impact on Acidification, Eutrophication, and Ozone Depletion than single-use paper and cotton bags. The impacts from paper and cotton bags will be reduced when being used for multiple times. From these findings, a set of recommendations for policy-makers as well as guidance on how to interpret results from comparative LCAs were developed. Overall, reducing environmental impacts of bags is not just about choosing, banning, recommending or prescribing specific materials, but also about changing the design of the bags, encouraging more sustainable consumer behaviour, and establishing proper waste management system to improve circularity.

5.06P.5

Marine litter contribution of plastic packaging in Germany.

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This master thesis study targets the estimation of littering to quantify the share of plastic packaging entering the aquatic environments in Germany. The severity of environmental impacts, such as water pollution and endanger of biodiversity caused by littering reveal the urgent need for action. Plastics in the ocean are mainly caused by irresponsible use and lack of integrated waste management. In the European Union, most plastic is consumed for packaging production, with Germany having the highest plastic demand. Often designed for single-use, packaging rapidly transforms into dispensable waste. A missing design for circularity leads to most plastic waste being incinerated, landfilled or disappearing from a controlled infrastructure by being littered. Environmental impact assessments such as Life Cycle Assessment (LCA) with the current regulated End-of-Life scenarios exclude littering and the resulting environmental impacts. Using life cycle thinking, this study presents a sustainability assessment of the primary packaging occurrence at the point of sale. In a case study on tomatoes from German supermarkets, the marine litter contribution of 10 available packaging types is calculated. The results are presented as a best practice and worst option depending on the mass, recyclability and overall environmental impact of the packaging. The inclusion of ML into a comparative assessment of packaging types enables the consumer to evaluate different environmental contributors and provides a broader picture of the impact, not only dependent on the selected material or the pressing marine litter issue. This is crucial to avoid shifts in burden and counterproductive behavior. The goal of the study is to develop a baseline for an inventory-level indicator expressing the likelihood of an item to enter the environment as littered waste. The proposal of plastic leakage as a midpoint indicator in Life Cycle Impact Assessment, and a plastic footprint as an area of concern is further discussed, finally including impacts of plastic litter into LCA.

Sustainability and Risk Assessments and Circularity Indicators for the Transition to a Circular Economy (P)

5.07P.1

Accounting for material properties and technology choices to assess effective recycling strategies

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To improve material and technology choices in the recycling industry it is essential to understand the complexity of material properties and their possible applications. We propose to assess a recyclind industry as a whole to undestand material flows, reveal cascading effects and consider trade-offs between different possible applications and environmental impacts. Cascading in the recycling industry needs to be adressed to sucessfully shift to a circular economy. In this contribution, we propose a methodology that allows optimizing material and technology choices in the recycling industry. We use constraints to account for limited availability of recycling material, for their specific properties and impurities, and also implement minimal recycling rates. Furthermore, we use multi-objective optimization to assess trade-offs between economic and environmental objectives. The methodology we present with an aluminium case study can be generalized and applied to other contexts in which both technology an material choices need to be simultaneously assessed.

5.07P.2

Coupling an economic time-series M-P chain model to CLCA: the case of post-consumer glass waste loops

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Consequential life cycle assessment (CLCA) is a relevant tool to assess the global efficiency of a transition to a resource-efficient circular economy. It examines the potential environmental impacts of all processes that are "affected" as a result of possible decision-making. To uncover environmental consequences when dealing with a transition to circular supply chains, single-region macroeconomic models are widely being used. However, their inherent framework and financial representation of flows carry a high level of aggregation. They fail to track the affected processes within a complex dynamic intersectoral and interregional interactions while ensuring mass conservation for circular supply and demand activities. To tackle these issues, this paper presents an integrated CLCA approach built on the foundation of an economic time-series material-product (M-P) chain model. It combines relevant elements of partial equilibrium thinking, material flow analysis and environmental life cycle assessment that single-region macroeconomic models fail to address. Through this novel modelling approach, we assessed the potential environmental consequences of post-consumer glass waste management options (reuse, closed-loop and open-loop recycling, and landfilling) in the province of Quebec, Canada. We examine the robustness of the economic flow optimality using different prospective state of the interlinked industrial ecosystem in north-eastern America and price sensitivity analysis. Preliminary results uncover a complex dynamic and systemic interactions in circular M-P flow optimality leading to region-specific economic and environmental trade-offs. Outcomes are controlled by concurrent driving parameters and market conditions that a generic framework or single-region models fail to address in “affected” process identification for a consequential assessment. The new insights for decision makers for a transition towards circular economy are compared to a single-region analysis.

5.07P.3

Consequential life cycle assessment of different valorisation alternatives for forest biomass residues: electricity, heat and bioethanol

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The production of bioenergy from forest biomass residues has been increasing in the last years, mainly as a consequence of concerns related to energy demand and climate change. Indeed, forest biomass potentially contributes for reducing emissions of carbon dioxide by displacing fossil fuels, since it can be considered carbon neutral. However, forest residues are limited and can be used to produce alternative bioenergy services, such as electricity, heat and biofuels. Therefore, comparisons between the different valorisation alternatives become imperative to support decision-making. In this context, a consequential life cycle assessment was applied to evaluate the environmental consequences of implementing the governamental strategy of increasing 60 MW of installed capacity for electricity production from forest biomass residues in Portugal and the consequences of losing alternative uses for the forest biomass residues, in particular, heat and bioethanol production. The study considers a short-term time scope and the geographical scope is Portugal. The system boundaries include the collection, processing and transportation of the forest biomass residues, the bioenergy conversion, the final destination of ashes, the avoided forest residues decomposition in forest soil, the decrease in soil carbon stocks, the mineral fertiliser compensation due to nutrient losses, and the fuels displaced. The characterisation factors used in this study for the impact assessment were those suggested in the International Reference Life Cycle Data System (ILCD). The comparison of the three scenarios under study shows that the production of electricity using forest biomass residues is advantageous in relation to leaving the forest residues in the forest soil and using fossil fuel sources to produce electricity only for some environmental impact categories. The scenario of cogeneration of electricity and heat production is the most advantageous among the three scenarios, while the scenario of bioethanol production is the least beneficial. However, for the impacts associated with particulate matter and marine eutrophication, the cogeneration scenario would only be better than using fossil fuels if measures are adopted to decrease the impacts.

5.07P.4

Assessing the environmental benefits of utilising residual flows: the case of urea production from basic oxygen furnace gas

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To promote a more circular economy, residual material and energy flows that were previously considered waste are increasingly utilised. To answer the question what the most environmentally beneficial use of the residual feedstock would be, a systematic implementation of two adjustments in life cycle assessment (LCA) was proposed: i) a shift from a functional unit (FU) based on the residues-based product towards a FU based on utilising a residual flow, and ii) estimating what the residues-based product would replace in the ‘conventional’ economy, i.e. defining its counterfactual. Basic Oxygen Furnace gas (BOFG) is a multi-component residual flow from integrated steel mills, which can be used for different purposes. The goal of this research was to quantify the environmental impacts of five alternative applications of BOFG: heat for internal use in the steel mill, electricity production, urea production, urea production combined with CO_2 storage, and flaring. Urea can be produced from solely BOFG components, making use of the novel sorption enhanced water-gas shift (SEWGS) technology. Our FU was the utilisation of 1 tonne of BOFG, and the impacts of each application were compared in light of the impacts of its respective conventional way of production, i.e. its counterfactual. Greenhouse gas (GHG) emissions were estimated to be largest with 1088 kg CO_2-eq/t_{BOFG} when BOFG is flared. With 691 kg CO_2-eq/t_{BOFG}, urea production has lower net GHG emissions than electricity or heat production. When the excess CO_2 is captured and stored, environmental benefits are reached. The sensitivity analysis showed that the results are largely dependent on the electricity mix used. When impacts were determined with a FU of the production of 1 tonne urea, we found that GHG emissions of urea production from BOFG were 0.4 t CO_2-eq/t_{urea}, accounting for the fact that urea would likely replace BOFG-based electricity, while conventional urea production causes 1.0 t CO_2-eq/t_{urea}. This FU, however, does not allow for a comparison between different uses of a waste stream as this would require different FUs. We conclude that the use of a waste-utilisation focused FU in combination with counterfactuals is beneficial to make informed decisions in terms of a circular economy. The importance of the electricity grid mix also implies that the relative environmental benefits of urea produced from BOFG are further enhanced under future power decarbonisation.

5.07P.5

Application of the GeoPolRisk method at Midpoint and Endpoint level: The case of Li-ion batteries for the EU

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Energy storage has become a key concern on the efforts for reducing greenhouse gas emissions in the mobility sector, and a significant presence of Li-ion batteries (LIB) in the market resulted on a growing interest in quantifying the impacts associated to their use. Concerns about access to critical materials show challenges in terms of the assessment of abiotic resources accessibility in LCA; selected materials in LIB were assessed with the aim to understand which of these have a higher contribution to impacts analyzed from the criticality perspective in the European Union (EU) at midpoint and endpoint level. The use of the Geopolitical Supply Risk Method (GeoPolRisk) has been recognized as one of the suggested practices by the UNEP Life Cycle Initiative to quantify supply risk in LCA. The recent introduction of recycling considerations into updated characterization factors of the GeoPolRisk method makes it possible to model recycling scenarios for selected materials used for LIB in the EU. Additionally, a recent extension of the GeoPolRisk method to an endpoint indicator allows to assess the economic implications of using these materials considering price indicators. **1. 2. 3.** The methodology is applied to four relevant materials in the supply chain of LIB from the perspective of the European Union. Aluminum, Cobalt, Copper and Nickel were selected from the inventory of cells of lithium-ion batteries with nickel-cobalt-aluminum (NCA-C) and nickel-manganese-cobalt (NMC-C) cathode active material. GeoPolRisk values were calculated for the EU from 2015 to 2017; these midpoint level results consider the effect of domestic recycling practices in the EU and are the base for the obtention of the endpoint level indicator. The GeoPolEndpoint method was used to assess the relative socio-economic damage attributable to the use of Al, Co, Cu and Ni in LIB. Results show that Cu and Ni dominate the mass share for both types of LIB; however, Co stands out at the midpoint level due to mainly being extracted from high risk countries outside of the EU; at endpoint level, Al and Cu lose relevance due to their relatively low price and low supply risk. A predominant role of Co and Ni at midpoint and endpoint level confirms the importance given for these materials deemed critical for the EU; future work should address how these results compare to environmental impact categories in order to complement decision making processes based on LCA.

5.07P.7

EU fertilizer regulation: Moving forward or backward

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Organic fertilizers are accepted to provide soil conditioning with competitive to the mineral fertilizers performance, and thus are able to partially replace the latter in the market. However, circularity (from organic waste to organic fertilizers) is not coming without shortcomings and weaknesses. In the view of the Regulation (EU) 2019/1009, three organic fertilizers were assessed, exemplified by their content in phosphorus (P) and Cadmium (Cd) for their suitability of meeting the regulatory standards. The organic fertilizers were assessed by modelling three organic feedstocks based on the conversion of volatile solids (VS), and total solid (TS) accordingly, into biogas. Biopulp, with 0.14 mg Cd/kg TS, was found to have the lowest concentration of Cd, followed by source sorted organic fraction (SSOF), with 0.31 mg Cd/kg TS, and mechanically separated organic fraction (MSOF), with 0.7 mg Cd/kg TS. Moreover, the P content in biopulp, SSOF and MSOF was 0.38, 0.26 and 0.34 %TS. Based on the modelling results, none of the digestates exceeds the regulatory limit of 1.5 mg Cd/kg TS, except for the one deriving from the mesophilic AD. Although, the methanation of VS leads to exponential increase of Cd per unit of TS. In reality, the application of fertilizer on farmland is dosed according to the nutrient plant demand, but only the German regulation has limitations on the amount of TS allowed to be applied on farmland while the Danish regulation has a limit of 0.8 mg Cd/kg P for fertilizing products. The digestates deriving from SSOF and biopulp also fulfil the required P content to be classified as fertilizer products. Nonetheless, digestates can be applied on farmland as soil improvers without the need to meet minimum requirements for minerals and with higher tolerant amounts of Cd concentration (2 mg Cd/kg TS). The results indicate that while all three digestates are acceptable for application on farmland there is no framework in the Regulation (EU) 2019/1009 for controlling the total amount of Cd applied on agricultural soil. Considering the low content of P in organic fertilizers in parallel with the gap of regulating the maximum amount of TS to be applied on farmland, the EU fertilizer regulation allows for risk of accumulation of micro-pollutants on agricultural soils.

5.07P.8

Environmental and economic assessment of MEMs in-line inspection system: CITCOM Project

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Modern electronic devices, like smart phones, consumer electronics or healthcare applications offer a high level of comfort and functionality in reduced space. Micro and nano-manufacturing are driven by micro-electro-mechanical systems (MEMS). A major task in micro-manufacturing is the quality inspection respect to specific defect classes. These micro components are susceptible to surface and internal impact damage, not visible with an un-aided eye at the surface, being unintentionally introduced into the final product. In this context, there is a need of more reliable and efficient system to reduce rate of rejection of high value parts in micro-manufacturing. CITCOM project aims to develop an efficient MEMs inspection system to inspect high volume MEMs component production which is reliable, accurate, cost-effective and low waste generation. Environmental and economic impacts associated to CITCOM system compared to the current manual system has been analyzed. Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) methodologies have been used for this purpose. The LCA results show tangible differences in both environmental and economic terms between the two systems under study, CITCOM and current system. By improving product quality control, increasing efficiency and reducing waste generation by up to 15% through CITCOM in-line inspection system, environmental and cost benefits are observed. In conclusion, the CITCOM system, an efficient technology that detects defect MEMs in a shorter period and more effectively than a human eye, preventing them from reaching the final product, provides environmental and economic benefits. CITCOM will help EU industrial sector to meet consumer demand in terms of high-quality manufacturing, 100% reliable, efficient products with minimum waste generation and efficient resource utilisation, thus following a circular economy approach.

5.07P.9

Assessment carbon footprint of citrus waste valorization to D-limonene

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Food production has grown in recent years to meet the demands of a growing world population and, consequently, the volume of food waste has also increased. Citrus fruits are the most abundant in the world and it is estimated that the products derived from their production (such as juices or jams) account for around 1 million tonnes of waste per year in Spain. There are several practices for the treatment and/or use of this waste, such as cattle feed or landfilling, but the latter needs pretreatment according to the requirements of the European Parliament Directive. Furthermore, keeing in mind the UN Sustainable Development Goal 12, waste production must be reduced through activities such as recycling or reuse. In this context, waste recovery is part of the circular economy strategy. An interesting alternative is proposed for the extraction of D-limonene from citrus residues such as peels and citrus pulp, considering that this product is one of the most widely used monoterpenes in industry, whether in the manufacture of food, perfumes, detergents, biodegradable solvents and even in the pharmaceutical sector. Moreover, once the terpenes content has been extracted from the waste, the new residual stream can be treated by anaerobic digestion to obtain biogas. The main goal of this study is to quantify the carbon footprint derived from the valorization of citrus waste into D-limonene via conventional extraction with ethanol, following the Life Cycle Assessment perspective. To this end, the biorefinery strategy has been developed and simulated using Aspen Plus software. The scenario, divided into three sections (pretreatment, extraction and purification), was assessed, identifying the main inputs and outputs through mass and energy balances. Bearing in mind the preliminary results, the purification section is considered to be the environmental hotspot due to the energy requirements, mainly associated with the solvent recovery process. This is why the main line of action for process improvement should focus on alternative extraction techniques that may result in a lower carbon footprint.

5.07P.10

Sustainable bioenergy systems: an environmental profile of wood pellet and wood logs for residential heating

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Urban areas are among the most vulnerable to climate change. Nowadays, more than half of the world population lives in urban areas and it is expected to increase to around 66 %. The use of bioenergy, based on wood feedstock, has been identified as an adaptation measure for climate change due to its potential to reduce fossil fuels dependency and greenhouse gas emissions. In 2015, 46 % of the total renewable energy produced in the EU came from woody biomass. Wood-fuelled systems are commonly used for residential heating and recently wood pellets have been replacing traditional firewood. Wood pellets are burned in pellet stoves (with higher combustion efficiency than wood stoves and fireplaces), have higher energy density and thus require less space for storage than firewood. However, pellets production requires a more complex industrial processing. This study applies life cycle assessment to compare the environmental impacts of three wood-based combustion systems for producing thermal energy for residential heating: i) a pellets stove using maritime pine pellets as feedstock; ii) a wood stove using eucalyptus and maritime pine logs as feedstock; and iii) a fireplace using eucalyptus and maritime pine logs as feedstock. The functional unit is 1 MJ of thermal energy produced for residential heating. System boundaries include four stages: (1) forest management from both eucalyptus and maritime pine species; (2) pellets and wood logs production; (3) distribution of pellets and wood logs, and; (4) thermal energy conversion, also including ashes disposal in landfill. Inventory data are representative of the current typical technological systems in Portugal. Environmental impacts were calculated for seven impact categories from the ReCiPe 2016 midpoint method: global warming, fossil resources scarcity, terrestrial acidification, freshwater eutrophication, marine eutrophication and ozone formation. From all the heating systems analysed, the fireplace presents the worst performance for all the impact categories with the exception of freshwater eutrophication and marine eutrophication, when maritime pine split logs are burned in the fireplace. Comparing the pellet stove with the wood stove, none of the systems is better than the other, for all the impact categories analysed. This study can support climate and air quality-oriented policies, highlighting the most suitable system and biomass feedstock to residential heating, depending on the impact category analysed.

5.07P.11

Life cycle assessment of hard carbon manufactured from organic and anthropogenic waste precursors

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The good technical performance of hard carbon is the reason why they are used for the manufacture of sodium-ion batteries (SIB). However, hard carbons are also interesting because of potentially low cost and environmental benefits. These attributes are associated with this novel material because its precursors can be waste, including waste tires and bio waste. However, there is little information about the environmental impacts of these materials. Therefore, to close this knowledge gap, an attributional life cycle assessment (LCA) has been used in this work to quantify the impacts associated to hard carbons based on pyrolized waste tires, coconut or peanut shell and apple waste. Given the origin of the precursors, the life cycle perspective allows the analysis of resource demand (energy and water) and climate change, identifying the hot spots of fossil depletion potential (FDP), water depletion potential (WDP), cumulative energy demand (CED) and global warming potential (GWP). These results are analyzed to quantify their contribution in the environmental impacts associated with the SIB.

5.07P.12

Freshwater eutrophication from forest management: the case of eucalypt in Portugal

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Phosphorus emissions to agricultural and forest soils due to fertilization cause freshwater eutrophication due to nutrient enrichment of inland waters. Indeed, eutrophication is still a problem in Europeans waters, being identified by the Water Framework Directive as a priority issue for water protection. Site-specific information on phosphorus emissions, which is most often the limiting nutrient for inland waters, should be considered to better reflect and characterize potential eutrophication peaks and hotspots. This work develops spatial and temporal differentiated midpoint characterization factors for phosphorus emissions in eucalypt-dominated catchments that reach Vouga River in Central Portugal. Eutrophication of Vouga River is a problem due to its use for public water supply. The midpoint factors represent the 'persistence' of phosphorus in the water column and were developed based on the methodology proposed by Helmes et al. (2012). The methodology considers spatially differentiated phosphorus concentrations simulated by the Soil and Water Assessment Tool (SWAT), as well as parameters related to phosphorus advection by water river, retention by vegetation and soil, and removal caused by the water capitation to human use. The presented life cycle impact assessment method is useful for forest owners/managers and policymakers. It provides detailed information on water quality for supporting decision-making towards better-informed choices to reduce eutrophication vulnerability. References Helmes, R. J. K., Huijbregts, M. A. J., Henderson, A. D., Jolliet, O. (2012). Spatially explicit fate factors of phosphorous emissions to freshwater at the global scale. The International Journal of Life Cycle Assessment, 17(5), 646-654. doi: 10.1007/s11367-012-0382-2

Sustainability Dietary Patterns: Nutritional and Environmental Implications (P)

5.08P.1

Classification of Belgian consumers based on protein source consumption frequency in relation to dietary guidelines

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Food production and consumption lead to major environmental impacts and protein sources play a key role in this. Dietary changes have the potential to lower environmental impacts, e.g. by moving from animal-based to plant-based protein sources. Meat products, in particular red meat and processed meat, induce large environmental impacts and are related to health risks. Within Belgian dietary patterns, the average intakes of these products are well above the recommended amounts, leaving a large scope for improvement. This research identifies typologies of Belgian consumers based on their consumption frequency of protein sources in relation to nutritional guidelines, revealing the largest potentials for environmental impact reduction. It allows proposing targeted dietary advices for each typology, which are more likely to be followed by individual consumers than the general advices that are usually provided. We apply a two-step classification based on consumption frequencies of protein sources retrieved from the Belgian National Food Consumption Survey (2014). Step 1 identifies "large meat eaters" (eating more than once per day meat), "moderate meat eaters" (5 to 7 times per week), "small meat eaters" (less than 5 times per week) and "no meat eaters" (never). The Belgian Food Based Dietary Guidelines (2019) provide advices on the consumption frequency of red meat, processed meat, dairy, (shell)fish and meat replacers. These frequencies are used to refine the main groups identified in step 1, by identifying consumers with a higher current consumption frequency than recommended. Hence, a possible typology is "moderate meat eater, with high consumption frequency of dairy." Primarily results show that 13.0% of the Belgian population are "large meat eaters", 81.4% "moderate meat eaters", 4.3% "small meat eaters" and 1.3% "no meat eaters". 60.9% has a high consumption frequency of red meat, 77.6% a high consumption frequency of processed meat, 31% a high consumption frequency of dairy, 19.0% a high consumption frequency of fish and 30.8% a high consumption frequency of meat replacers. These results will be combined to identify the typologies, which are further characterized in terms of usual intakes of protein sources (g/day), personal characteristics, nutritional adequacy and environmental impacts. The characterized typologies will allow to formulate specific advices to lower environmental impacts without compromising health aspects.

5.08P.2

Food Systems Sustainability Through Surplus Food Redistribution: Scenario Analysis and Recommendations from a Case Study

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Improving food systems sustainability has a pivotal role on the way towards the achievement of sustainable development goals for many reasons. First, food production and distribution systems should be able to ensure a fair distribution of food, especially for the poor and most vulnerable people. This closely relates to the optimisation of production and consumption patterns and, in particular, to food waste prevention along the supply chains, from food production to retailing and consumption.Developing strategies to address food waste generation carries major economic, social, and environmental benefits. In this sense, redistribution of surplus food is becoming a rather common and effective practice. This work focuses specifically on studying the environmental burdens of food waste generation downstream of the retailers, and the environmental implications of surplus food redistribution.Life Cycle Assessment (LCA – ISO 14040-44:2006) has been applied to the activities of an Italian, regional network of charities (*empori della solidarietà* – literally *solidarity emporiums*), that recover food surplus from organised large-scale distribution and from production companies, to redistribute it to the people in need. The estimated number of people taking advantage of this initiative is around 130000 per year.Primary data have been collected from six emporiums located in the Veneto region, Italy. A surplus food disposal scenario and a recovery scenario have been assessed, taking into account food production, distribution, and surplus disposal/redistribution. The functional unit was the management of 1 kg of food (wet weight basis), without the packaging since it accounts for 2% of the total weight only. The chosen impact categories were those included in the ReciPe method (Midpoint, Hierarchist). All LCA modelling has been carried out in SimaPro.The recovery scenario showed overall better environmental performance than the disposal scenario for all impact categories. There are however few exceptions to be considered, mainly related to transport activities. Food waste recovery and redistribution has to take into account geographical locations and transport distance from the retailers to the distribution hub of recovered food. This study represents a first step supporting the development of sustainable food systems and should be complemented with a comprehensive assessment of economic and social benefits deriving from food waste prevention through redistribution.

Increasing the Utility of Non-standard Studies in Weight of Evidence Evaluations (P)

6.01P.1

How non-standard studies and their weight of evidence evaluation may be used within Environmental Quality Standards derivation: a case study with endocrine disrupting assessment of tebuconazole

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Within the Water Framework Directive Common Implementation Strategy (WFD CIS) context, the derivation of Environmental Quality Standards (EQS) is framed by recommendations given in the European Technical Guidance Document for Deriving EQS (TGD EQS, E.C., 2018). This guidance recommends the use of data that are deemed relevant and reliable. Those are most often generated using standardised test methods, but not confined to standard studies and species. The concern raised by endocrine disruption should be tackled in diverse regulatory frameworks, among which the WFD. In this context, endocrine disruption (ED) is quoted several times as an issue in the TGD EQS but recommendations as this regards are not prescriptive. Our study attempts to assess how far can the ECHA/EFSA Guidance for the identification of endocrine disruptors (ECHA/EFSA, 2018) and its weight of evidence approach may be used within the context of the derivation of EQS for tebuconazole.The work consisted in (i) gathering ED toxicity data for aquatic wildlife and mammals not included in the initial dataset underlying the current EQS, (ii) assembling them in a database as lines of evidence of toxicity adressing endocrine activity in order to assess as rigorously as possible the ED potential of tebuconazole and finally (iii) analysing these data to evaluate how far they allowed to improve reliability of the current EQS for tebuconazole.The collection of data allowed to assemble more than 200 lines of evidence consisting in vivo mammals data (52%), in vivo wildlife data (34%) and in vitro mechanistics data (14%).The assessment did not allow to conclude on the ED potential of tebuconazole for wildlife strictly applying the guidance, whereas this ED potential was demonstrated for mammals for which at least two modes of action analysis enabled to conclude on the link between endocrine activity and adversity.Wildlife toxicity assessment would need more in-depth analysis, inter alia to collate and analyse invertebrates toxicity data in order to assess other taxa than fish and amphibians which are already well-represented in the dataset.This analysis allowed implementation of the weight of evidence approach of non-standard toxicity test within the assessment in order to strenghten relevancy and reliability of its EQS. It gives a good opportunity to evaluate how ECHA/EFSA guidance may be used in another regulatory context for which it is meant and allows highlighting the pros and cons of this exercise.

6.01P.2

Modification of CRED system for study evaluation in a regulatory context

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The CRED system for evaluation of aquatic ecotoxicity studies was developed in order to ensure a transparent evaluation of studies, both those submitted for regulatory purposes (mainly GLP studies) and those from grey literature or from public literature [1]. The system puts somewhat less emphasis on the GLP status of a study than the Klimisch system [2], while still allowing the evaluator to be transparent about the considerations which went into determining the eventual utility of the study for a regulatory purpose. In addition to a consideration of the reliability of the study, the CRED system considers the relevance of the study in question for the regulatory purpose in question. In order to make use of the CRED system for our regulatory assessments, we slightly modified the parameters dictated and tested by the original CRED methodology in order to be able to utilize the system for all study types. We will present our modifications and how we use the system, as well as our pros and cons from working with the modified CRED for several years. In general, although there is some resistance to the use of the modified CRED system, particularly for very straight-forward study evaluations (due to the extra time required to fill in the separate tables), we are pleased with the results of the modified system and its implementation. Once evaluators are comfortable with the system, its use becomes more routine and harmonization improves. The generic system provides us with a “fit-for-purpose” manner in which to transparently communicate study evaluation decisions and show how they contribute to weight of evidence considerations in regulatory risk assessment.

Integrated Effect Assessments to Enable a Sustainable Future for the Marine and Coastal Environment (P)

6.02P.1

Addressing sustainability needs with isothiazolinone chemistry: the favorable environmental profile of DCOIT

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In critical end-uses such as wood preservation and antifouling paints, biocides are required to meet highly stringent product stewardship and regulatory assessment standards. Ideally, sustainable preservatives must be efficacious, ideally to control a broad spectrum of target organisms, whilst posing minimal risk to non-target organisms and the environment. Where environmental exposure can be predicted either during manufacturing or service life, subsequent leaching and ultimate environmental fate are key factors that must be considered if a chemical is to be truly considered sustainable. The organic, non-metal-based active ingredient 4,5-Dichloro-2-octylisothiazol-3(2H)-one (DCOIT), currently used in many environmentally-sensitive preservation applications globally, meets all of these requirements. By examining key aspects of DCOIT’s environmental toxicology, fate (e.g. biodegradation, soil mobility), minimal bioaccumulation potential and ultimate impact (i.e. risk) in various practical applications, we demonstrate the important role that DCOIT can play to deliver environmentally sustainable solutions.

6.02P.2

Ensuring the environmental sustainability of Production of primary aluminium (ESPIAL): the Marine environment

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The ESPIAL (2018-2021) project is an industry funded research project revisiting an effects study conducted 25 years ago looking at Norwegian Aluminum smelters and the local environment. The new ESPIAL study looks at the impact of pollution on the areas around aluminum smelters in Norway, Sweden and Iceland and looks at changes since the original study as well as assessing todays environmental status. The study encompasses effects on the marine environment, air, wildlife and vegetation in and around these smelters. Through the years, NIVA has been involved with research and monitoring regarding discharges from the aluminum smelters in the marine environment. Therefore, NIVA have been leading the part of ESPIAL related to this compartment. Other partners include NIBIO (vegetation), NILU (air) and the veterinary institute of Norway (wildlife). ESPIAL for the marine environment has three main objectives. Objective 1) aims to look at today’s status and identify and describe changes since the original effect study. Specifically, data was gathered from numerous sources on pollutant levels in biota, sediment and water in the local environments around the smelters. In addition, a database tool for visualizing and investigating historical and new data on pollutants in the local environment in these matrices was created. Objective 2) aims to quantify environmental effects of changes in emission volumes and parameters, quantify emission levels if environmental standards (EQS) are to be met, forecast time to reach EQS s if remedial actions are not implements (This was done employing a Bayesian network modelling approach) and finally objective 3) aims to assess the effects and risks today in a holistic manner coupling novel analytical and toxicological methods at specific case study sites to look at risks from historical compounds, identify unregulated/unmonitored compounds and use this information to stay one step ahead of any future changes in legislation.

6.02P.3

First Evaluation of the Status of Marine Invertebrates in North Corsica Ports

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Coastal areas are the interface between terrestrial and marine ecosystems and are a habitat for 90% of the marine biodiversity. However, these waters are the first impacted by anthropic activities, especially in port areas, due to the maritime traffic or accidental pollution. Following the recommendations of the Marine Strategy Framework Directive, the first objective of the QUAMPO (QUAlity of the marine environment in the Mediterranean POrt areas) project is to evaluate the environmental status of several ports in north Corsica. Several benthic organisms (mussel Mytilus galloprovincialis, sea cucumber Holothuria tubulosa and limpet Patella caerulea) were sampled in four sites (reference site in Revellatta Bay, ports areas in Calvi bay, Île Rousse and Saint-Florent) and biomarker analyses were performed. Biomarkers of oxidative stress (catalase, glutathione peroxidase, malondialdehyde…), detoxification (glutathione-S-transferase…), energetic metabolism (pyruvate kinase, phosphoenolpyruvate carboxykinase…) and immunity (laccase) were analysed in the digestive gland of mussel and limpet and in the respiratory tree of sea cucumber while bioaccumulation of trace elements and organic contaminants was measured in the edible part of the organism (soft tissue for mussel/limpet and body wall for sea cucumber). Moreover, development of biomarker reference values will be associated with the monitoring for other parameters such as water physicochemical assessments (temperature, salinity, turbidity, chlorophyll and dissolved oxygen) will be measure in each station, and the concentrations of chemical contaminants (inorganic and organic pollutants) in animals could be also quantified to carry out a qualitative correlative approach. Finally, several biometric parameters (length, thickness and width) will be done to (1) establish results to evaluate the health status of the organisms according the type of ports (marina or fishing harbour) and (2) select the best biomarkers for monitoring programs in order to confirm, for the first time, their ability to appraise the environmental quality in north Corsica coastlines, such as port areas. Here, the firsts steps of the QUAMPO project and our first results will be presented.

6.02P.4

Classification of coastal sites in North western of Algeria using biomarker index and the Norwegian classification system (KECS) for monitoring pollution

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In this work, we studied the potential toxic of different coastal sites in the north-western of Algeria using native mussels *Mytilus galloprovincialis* as bioindicator organisms. The concentrations of some persistent chemicals such as, polychlorobiphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals were analyzed in the mussels. These pollutants can accumulate in sediments and organisms, and they biomagnify through the food chain leading to high concentrations in top predators. Moreover, biochemical and physiological responses of sentinel organisms were measured to assess the impact of pollutants. The biomarkers glutathione s-transferase (GST), catalase (CAT), as well as the condition indices were determined. GST and CAT activities are often used as biomarkers of pollutant-mediated oxidative stress in mussels, and GST has a second function as a Phase II (conjugation) enzyme involved in the detoxification of organic xenobiotics. Site classification indices based on biomarkers data, biomarker index (IB), and on concentration levels of measured pollutants, Norwegian classification system (KECS), were combined to assess the toxic potential of the different six studied coastal areas. The results show that mussels of Oran Harbour are extremely polluted by PCBs and PAHs, i.e., 97.6 and 2892.1 µg/kg d.w., respectively. Other sites present low levels of pollution. Furthermore, high concentrations of zinc, lead, and cadmium are found in mussels from fishing, agricultural and estuarine sites, respectively, while low concentrations of copper are found in all of the sites studied. CAT activity is negatively correlated with Cd and Cu, and Zn is positively correlated with GST and CAT. The KECS classification based on concentrations of measured pollutants reveals a gradient of toxicity along the studied area and mixed pollution from trace metals and organic compounds in Oran Harbour. The IB classification from the biomarkers data shows similar results incriminating Oran Harbour as the most heavily impacted area. The site classification tools reveal the potential toxicity of studied coastal areas on mussels exposed to anthropogenic pressure. It also highlights a gradient of toxicity along the Algerian west coast.

6.02P.5

Mapping Stressor Effect of Sediment Nutrients on Macrobenthic Invertebrate Distribution in Estuarine Tropical Water Southwestern Nigeria

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The coastal ecosystem has enormous benefits to humans which includes transportation, fisheries resources and recreational activities. Despite the significant importance, it is identified as one of the most vulnerable environment to human-induced contaminants. Lagos lagoon has been documented as one of the most utilized lagoon systems in southwestern Nigeria. This study aimed to map the benthic macroinvertebrates distribution in responses to nutrients level in the sediment of Lagos Lagoon. Sediment samplings were collected bimonthly with a 0.1m2 Van –Veen grab at twenty-four selected stations covering the wet and dry seasons over a two year period. Standard APHA methods were employed to determine the phosphate, nitrate and silicate concentrations in the sediment. Canonical correspondence analysis (CCA) was used to verify the influence of nutrients on the spatial distribution of benthic macroinvertebrate community structure. The spatial abundance response of each of taxa was associated with phosphate and silicate loading in the Geostatistical Analyst of ArcGIS 10.3.1 version. The optimum abundances for macroinvertebrate taxa were observed at relatively low spatial concentrations of nutrient which highlights the vulnerability of Lagos Lagoon with increasing stressor loads. These observation documents nutrients as an indicator of stress in macroinvertebrates abundances. Therefore, benthic macroinvertebrates are considered an important biological indicator of lagoon system because of their sensibility to environmental changes.

6.02P.6

Climate-change associated floodwater as a pathway for microplastic and tire wear particle transport to coastal waterways: A case study of Charleston, SC (USA)

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The streets of Charleston, South Carolina (USA) experience nuisance flooding more than 50 days annually due to encroaching perigean spring tides (or "King Tides") and extreme rainfall events, both of which are exacerbated by climate change. Street floodwaters are not only hazardous to drivers, but also serve as a potential pathway for anthropogenic pollutants, such as microplastics, into local waterways. Tire wear particles (TWP), a type of microplastic produced by mechanical abrasion of tires on streets, have been documented in waterways near urbanized areas. Surveys of Charleston Harbor have indicated high concentrations of TWP in intertidal sediment and surface waters. This research aims to establish baseline data on microplastic and TWP abundance found in street floodwaters and to investigate how nuisance flooding events may contribute microplastics and TWP from the streets to the harbor via adjacent tidal creeks. Four street locations prone to regular nuisance flooding and their adjacent tidal creeks were identified on the Charleston peninsula for this year-long study. During each flood event, tidal creek surface water and floodwaters were sampled, then sieved at 63 and 500 µm. Retained particles were visually identified as microplastic or TWP under a dissecting microscope. Preliminary data suggests that tidal creeks have lower microplastic abundances before flooding (8.75 microplastics/L) and higher counts after flooding (14 microplastics/L). Total microplastic abundance in floodwater ranged from 9-607 microplastics/L, of which the majority were clear fibers (69%) and TWP (29%). The high variability in microplastic abundance amongst floodwater samples is potentially due to differences in land use and/or roadway conditions and warrants further investigation. Additional sampling is planned and will occur opportunistically when flooding occurs. This research is the first to investigate climate change-induced coastal flooding as a pathway for land-based microplastic pollution, particularly TWP, into our coastal waterways. Understanding these pathways is critical to informing policy and management decisions to minimize the environmental and economic impacts of this type of debris in the future.

6.02P.7

Developmental effects of fuel oils on early life stages of Atlantic cod (Gadus morhua)

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The ban of using heavy fuel oils in the Arctic and demands for low sulphur content fuels to reduce air pollution have resulted in a drive towards development of new generation fuel oils. In the present study, we studied the toxicity of three fuel oils; two marine diesels with sulphur contents of 0.05 (MGO500) and 0.1 % (MGO1000), respectively, and a bunker oil (IFO-LS) as reference. Effects of dissolved fraction of the fuel oils were tested on developing cod embryo and larvae. The effects of exposure on survival, hatching, development, deformations, heart activity and gene expression of transcripts related to vascular, angiogenesis and osteogenesis differentiation were investigated. The fuel oils produced oil-type specific morphological alterations (such as cranial deformation and pericardial edema) on cod larvae. On the transcript expression patterns, we observed that, all fuel oils produced concentration-dependent increases on genes belonging to the AhR gene battery (e.g. *cyp1a* and *ahrr*). However, on genes related to vascular development (*vegf*), angiogenesis and osteogenesis (*bmps, col1a1, runx, osteocalsin*, among others), the fuel oils produced concentration- and oil-type specific modulations on these transcripts. Even after diluting WAFs to 6%, we were unable to reach an exposure level that produced zero toxicity. Of the three fuel oils tested, the MGO1000 displayed the highest toxicity. In order to assess potential environmental effects of acute fuel oil spills in the Arctic, the Oil Spill Contingency and Response (OSCAR) model was used, and oil spill simulations in Isfjorden (Svalbard) were performed for all three fuel oils. These simulations indicated that, given identical spill properties, the potential for toxicity to early life stages of fish were significantly higher for MGO1000, compared to MGO500 and IFO-LS. The chemical composition of the MGO1000 facilitates dissolution as it contains high level of dissolvable components which are expected to cause developmental effects on fish early life stages. In conclusion, a marine diesel (MGO1000), approved for use in the Arctic, displayed higher toxicity to fish ELS than a heavy bunker fuel oil. Our study highlights the important to assess environmental fate and effects of spills of new fuel oils entering the market.

6.02P.8

Characterization and basal physiological status of Mytilus edulis haemocytes: a flow cytometry approach

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The mussel *Mytilus edulis* is a well-recognized bioindicator species in ecotoxicological and biomonitoring studies for assessing the health status of the aquatic environment. Haemocytes, the main cellular components of bivalves' haemolymph, play an important role in several vital functions (immune defence, growth, reproduction) and have been identified as a reliable target for investigating the effects of contaminants at cellular, biochemical, and molecular levels. The aim of this study was to develop an integrated effects methodology using flow cytometry to quickly assess the impacts of contaminants in mussel haemocytes at conditions close to an *in vivo* state. In a first approach, the morphological and structural characteristics of haemocytes subpopulations were studied using a combination of light microscopy and flow cytometry. Three major haemocytes subpopulations were identified visually according to their morphological aspects. Granulocytes were the largest cells (23.64 ± 7.37 µm) containing numerous granules of various size, while hyalinocytes were smaller agranular cells (17.14 ± 5.71 µm). Blast-like cells were homogenous cells characterized by a smaller size (8.8 ± 2.85 µm). The presence of different haemocytes subpopulations in *M. edulis* was also identified using flow cytometry. Granulocytes and hyalinocytes were recorded as the main population composed by cells with a high and intermedia internal complexity and larger size, while a smaller population composed of blast-like cells was characterized by a smaller size and complexity. In a second step, haemocytes' basal functional, physiological and immunological responses were evaluated in terms of haemocyte mortality, cell viability, reactive oxygen species (ROS) formation, cellular and mitochondrial membrane potential and lipid peroxidation (LPO) using flow cytometry. Results obtained showed that in general hyalinocytes exhibited higher oxidative (ROS formation and LPO), metabolic and membrane activities (cellular and mitochondrial), followed by granulocytes and blast-like cells. Overall, the methodological approach used in this study was successful in differentiating the different morphological, biochemical and physiological characteristics of mussel haemocytes subpopulations. In addition, these results will serve as a first step to develop fast, accurate and reliable biological endpoints for use in environmental hazard assessment covering a wide array of cellular functions in *M. edulis* haemocytes.

6.02P.9

Effects on marine planktonic species under temperature changes and oxyfluorfen exposure

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The exponential increase of human population demands an over usage of fertilizers and pesticides in agriculture practices to supress the food production needs. Still, the excessive use of these compounds can comport deleterious effects to the ecosystems (e.g. biodiversity losses) and damage to the public health. Herbicides are highly used and have the ability to bioaccumulation on primary producers with proliferation along the trophic chain. Oxyfluorfen is a fluorine-based herbicide that acts by inhibition of protoporphyrinogen (PP) oxidase (PPO). The oxyfluorfen application has increased, since nowadays these non-selective and of broad-spectrum herbicide is seen as an alternative to control the glyphosate resistant weeds. The PPO activity inhibition and consequent accumulation of PP in the chloroplasts with leakage to the cytosol lead to a great production of single oxygen. Hydrogen of the unsaturated lipids is extracted, lipid radicals are produced and a chain reaction of lipid peroxidation begins, resulting in damage on cell membrane, occurring fast disintegration of organelles and cells and in last state the cell death. Few studies are dedicated to the evaluation of the oxyfluorfen effects on a set of species. Thus, this work pretends to evaluate the effects of temperature variations and oxyfluorfen exposure on the sensitiveness of two marine species, a diatom *Thalassiosira weissflogii* and a copepod *Artemia franciscana*. Acute bioassays were performed during 96h to the diatoms and 48h to the copepods, both at 15ºC, 20ºC and 25ºC. The organisms were exposed to a range of 8 concentrations (1-18 µg/L and 0.05-1.2 mg/L, respectively) plus a negative and a solvent control. Lethal concentrations were determined to both species and to different temperatures (*T. weissflogii*: LC_{50}(15ºC)=2.31(1.44-3.17) µg/L; LC_{50}(20ºC)=3.22(2.60-3.84) µg/L; LC_{50}(25ºC)=8.74(7.98-9.49) µg/L; *A. franciscana*: LC_{50}(15ºC)=1.107 (0.942-1.383) mg/L; LC_{50}(20ºC)=1.019(0.708-1.939) mg/L; LC_{50}(25ºC)=0.853(0.548-1.832) mg/L). Results show the diatoms are more tolerant to oxyfluorfen under higher temperatures, whereas the brine shrimp present an opposite, trend. Main conclusions are that with the raise of temperature, and under chemical stressors, primary producers seem to be more tolerant than primary consumers with severe consequences to the trophic chain and to ecosystem water quality in cases of long exposure.

Marine and Freshwater Pelagic and Benthic Harmful Algal Blooms: Toxins Production, Detection, Fate, Effects, Monitoring and Management (P)

6.03P.1

Early detection of cyanotoxin threat in freshwater bodies by quantitative PCR

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Increased frequency of cyanobacterial blooms, caused by rising temperatures and eutrophication, is creating a growing need for reliable methods for early cyanotoxin threat detection in water bodies. Current methods are time-consuming and sensitive to taxonomic inconsistencies. On top of that, they only enable detection of potentially toxin-producing cells when they have already multiplied to a relatively large number. To develop a tool for earlier detection of potentially toxic cyanobacterial strains, we are using qPCR to target genes involved in crucial parts of cyanotoxin synthesis. Detection and quantification of these genes, if incorporated in regular monitoring, might serve as an early warning in case of increased potential for cyanotoxin production. Toxins and genes of interest in this study are microcystins (mcyE), saxitoxins (sxtA) and cylindrospermopsins (cyrJ). The analyses will be carried out on 28 phytoplankton and 23 phytobenthos samples from 15 different freshwater bodies in Slovenia. This way we will include also understudied benthic cyanobacteria and their ability to produce toxic metabolites. Results will be compared with taxa list obtained by traditional microscopic identification methods and with cyanotoxin content evaluated by LC-MS/MS. The study has a potential to prepare the ground for improving current monitoring programs by complementing them with highly sensitive molecular methods. This can provide more in-depth information about dynamics of toxic cyanobacterial populations in water bodies and thus help with adopting appropriate mitigation strategies.

6.03P.2

Experimental approach to understand aerosolization mechanisms of toxins produced by the microalga Ostreopsis cf. ovata

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Blooms of the benthic dinoflagellate *Ostreopsis* cf. *ovata* have been related to mild but acute respiratory symptoms on people exposed to marine aerosols in some Mediterranean and Brazilian beaches. These disorders have been attributed - but not proven yet- to palytoxin (PLTX) analogues (ovatoxins -OVTX- and isobaric palytoxin -isoPLTX-) produced by *Ostreopsis*. However, these compounds have been seldom found in the aerosol and when detected it was not associated to human health impacts. To shed light on the mechanisms of toxin production and transfer to the atmosphere five laboratory experiments were run using an aerosol generation tank with microbial communities obtained during the peak of an *Ostreopsis* bloom.Seawater and aerosol samples were analyzed for i) toxin concentration (with an UHPLC system coupled to a Q–Exactive Orbitrap Fourier–Transform Mass Spectrometer (FTMS) equipped with a heated–electrospray ionization source (H-ESI) operating in positive ion mode using a Hypersil GOLDTM C18 column (100 mm x 2.1 mm id., 1.9 µm particle size) packed with totally-porous silica particles, under a gradient elution of acetonitrile:water (0.1% formic acid) mobile phase), and ii) offline spectroscopic analysis performed by nuclear magnetic resonance (NMR).Toxins were detected in seawater samples and in atmospheric aerosol generated in all experiments. Unexpectedly, higher toxin concentrations (49-69 pg·Lair–1) were found in the aerosols generated by bubbling relatively low *Ostreopsis* cells abundances and toxins in the water (respectively, ca. 105 cells·L–1, 4 x 106 pg·Lwater–1), compared to the ones (3-4 pg·Lair–1) obtained on higher biomass abundance experiments (1 x 106 cells·L–1 and 2 x 108 pg·Lwater–1, respectively). The toxin profile in the air contained OVTX-a and b only, while other forms (OVTX-c, d, e and isoPLTX) were also present in the water. Moreover, NMR analysis showed different composition of the particulate organic carbon in the water (*Ostreopsis* cells and accompanying microbiota) and bubble bursting aerosols, suggesting selectively transfers of organic compounds from seawater into the atmosphere. High foam content was also observed in the water surface of the tank. Overall, these experiments constitute a first step to understand the ecophysiogical microalgae responses to bubble bursting as well as the complex air-sea dynamics in the surface microlayer likely regulating aerosol production and diffusion of the marine toxins in the atmosphere.

Modern Approaches to Assessment and Management of PFAS: A Science-Policy Dialogue (P)

6.04P.1

Environmental Sources, Analyses, Chemistry, Fate & Transport of Per- and Polyfluoroalkyl Substances - Workgroup Summary, Output, Conclusions and Implications from the 2019 SETAC Focused Topic Meeting

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The overall objectives of the SETAC focused topic meeting (FTM; August 2019) were two-fold: 1) to review new and emerging information on PFAS chemical classification and grouping, environmental chemistry, detection technology, fate and transport, exposure potential, human health toxicity, and ecological toxicity and 2) to harness the expertise of eminent scientists from around the globe with the goal of developing a risk assessment approach that considers mechanistic (including computational) approaches for extrapolating exposure and effects across different scenarios/species and compounds within environmental pathways for exposure. This workgroup included several presentations and a subsequent breakout group that identified fundamental aspects of the current state of the science, critical knowledge gaps, and future research needs. The major workstreams in our workgroup (sources, analyses, chemistry, fate & transport) were as follows: 1. Key challenges and strategies for source-pathway evaluation and prioritization 2. Existing analytical techniques and areas of need for detecting and measuring PFAS 3. Identification of PFAS in the environment 4. Physical-chemical properties of PFAS, patterns that can be used to develop empirical models of environmental fate and transport, and application of physical-chemical properties towards defining source zones and source strength 5. Addressing long-term transformation of PFAS precursors in the environment 6. Current state of the science and advances in the systematic characterization and categorization of PFAS 7. Classification and grouping of PFAS for environmental risk assessment This platform presentation will highlight workgroup outcomes from the ten (10) major talks given at the FTM and significant subsequent work the extended team has done in creating a manuscript for publishing the work discussed, research needs, challenges, conclusions and primary path forward items.

6.04P.2

A comparison of perfluorooctanoic acid (PFOA) and hexafluoropropylene oxide dimer acid (HFPO-DA, or GenX) effects in target tissues of exposed mice.

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Perfluorooctanoic acid (PFOA) is a perfluoroalkyl substance (PFAS) associated with adverse biological effects in numerous tissues, with concordance across species for many of the targets, but little is known regarding health effects for one of its replacements, hexafluoropropylene oxide dimer acid (HFPO-DA or GenX). GenX was developed to replace PFOA due in part to its quicker elimination in both human and rodents but has received public scrutiny in the US and Europe after reports of GenX contamination in drinking water sources. The purpose of these studies was to compare changes in known PFAS-target tissues following *in utero* exposure to PFOA and GenX in a blinded study design. Time-pregnant CD-1 mice were dosed with PFOA (0, 0.1, 1.0, or 5.0 mg/kg) or GenX (0, 0.2, 1.0, 2.0, or 10.0 mg/kg) from gestation day (E) 1.5 to 17.5. Maternal clinical chemistry, maternal liver, mammary and placental histopathology, embryo and placental weight, internal dosimetry, and placental thyroid hormones were measured at E11.5 and E17.5. Body weight was measured daily. Birth outcomes, mammary glands, weight gain, internal dosimetry and other metabolic data were collected from offspring at multiple developmental time points. Although GenX did not accumulate in the dam liver over time like PFOA, it recapitulated many known effects of PFOA on the liver/metabolic system of the dams, including significantly increased gestational weight gain, reduced serum triglycerides and 100% incidence of hepatocellular cytoplasmic alterations. PFOA and GenX both induced lesions in more than 68% of placenta evaluated at the highest doses, but lesion subtype incidences were compound-specific. Placenta labyrinth atrophy was more common in GenX-exposed placentas, whereas labyrinth congestion was more common in PFOA-exposed placentas. Although there was no sex-specific accumulation of GenX or PFOA in the E17.5 embryos, there were sex-specific effects in the offspring. There were significant dose-dependent reductions in developmental score for female mammary glands exposed to PFOA and GenX ($p< 0.01$). All female dose groups except 0.2 mg/kg GenX had mean scores significantly lower than control glands but no male dose groups differed from the control. Although similar in weight at birth in all groups except 5 mg/kg/d PFOA (reduced), male offspring exposed to GenX in utero exhibited enhanced weight gain over time and other metabolic shifts consistent with metabolic disease in our preliminary analyses. In summary, although GenX and PFOA share some effects on the liver and mammary gland, there are also target tissues with unique sensitivities to these PFAS that may be sex-dependent and may vary in their modes of action.

6.04P.3

Biosorption and permeabilities of PFAAs and four of their alternatives and the effects on toxicokinetic behaviour

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Perfluoroalkyl acids (PFAAs) have been in focus of authorities and researchers due to their persistent and bioaccumulative behaviour for the last 20 years. Though the production of long-chain PFAAs like PFOA and PFOS has been partly restricted, a diverse set of alternatives is now commercially used. These alternatives comprise short-chain PFAAs but also structurally modified PFAAs which are still highly fluorinated. Human risk assessment for PFAAs including the alternatives is challenging since existing models are missing accurate experimental input parameters such as partition coefficients. If partition coefficients to relevant biological matrices are known they can be used to improve the prediction of bioaccumulative behaviour in an organism. In literature, two matrices have been discussed to contribute predominantly to the bioaccumulative potency of PFAAs: the transport protein serum albumin and phospholipid membranes. We determined albumin/water and membrane/water partition coefficients for a series of PFAAs including seven perfluoroalkyl carboxylates (PFCAs) and three perfluoroalkane sulfonates (PFSAs), as well as the four alternatives tetrafluoro-2-(heptafluoropropoxy)-propanoate (HFPO-DA), 4,8-dioxa-3H-perfluorononanoate (DONA), perfluoro-4-ethylcyclohexanesulfonate (PFECHS), and 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (9Cl-PF3ONS) by dialysis experiments. The sorption of PFAAs to albumin and to membranes increases with increasing number of perfluorinated carbons and ranged from log $K_{albumin/water}$ 2.5 – 4.8 (K in units of L/kg), and log K<sub>membrane/water 99.999% in anionic form at pH 7.4), they were expected to be transported only actively. However, our experiments show that passive membrane transport along a concentration gradient could be the dominant pathway for the uptake of PFAAs and their alternatives into organisms. We discuss the results of our experiments considering the consequences for the toxicokinetic behaviour of PFAAs and of their alternatives.

6.04P.4

A comparative assessment of estimation methods for property estimation of per- and polyfluoroalkyl substances (PFAS)

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Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals widely used in a variety of industries. Once introduced to the environment, PFAS are resistant to degradation which increases their bioaccumulation potential and exposure to the environment. For the purposes of environmental remediation and risk assessment, characterizing PFASs by their physicochemical properties can provide insight into their transport and fate within the environment. An important requirement for accurately modelling chemical fate is the availability of reliable physicochemical property data. Attempts have been made to experimentally measure various physicochemical property data of PFAS; however, their surfactant-like nature makes it difficult to obtain certain physicochemical properties. Also, inconsistency has been found in the literature for physicochemical property measurements. Therefore, the assessment of the accuracy of existing property estimation models is required. The quality of predicted physicochemical data for a set of 25 PFASs is examined using COSMOtherm, EPI Suite, and the estimation models accessible through the US EPA's CompTox Chemicals Dashboard. This research aims to support regulatory efforts in characterizing the large amount of currently non-regulated PFAS by their physicochemical properties, and to provide an up-to-date assessment of physicochemical property estimation models. Preliminary results indicate that COSMOtherm and the OPERA model estimates from the Dashboard largely provide physicochemical property estimates with the lowest deviations from experimental measurements.

6.04P.5

Exposure of lettuce to perfluoroalkylated acids (PFAAs) in naturally contaminated soil: accumulation patterns and toxicokinetics

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Perfluoroalkylated acids (PFAAs) can enter the food chain due to their persistence, widespread use and global distribution. Field research has demonstrated that these substances can bioaccumulate and biomagnify in wildlife. Hence, it is very plausible that PFAAs can biomagnify to high concentrations in humans. For these reasons, PFAAs may pose a significant risk to human health as residents may inadvertently consume PFAA contaminated food. Over the last decade, the consumption of food products from self-cultivated origin by humans has become a remarkable trend in rural, urban and even industrial environments. For instance, growing edible crops in private gardens has gained popularity as plants constitute an important component of the human diet. At the same time, the dominant exposure source of PFAAs to humans is the diet. Due to the partial water solubility of PFAAs, water can be considered the main vehicle of transfer for PFAAs through the terrestrial food chain. Within this scenario, it has been demonstrated that some PFAAs accumulate to great extent in plants, from which they can be transferred to humans. However, little is known on the uptake rate of PFAAs in plants and the influence of different ontogenetic stages in this regard. Nevertheless, this is crucial information when considering the exposure risk of PFAAs through vegetables to humans. The main objective of this study was to assess the uptake rate of PFAAs in different (edible) parts of an edible crop species grown on a naturally PFAA contaminated soil. Secondly, we examined whether different ontogenetic types (primary versus secondary roots) and stages (young versus old leaves) of the plant were related with diverging uptake patterns. Preliminary results show that short-chain PFAAs bioaccumulate to relatively high concentrations in all plant parts. Kinetic modelling will reveal which PFAAs and to which extent they are taken up. For instance, PFOA accumulated to a greater extent and faster than PFOS in the leaf parts. Additionally, roots accumulated long-chain PFAAs to a greater extent than leaves. Total PFAA concentrations for all the detected compounds saturated over time, which has important implications regarding timing of harvesting crop plants in relation to potential human exposure to PFAAs.

6.04P.6

Analysis of Northern Bobwhite Quail Liver Bioaccumulation and Egg Deposition from Chronic Exposure to Perfluorooctane Sulfonate (PFOS) or a Mixture of PFOS and Perfluorohexane Sulfonate (PFHxS)

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Per- and Poly-fluoroalkyl substances (PFAS) are a broad class of potentially toxic synthetic organic molecules that include thousands of environmentally persistent compounds. There is a lack of terrestrial toxicity data on individual PFAS for comprehensive risk assessment, and therefore a lack of data to inform regulations of these chemicals. Recent research attention has focused on the toxicity and bioaccumulation potential of select per- and poly-fluoroalkyl substances (PFAS) producing new data which suggests lower dietary toxicity values over chronic exposure than previously derived for Northern Bobwhite quail. In this study, we correlated tissue and egg residue data as well as biometrics and liver lipid content to results from chronic toxicity data previously reported by our lab. We then calculated average daily intakes (ADIs) based on residue data for a more thorough investigation of the toxicity of perfluorooctane sulfonate (PFOS) and a mixture of PFOS and perfluorohexane sulfonate (PFHxS) to avian receptors. Specific variables analyzed were wing band and tarsal length, head size, relative liver weight, liver lipid content, and residues in liver tissue and egg homogenates. Residues for only the high exposure level groups were quantified because this is the concentration at which adverse health effects were observed in our chronic toxicity study. Egg residues for PFHxS and PFOS ranged from 1.3 to 223 ng/mL and from 2.7 to 607 ng/mL, respectively. Controls displayed trace amounts of both chemicals as well, likely due to environmental exposure pre-acquisition which was subtracted as background concentrations from treatment results. Feed supplies were also tested to ensure delivery of test chemicals was solely through drinking water. Liver lipid content in the adults was not significantly associated with treatment; however, the same trend did not hold true for chick liver lipid content which was significantly associated with treatment. Results from this study can be used to update and/or derive avian toxicological reference values (TRVs) for PFOS and this simple PFOS:PFHxS mixture to better estimate ecological and human health risk.

6.04P.7

Bioaccumulation and biomagnification of perfluorinated alkyl substances (PFAS) in marine biota of the Belgian North Sea and their potential risk for human consumption

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Perfluorinated alkyl substances (PFAS) are synthetic chemical compounds with a highly persistent character and a widespread usage, making them ubiquitous in the environment. Biomonitoring of PFAS is of critical importance because of their bioaccumulative potential and known adverse health effects on biota, including humans. Due to the proximity of a fluorochemical plant near the port of Antwerp (Belgium), the Western Scheldt estuary is an interesting area to study the occurrence, fate and effects of PFAS in an aquatic environment. Previous studies have been performed on PFAS contamination in biota from the Western Scheldt, yet little is known on the occurrence of PFAS in marine biota of the southern North Sea. The first objective of this study is; (1) to identify whether a PFAS pollution gradient can be found from the Western Scheldt towards the Belgian North Sea. Therefore, PFAS contamination profiles are analysed in muscle and liver tissue of marine biota sampled at 10 locations expanding from the mouth of the Scheldt estuary towards open sea and along the Belgian coast. Secondly, (2) the biomagnification potential of PFAS compounds throughout the marine food web of the North Sea is studied using stable isotope analysis of seven fish species and four invertebrate species. Since the Belgian North Sea is a commercial fishing area and PFAS tend to accumulate in muscle tissue of fish, a third objective (3) is to determine the potential risk to human health by consumption. Based on the Minimum Risk Levels (MRLs) determined by the Agency for Toxic Substances and Disease Registry (ATSDR), the maximum daily consumption of perfluorooctane sulfonate (PFOS) contaminated fish is calculated in relation with human body weight. With this study we aim to provide more in-depth knowledge on PFAS pollution along Belgian coastal waters in order to assess the potential risks to marine biota and humans.

6.04P.8

How much chemicals with PFAS do we use in Norway? A data register study based on data from 2009-2017

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A data register study was performed to identify the amounts of PFAS in chemicals imported to Norway during 2009-2017. The Product Register in Norway is the Norwegian authorities' official register of chemicals imported and produced in Norway. According to legislation, manufacturers or importers who produce and/or place on the market 100 kg/y or more of a chemical classified as hazardous are obliged to submit a declaration to the Product Register. The data from the years 2009-2017 was searched for substances from PFAS on the OECD list and on the KEMI list (short chain PFAS). During the period, 72 CAS from the OECD list and 5 from the KEMI list was used in products in Norway. Most of the PFAS were telomers, sulfonyls precursors or polymers. The tonnages imported (since Norway has no production) were between 15-28 tons/y and was 249 tons in the period. During the years, the amount of telomers was highest (150 tons), but also the amounts of polymers, short chain PFAS and precursors were high (51, 26 and 16 tons respectively). Since 2014, the yearly percentage of use of telomers have decreased from >75 to 25% of yearly import, while the use of polymers, short chain PFAS and precursors have increased. A telomer with chain length of 6 was the PFAS used in highest amount (110 tons) in the period for fire fighting purposes, followed by PTFE (49 tons) used in very different products such as e.g. paint, reducing friction, lubricant and more. A silicon with 9 Fluor atoms was used as an defoamer by the oil industry. The use of PFAS compounds was quite diverse, but fire-fighting purposes was using most PFAS (130 tons). However, more than 71 tons of PFAS in the period was used in paint, as defoamers, friction reducers and paper industry (each group >14 tons). In the period the yearly percentage of PFAS used for fire fighting purposes has been reduced (>50% to 25%) while other uses have increased. We believe that the amounts that we have reported are minimum data since Norway currently do not have an obligation to declare PFAS to the product register. We recommend Norwegian authorities to amend legislation to include such obligation in line with Sweden. Our conclusions are that the Product Registry is a very useful tool showing that PFASs are used in high amounts. Fire-fighting purposes has been the major use, but PFAS compounds used for other purposes are increasing.

6.04P.9

Distribution pattern of perfluoroalkyl substances in Swiss riverine sediments

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Perfluoroalkyl substances (PFAS) are synthetic chemicals that have been used for decades in numerous products due to their specific chemical properties, including their resistance to degradation, and that are of particular concern because of their persistence and their bioaccumulative properties. Due to the low volatility and high solubility of PFAS, aquatic environment is an important sink for them and their distribution between water and particles is considered as an important process in controlling their transport and fate. The aim of the present study was to examine the distribution pattern of PFAS in different sediment collected in 19 Swiss streams. Total Organic Carbon (TOC), dry matter level, and fine fraction content (mass percent fraction of the < 63 µm fraction over the < 2 mm fraction) were determined in each sediment. 11 perfluoroalkyl substances (9 perfluorocarboxylic acids - PFCA, 1 perfluorosulfonic acid - PFSA and perfluorooctanesulfonamide - FOSA) and the perfluorooctane sulfonate (PFOS) isomers were investigated in the riverine sediment samples. Analyses were carried out on < 2 mm and < 63 µm fractions. Extraction of the freeze-dried sediment (2-10 g) was performed by sonication with ACN/H_2O mixture enhanced by salting out and acidification; extracts were filtered (nylon 0.2 µm) before injection. Perfluoroalkyl compounds were determined by liquid chromatography tandem mass spectrometry (HPLC-MS/MS) coupled to an on-line turbulent flow chromatography (TFC) for on-line purification of the extracts. The ∑PFAS concentrations ranged between < LOD µg/g dw and 43 µg/g dw. PFOS and perfluorohexanoic acid (PFHxA) were the dominant PFAS with an average percentage of 49 and 15 respectively. Generally the higher concentrations were measured in the < 63 µm fraction. Most of the samples have low PFAS concentrations that can represent the anthropogenic background concentration. Some samples showed higher PFAS values (particularly PFOS) suggesting the presence of point sources in the river basin. We observed a composition of PFOS isomers similar the manufactured product with 64% and 36% for n-PFOS and br-PFOS respectively. Correlation with TOC content was also investigated.

6.04P.10

Perfluoroalkyl acids (PFAAs) in the Pra and Kakum River basins and associated tap water in Ghana

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Perfluoroalkyl acids (PFAAs) are persistent environmental pollutants that have been detected in various media including human serum. Due to concerns regarding their bioaccumulation and possible negative health effects, an understanding of routes of human exposure is necessary. PFAAs are recalcitrant in many water treatment processes, making drinking water a potential source of human exposure. This study presents the first report on contamination from PFAAs in river and drinking water in Ghana. The targeted PFAAs were perfluoroalkyl carboxylic acids (PFCAs) with C4–14 carbon chain and perfluoroalkane sulphonic acids (PFSAs) with C6, 8, 10. Five PFAA congeners – PFOA, PFOS, PFHxA, PFDA and PFPeA – were commonly detected in river and tap water. The mean concentrations of ∑PFAAs in the Kakum and Pra Rivers were 281 and 398 ng/L, while tap water (supplied from the treatment of water from those rivers) contained concentrations of 197 and 200 ng/L, respectively. PFOA and PFOS constituted about 99% of the ∑PFAAs. The risk quotient (RQ) attributed to drinking of tap water was estimated at 1.01 and 1.74 for PFOA and PFOS, respectively. For a country that has not produced these compounds, the RQs were unexpectedly high, raising concerns particularly about contamination from such emerging pollutants in local water sources. The study revealed limitations of local tap water treatment in getting rid of these emerging pollutants.

6.04P.11

Monitoring of PFAS in edible crops of an area impacted by a fluorochemical plant

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In 2013, large-scale contamination with PFAAs was discovered in Veneto region, Northern Italy, as a consequence of the emissions from a fluorochemical plant located in the province of Vicenza. PFAS emitted by the plant for decades diffused into the groundwater used as potable water resource because of their mobility and persistence characteristics. Nowadays, the total catchment area cover more than 540 km2 and 350,000 inhabitants, Veneto region matched the results of the biomonitoring study to those of chemical analyses in order to define 3 areas with different health impacts (i.e.: Red, Yellow and Green). Even though elevated serum PFAAs concentrations were detected in the residents of the contaminated areas in Veneto, connected with contaminated drinking water consumption, comprehensive health risk assessment and research considering the food consumption are still lacking. For that reason, the EU LIFE PHOENIX project envisages an extensive monitoring program to assess the distribution of PFAS in different environmental matrices (water, soil, plants, animals) in agriculture areas in the three areas, with a specific focus on more mobile but shorther chain PFAS. These sites are all characterized by the presence of a water body and enough soil to characterise the area of plants growth, moreover the selected stations are all featured by the presence of one ubiquitous aquatic vegetal species (Phragmites australis) and edible vegetables (Zea mais, Lactuca sativa, Cichorium intybus, Allium cepa). Water, soil and biological samples have been collected in 10 areas, including uncompromised site. The monitoring activity started in May 2018 and will last in March 2020. On the selected sampling sites surface and ground-waters, vegetables (3 different periods of the irrigation season for 24 months), soil, earthworms and water snails (3 periods every 12 months) will be analysed. Preliminary data show that PFAS concentrations in irrigation water do not exactly match with the exposure levels of the population, because both surface and groundwaters are used for irrigation in these areas. Nevertheless, soil contamination more reflects the exposure areas, but in soil long-chain PFAS prevail. Both aquatic and edible plants are not significantly impacted by the PFAS contamination, even if plants from red area show a larger number of positive results, especially for the aquatic species Phragmites, which are directly exposed to the contaminated waters. Positive findings in edible vegetables are very few and connected risks for population are very limited.

6.04P.12

LIFE PHOENIX project: a new project for the management of water pollution from short chain PFAS in Veneto region (Italy)

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In 2013 a significant episode of PFAS pollution of surface, ground- and drinking water has been discovered in a large area of the Veneto region (Northern Italy). The most important source of pollution was identified in a fluorochemical plant, sited in an area of groundwater recharge which impacted for decades groundwater, used as potable water source, in a large area (about 540 km2), because of the persitence and mobility characteristics of PFAS. The Veneto Region immediately put in place mitigation actions that were more effective for long chain PFAS than for short chain ones. Within the framework of the Community Life Program, a project on the management of short-chain PFAS, coordinated by the Department of Health Protection, Food and Veterinary Safety of the Veneto Region, in association with CNR IRSA, ARPAV, Azienda Zero and University of Padua, has been funded. The "Perfluorinated compounds Holistic Environmental Institutional eXperience" (LIFE-PHOENIX) project started in September 2017 and will end in March 2021. The approach requires the involvement of regional authorities, developing tools, such as shared common datawarehouse, transferable to other geographical contexts characterised by episodes of similar environmental contamination but also proposes mitigation strategies based on innovative technologies. The activities are focused on a real scale case constituted by the PFAS pollution episode. The project includes the field monitoring of PFAS in abiotic (water, soil) and biotic (vegetables, animals) in agricultural field. Meanwhile, some innovative technological tools for mitigation of PFAS concentration in water are being validated and compared for irrigation and drinking water purification, using full-scale plant (wetland system) and physico-chemical plans breakdown system (filters). Finally, a typical local crop (red chicory) was cultivated in a greenhouse as model crop, under varying concentrations of pre-contaminated soil and irrigation water. The obtained results are a valuable empirical base for the validation of the existing plant uptake models and can support a mechanicistic understanding of contaminants transport from soil to crop. All project activities offer well-timed and innovative environmental tools for the estimation of PFAS distribution and risks based on multidisciplinary datasets aimed to implement policy measures to prevent or limit problems related to the diffusion of persistent mobile organic contaminants.

Nanotechnology Regulatory and Assessment Frameworks and Nanoremediation Approaches (P)

6.05P.1

Database applications could support the registration of similar nanomaterials

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From 1st of January 2020 on all chemical registrations need to be updated regarding the nanoform. The ECHA (European chemical agency) recently informed about adaption options for this procedure. Further, in 2020 there will be the need for an update of the ecotoxicological part of the REACh dossiers. This may entail that new studies need to be conducted for already registered chemicals to gather the nano information. To support such dossier updates grouping and/or read-across approaches may be useful techniques for avoiding additional laboratory studies. In this respect, one powerful source of comprehensively gathered, but frequently underestimated data are peer-reviewed research papers, which represent a big data pool but with only limited accessibility. Thus, a conventional online literature search of ecotoxicological endpoints can be very time consuming. Online databases need to be developed, which allow users to get a quick overview about existing EC50 values for different test organisms along with all important information regarding e.g. experimental environmental conditions, particle properties and other information (characterization, material composition etc.). The search mask of such database applications should be rather simple, to allow for fast and easy searches. For example, only the test substance and the particle size range (min-max) must be entered in order to very quickly get to first findings. Generally, the gained search results should be sorted in a proper way, e.g. by coating and test organism, displaying an overview of results (EC50: median, min, max and N) for these groups. An example database allows, for instance, to draw the conclusion, that citrate coated silver nanoparticles (nAg; N=25 studies) are 30-times more toxic than polyvinylpyrrolidone coated nAg (N=8 studies) for the water flea *Daphnia magna*. In comparison, for the fish *Danio rerio* the difference between citrate and PVP coated nAg was only as high as factor 1.5 (N=10 and N=1 studies). A search for zinc nanoparticles (nZnO) would come to the conclusion, that algae (*Pseudokirchneriella subcapitata*) is the most sensitive (EC50: algae< water flea< fish) organism. However, more data need to be uploaded to increase the completeness of the picture. Study quality information parameters displaying the plausibility of the results are required to increase the power of database tools.

6.05P.2

Identification & characterization of nanoparticles

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Nanomaterials are an area of increasing scrutiny in a regulatory environment, with regulatory frameworks in development for multiple countries such as the EU, US and Canada. Recently a **specific revision of the REACH information requirements for nanomaterials has been adopted by the European Commission. The amendments clarify the information registrants will need to**

provide in their registration dossiers, if they supply substances as a nanoform. The new rules apply as of 1st January 2020. Nanomaterials may have different properties and thus different classification(s) for the relevant physicochemical, human health or environmental endpoint compared with the bulk of the same substance. Nanomaterials can be further categorised into individual nanoforms, with nanoforms then being grouped together based upon particle size distribution, shape, surface treatment and surface area. Hazard assessments, exposure assessments and risk assessments for each set of nanoforms will be required along with justification as to why the nanoforms can be grouped within their boundaries. While certain companies may be aware that they manufacture nanomaterials and have some expertise in the characterisation of them, it is possible that they will not have data in all areas to define a nanoform due to requirements of specialised equipment. Additionally, companies may be unaware they manufacture nanomaterials, therefore there is a clear need to demonstrate how companies can go about identifying and characterizing nanoparticles. This poster illustrates how companies can determine if they manufacture nanoforms and the characterisation methods available to allow determination of parameters required for grouping nanoforms e.g. particle size distribution, shape, surface treatment and surface area.

6.05P.3

Nanotechnology-based coatings: a framework for the anti-fouling efficacy, toxicity and risk assessment

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Marine biofouling in man-made metallic immersed structures is a global socio-economic problem. The protection of structures has been conventionally performed using antifouling (AF) paints, which toxicity towards marine organisms is surprisingly poorly studied. In opposition, most active ingredients used in AF coatings (i.e. organic and/or metallic AF biocides), which are well-characterized in terms of ecotoxicity, cause detrimental effects on non-fouling species. In order to circumvent this environmental problem and control the release of the chemicals over time, some AF biocides have been immobilized/encapsulated in engineered nanomaterials. Recent studies showed a noteworthy reduction of the toxicity and marine hazard of the nanostructured AF biocides comparatively to the respective free form of the biocide when dispersed in artificial saltwater. In order to increase the insight in terms of paints ecotoxicity and assess the potential use of those new nanomaterials as AF additives in coatings, the present study encompasses a holistic framework addressing the AF field efficacy, toxicity and release rate of state-of-art and novel nano-based biocides-release AF paints. This information is critical for future risk assessment and regulatory purposes. A total of nine AF coatings were tested. A non-commercial topcoat without biocides was modified to prepare seven systems by adding free booster biocides (Sea-Nine; Cu pyrithione (CuPT)), nanostructured biocides (Sea-Nine@SiNC; CuPT@Zn-Al LDH) and unloaded nanomaterials (SiNC; Zn-Al LDH; Cu-Al LDH). A commercial coating containing CuPT and Cu_2O and the “raw” topcoat were used as references (with and without biocides). The anti-fouling efficacy assessment was run in a marina for a complete year. Bacteria, microphytobenthos and macrofoulers communities were evaluated periodically. In the laboratory, coated plates were properly characterized and then, placed in aquaria to release their chemical compounds for 3 months. Water samples were taken from each aquarium and assessed in terms of toxicity towards three marine species (microalgae and crustaceans), elemental composition (ICP-MS/OES), DCOIT quantification (HPLC) and physicochemical characterization.Overall, this study showed that coatings with free biocides showed high antifouling efficacy, but extreme toxicity. The encapsulation of these biocides effectively reduced their toxicity while keeping good AF efficacy.

6.05P.4

Silver toxicity towards Daphnia magna affected by titanium dioxide nanoparticles and duration of aging

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Immense usage of titanium dioxide nanoparticles ($nTiO_2$) in consumer products (e.g., cosmetics, food, cleaning agents, filters) undoubtedly leads to their release into freshwater systems. There, $nTiO_2$ may interact with co-occurring heavy metals affecting their ecotoxicological effects. Additional parameters, such as presence of natural organic matter (NOM) and aging duration, may affect fate and toxicity of both $nTiO_2$ and heavy metals as well as their interaction. The present study aimed at assessing the effects of $nTiO_2$ on the toxicity of silver (Ag) over various aging durations using a factorial test design. The test design crossed three $nTiO_2$ levels (0.0, 0.6 and 2.6 mg/L) with two levels of NOM (0 versus 8 mg/L TOC) and seven nominal Ag concentrations (ranging from: 0 – 64 µg/L) with an aging duration of 0, 1, 3 and 6 days. Eventually, *Daphnia magna*, a freshwater pelagic microcrustacean, was exposed to these aged dispersions for 48 h largely following the respective OECD guideline. The results showed that presence of 2.6 mg $nTiO_2$/L elevated Ag toxicity significantly up to~6-fold with increasing aging duration as compared to the absence of $nTiO_2$. Similarly, the presence of 0.6 mg $nTiO_2$/L elevated Ag toxicity significantly by~5-fold after 1 day and~2-fold after 3 and 6 days of aging relative to $nTiO_2$ absence. Also without aging, 0.6 mg $nTiO_2$/L elevated Ag toxicity; this observation, however, was statistically non-significant. In presence of NOM, similar patterns in terms of increase in Ag toxicity triggered by $nTiO_2$ (independent of its concentration) were noted, again independent of the aging duration. While multiple other environmental factors can affect the interaction of $nTiO_2$ and Ag, it is evident from this study that $nTiO_2$ can elevate Ag induced toxicity – a pattern independent of the aging duration.

6.05P.5

REACTIVITY OF MICRONIZED HYDROXYAPATITES FOR THE REMOVAL OF GEOGENIC CONTAMINANTS IN WATERS

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This study shows the reactivity at lab scale of several hydroxyapatites (HAPs, $Ca_5(OH)(PO_4)_3$) as a possible alternative for the elimination of contaminants such as copper and zinc in mining wastewaters and fluoride in groundwater. Three materials were tested: 1) micronized commercial HAP (1-100 µm, mHAP), 2) nanopowder commercial HAP (0.4-150 µm, nHAP) and 3) synthetic HAP material (10-1000 µm, sHAP). BET area was similar for mHAP and sHAP but was three times higher than for nHAP. A SEM study showed that nanoparticles sized in nHAP were attached on bigger particles, explaining BET results. Equilibrium and kinetic batch studies were performed by maintaining in contact synthetic solutions of copper, zinc and fluoride with the HAPs from 1 minute to 24 h, at ambient temperature at constant stirring. Langmuir isotherms and second order kinetics showed to be very good models that described the removal of all the tested contaminants and solids. In the case of synthetic dissolutions of copper or zinc (5 to 120 mg/l), the best removal results were obtained with sHAP (q_{max}=60 mg Cu/g and 35 mg Zn/g for Langmuir fittings). Kinetics was very fast: an initial concentration of 50 mg/l solution of Zn or Cu in a suspension of 1 g of HAP/l reached the equilibrium in less than one hour. The pH of dissolutions in contact with solids increased from an initial pH of 4,7 to neutral-alkaline values. Adsorption was the main mechanism identified for metal removal, but precipitation of metals was also important. The application of sHAP for the treatment of pyrite mining wastewater from Aznalcóllar mine (Spain) (pH 2.3, 721.4 mg/l of Zn and 18.9 mg/l Cu) showed complete removal of copper and 10-20% removal of zinc. This low elimination is related to the adsorption or reaction competition of zinc with other metals in the real sample, such as iron, aluminium or copper. For the case of fluoride, batch experiments consisted in contacting a solution of 25 mg/l of F mixed with 5 g/l suspensions of the three HAP. The best results were obtained for nHAP (q_{max}=6.2 mg F^-/g for Langmuir fittings). Kinetics for the same conditions showed that equilibrium was reached after 24 h of contact. During the reaction pH increased from 9.8 to 11.2, showing that hydroxyl groups were replaced by fluoride, forming fluoroapatite, $Ca_5(PO_4)_3F$. Four samples of groundwater from the East African Rift Valley in Tanzania and Ethiopia (16 to 35 mg/l F) were also tested, showing 67-91 % of fluoride removal.

6.05P.6

Considerations for bioaccumulation studies in fish with nanomaterials

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Bioaccumulation studies in fish are needed under the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) to classify substances that have the potential to bioconcentrate. In addition, bioaccumulation is an essential part in a persistence, bioaccumulation and toxicity (PBT) assessment that is required in several regulatory frameworks. However, currently there is some ambiguity surrounding how to perform these assays with nanomaterials (NMs) to be accepted in regulation frameworks. A thorough bibliographic review of peer-reviewed literature from the Web of Science database has been conducted to identify the latest progress in NMs bioaccumulation studies in fish. A total of 38 studies were identified. The NMs tested included TiO2, ZnO, CuO, Ag, CeO2, FeO2, Nano-Se, CdSe/ZnS-QDs, Graphene and MWCNT. From the 38 studies, 26 were conducted using aqueous exposures. Only 8 used the dietary exposure route and 4 incorporated both aqueous and dietary studies. This critical review afforded some initial conclusions to be made. For example, although the dietary exposure route has been suggested as the most appropriate for assessing the bioaccumulation of NMs, the aqueous route appears to be valid for some NMs. Different accumulation was observed depending on the exposure route, exposure concentration and presence of organic matter. TiO2 NPs present a fast depuration in the whole fish but not from some organs. For ZnO NPs, concentration-dependent increases in tissue levels could be measured with little or slow depuration from the analysed target organs and higher levels in liver tissues compared to Zn salts. For AgNPs, it was observed that larger NPs predominantly accumulated in the intestine and liver, whereas smaller NPs were filtered and attached to the gill lamella. Some tissues rapidly and completely depurated total silver but some did not, most notably the liver. The amount of uptake for both large few layer graphene (L-FLG) and short few layer graphene (S-FLG) increased by a factor of 2.5 and 16, respectively, when organic matter was present. L-FLG were not absorbed whereas S-FLG could reach the systemic circulation. MWCNT accumulated mainly in the gut reaching low levels in blood and muscle with fast depurations. These early studies provide valuable insights into critical issues that need to be addressed when testing the bioaccumulation potential of NMs. Acknowledgement - H2020 project Gov4Nano (Grant Agreement nº 814401)

6.05P.7

The accumulation of nanoparticles in the pseudofeces of bivalves may play a central role regarding the ecological impact of engineered nanomaterials

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The increasing production and usage of engineered nanomaterials leads inevitably to a larger environmental burden. The main part of the nanoparticles (NPs) enters the aquatic environment by leachates of landfills or sewage treatment plant effluents. After reaching aquatic systems most NPs tend to sediment in water. Due to the sedimentation process, it is supposed that NPs are primarily taken up by benthic species in aquatic ecosystems. Filter-feeding bivalves may ingest considerable amounts of particulate materials including NPs that are concentrated in their feces or pseudofeces. Due to the fact that the feces and pseudofeces of bivalves represent an important part of the diet of benthic invertebrates, they may play a key role regarding the transfer of NPs into the aquatic food chain. In this study, the freshwater bivalve *Corbicula fluminea* was exposed several days to fluoresce polystyrol NPs, AgNPs and AuNPs suspended in water. The released pseudofeces of *C. fluminea* were collected and examined using fluorescence microscope, TEM and EDX and ICP-MS to determine the presence of nPS and the concentrations of Ag and Au, respectively. In a second step a feeding study with the benthic amphipod *H. azteca* was carried out to investigate the transfer of NPs from the bivalve (pseudo) feces to the amphipod crustacean. At the end of the study lasting 21 days, animals exposed to the fluoresce polystyrol NPs were examined for the presence and localization of the particles in the amphipods. Animals which were exposed to AgNPs and AuNPs were collected and the metal body burden was determined using ICP-MS. Magnification factors were determined. The studies have shown that filter feeding freshwater bivalves may release pseudofeces with a very high concentration of NPs. These particles are available for benthic invertebrates such as *H. azteca* and may enter the aquatic food chain where they could biomagnify. Therefore, the accumulation of NPs in the pseudofeces of bivalves may play a central role regarding the ecological impact of NPs.

6.05P.8

Investigations on the bioaccumulation of engineered nanomaterials in the benthic fresh water amphipod Hyalella azteca

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The identification and scientific assessment of compounds that bioaccumulate in organisms and biomagnify in food webs play a key role within the PBT-assessment. The bioaccumulation potential of compounds is commonly expressed in form of bioconcentration factors (BCF) determined in flow-through studies with fish according to OECD 305. Comparable studies with engineered nanomaterials (ENMs) are difficult to carry out due to the lack of suitable test systems that allow a permanent and constant exposition of the compounds. ENMs tend to sediment in water and are supposed to be primarily taken up by benthic species in aquatic ecosystems. Recently, a test protocol for bioaccumulation tests using *Hyalella azteca* was published. This method was developed to allow the investigation of bioaccumulation of soluble, non-particulate substances. Nevertheless, different studies have shown that the benthic fresh water amphipod *H. azteca* is also able to ingest ENMs if present in the water, sediment or diet. Several bioconcentration studies with *H. azteca*. were carried out with different ENMs. In addition, we prepared an experimental diet containing ENMs for biomagnification studies. ENMs with different characteristics were used to determine their bioaccumulation potential in *H. azteca*. Water, food and animal samples were taken during the studies lasting 7 to 28 days and examined using ICP-MS for the quantification of total concentrations. Due to the possibility of dissolution of ENMs or ion release, further media and animal samples were investigated using spICP-MS for the presence of particles. TEM and EDX were used to examine the experimental diet for the presence and grade of transformation of ENMs enriched in diet. The studies have shown that the bioaccumulation of ENMs in *H. azteca* is depending on the characteristics of the nanomaterials and that the bioaccumulation of metals from metal based ENPs seems to be mainly driven by the uptake of ions.

6.05P.9

Introducing a New Hydrophobic Magnetic Polymer Nano Composite for Selective Oil Absorption

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In this paper, we introduce a new super hydrophobic magnetic polymer nanocomposite for oil spill remediation. We developed an environmental-friendly magnetic polymer nanocomposite with inorganic core-shell structure surrounded by an organic brush by surface-initiated atom transfer radical polymerization (ATRP). To produce the aforementioned magnetic polymer nanocomposite, we first functionalized silica coated iron oxide nanoparticles by ATRP initiator. Then, hydrophobic polymer brushes were grown on the surface of the functionalized nanoparticles by applying the grafting-from technique. The produced magnetic polymer nanocomposite was characterized by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-ray Photoelectron Microscopy (XPS), and Thermogravimetric Analysis (TGA). Afterward, we used our developed magnetic polymer nanocomposite to remove oil from water in different magnetic polymer nanocomposite concentrations. The results showed a fast and efficient oil separation. Notably, magnetic polymer nanocomposite absorbed oil three times of its weight. Besides, the magnetic oil separation technique allowed us to easily collect magnetic polymer nanocomposite after oil absorption by using an external magnet. Also, the collected magnetic polymer nanocomposite could be reused in oil separation for at least five cycles with the same oil absorption capacity.

6.05P.10

Petroleum hydrocarbons degradation in diesel contaminated soil based on activation of peroxymonosulfate by zero-valent iron nanoparticles

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The application of nanoscale zero-valent iron (nZVI) has been increasing for the degradation of various organic pollutants. However, the use of nZVI, its combination with other systems and mechanisms of reaction system for degradation of total petroleum hydrocarbons (TPH) in diesel contaminated soil has been scarce. The objective of this study was to investigate the advanced oxidation process using peroxymonosulfate/nZVI (PMS/nZVI) system to treat diesel contaminated soil. The soil-TPH removal by nZVI, PMS and their combination to activate PMS by nZVI were evaluated at various dosages and reaction time. In addition, the effect of soil-to-solution ratio (w/v), soil initial pH, nZVI/PMS ratio (w/w), activator types were tested. After treatments with nZVI and PMS alone, the respective maximum TPH removal efficiencies of 30 and 45% were achieved. However, nZVI was found to be best activator for PMS and this combination resulted a higher TPH degradation as compared to either alone. Furthermore, the serial treatments of PMS/nZVI were used to increase the efficacy of TPH remediation and a more than 93% TPH degradation was achieved by this method. The enhanced rate of reaction is due to formation of reactive intermediates (OH., SO4-.) in PMS/nZVI system which was verified by radical scavenging tests and electron paramagnetic resonance (EPR) studies. The results successfully demonstrated soil-TPH remediation below Korean TPH regulatory limit for residential areas (500 mg/kg) and it shows that PMS/nZVI system could be an effective advanced chemical oxidation method to apply for the remediation of diesel contaminated soil. (This work was supported by the National Research

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6.05P.11
Solubility of different nanomaterials used in biomedicine
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Nanomaterials have become an emerging field that has shown great promise in the development of novel diagnostic, imaging and therapeutic agents for a variety of diseases, including cancer, due to their nanoscale size effects and increased surface area [1]. However, the release of ions from nanomaterials is discussed as one important mode of action. Due to this it is very important to analyse if nanomaterials which are used for human or environmental applications can release toxic ions. Therefore, the solubility of different nanomaterials which will be used in medical application were analysed using a continuous flow system. In this system, the nanomaterial is held between two filters and is subjected to a fixed flow-rate of 2ml/h over one week. During that time the eluate is collected and the amount of released ions is analyzed with inductively coupled plasma- mass spectrometry (ICP-MS). The solubility of the materials is tested in water with a pH of 3 and additionally in artificial fluids like saliva, gastric, duodenal and bile, dependent on the foreseen application. After the solubility experiments the materials on the filter are analysed with SEM to identify possible transformation processes influencing the applicability of the materials. [1] Abdel-Wahhab, Mosaad A., and Márquez, Francisco. Nanomaterials in Biomedicine. United States: N. p., 2015. Web. doi:10.4236/snl.2015.53006.

6.05P.12
Modelling the contribution of pigment-TiO2 to the released nanosized TiO2 fraction
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Most of the existing exposure models for engineered nanomaterials (ENMs) only consider a "generic ENM" in their assessment. Particle size, crystalline forms and coating materials that may potentially influence the fate, transport and toxicity of the material are not considered. Titanium dioxide is widely used as white pigment but also in nanoscaled form in a variety of commercial products. Because pigment-TiO_2 also contains a nanosized fraction, not all TiO_2 particles below 100 nm are actually released from nanoproducts, but may also be unintentionally released from pigment-containing products. Previous studies showed that the two primary crystalline forms of TiO_2, photostable rutile and photocatalytic anatase, have considerably different release amounts and adverse effects to the environment. These studies did, however, not consider the specific form of TiO_2 used in sunscreens, where the material is normally surface coated with silica, alumina and/or various polymers to reduce its photoactivity and increases stability in the cream. The ultimate goal of this study is to determine the contribution of nano-sized particles of pigment TiO_2 to the total budget of nano-scale TiO_2 released into the environment, distinguishing between the different TiO_2-forms (rutile/anatase/coating). A method was developed to incorporate the size distribution of TiO_2 into dynamic probabilistic material flow analysis (PDMFA). To allocate the total production volume to the different forms, the crystal forms are allocated to the corresponding product category based on material functionalty. Size distributions for each product category were subsequently collected from the literature, manufacturers and marketing reports. The size-dependency of the removal efficiency during wastewater treatment is incorporated into the model. The release percentage of particle aggregates from sunscreen composites during to the aging process is also included in the model. The final results show the size distribution (mass and number distribution) of TiO_2 released into the environment in different size ranges, considering the following five forms: (1) nano-rutile, (2) nano-anatase, (3) nano-coated composites, (4) pigment-rutile, and (5) pigment-anatase. The results from this work will forms the basis for a complete environmental risk assessment of all TiO_2-forms present in the environment.

6.05P.13
Application of Na-HMP to enhance aqueous phase delivery of remediation agents in soil.
P. Gautam, R. Bajagain, S. Jeong, Kunsan National University / Department of Environmental Engineering
Infiltration in the contaminated soil is a difficult phenomenon. This study enhanced water infiltration using sodium hexa-metaphosphate (Na-HMP) from the TPH contaminated soil. Contaminated soil was made in the laboratory by mixing diesel (120 mL/kg) on the soil. To contaminated soil, Na-HMP in combination with surfactant was used first to soak the soil and infiltration was conducted using water. Along with infiltration, residual TPH on depth basis was also calculated. Concentration of Na-HMP above 0.1% have induced lower infiltration due to the movement of clay. Compared to 0.1%, 1.81% and 3.16% more clay were present on bottom when 1% and 5% Na-HMP was used. Presence of residual TPH in the soil has no effect on infiltration when Na-HMP was added. High concentration of Na-HMP made the soil more hydrophilic. However, using only AOS, TPH has induced water repellent effect and it was increased with the increase in concentration. For 1.5% AOS, 0.4 mL penetration takes 7600 S which could also be associated with high adsorption (13586.0 mg/Kg for 1.5%) in the soil. This study suggest the use of 0.1% Na-HMP in combination with 0.5% AOS for the effectual infiltration from the TPH contaminated soil. *Key Words*: Sodium hexa-metaphosphate (Na-HMP), TPH contaminated soil, soaking, infiltration and water repellency.

PBT and Other Substances of (Very High) Concern: Policies, Practices and Challenges (P)

6.06P.1
PBT-Assessment in the EU - an Overview
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PBT/vPvB-substances are defined as substances being Persistent (P), Bioaccumulative (B) and Toxic (T) or very Persistent (vP) and very Bioaccumulative (vB). Based on these properties they are unwanted in the environment as they are supposed to have potential long-term adverse impacts on the environment and human health. In the EU a PBT/vPvB-assessment is relevant under different legislations: REACH (Regulation (EC) No 1907/2006), the Biocidal Products Regulation (Regulation (EU) No 528/2012), Plant Protection Products Regulation (Regulation (EC) No 1107/2009) and Human/Veterinary Medicinal Products Regulations (Directive 2001/83/EC; Directive 2001/82/EC). At present, the goal to prevent exposure of humans and the environment to PBT/vPvB substances is shared among all EU regulatory frameworks. However, the technical criteria, assessment procedures, availability of data and the subsequent regulatory follow-up partly depend on the regulatory framework under which the substance is evaluated, leading to differences in results and regulatory action This poster highlights the current similarities and differences in PBT/vPvB-assessment among the different EU legislations and gives an overview on the regulatory consequences.

6.06P.2
Variability and discrepancies in the interpretation of simulation tests for Regulatory purposes
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Simulation tests are required as higher-tier methods when there is a need to obtain a half-life for comparison against the “P” (Persistent) and “vP” (very Persistent) criteria. A tiered approach for assessing persistency is proposed in the Integrated Testing Strategy of the REACH Guidance in its Chapter R.11 (PBT/vPvB assessment, June 2017). However, the selection of the relevant simulation tests to characterize persistence of chemicals remains subject to interpretation. Not only do the technical feasibility of the simulation tests and the environmental relevance of the media selected constitute critical points to address at an early stage of a testing strategy, but also analysis and interpretation guidance needs to be defined. Not predefining these may lead to contradictory outcomes even though the test methods are standardized. For example, the interpretation of chromatograms of radiolabeled samples is challenging as the determination of the baseline and the selection of the “significant peak” that would require further identification are closely dependent on the analyst expertise and familiarity with such tests, as well as with the materials being tested. The kinetic modeling of the degradation processes may also be a source of variability. It requires expertise in both modeling and statistics, as well as knowledge of eco-relevant parameters and their impact (e.g. pH, temperature, organic matter content, diversity of biological communities, …) in order to avoid improper predictions and interpretation of results. In this poster we provide examples of studies where such difficulties were encountered, and which emphasize the need for a more holistic approach substantiated with more expert judgment and robust weight-of-evidence analysis.

6.06P.3
Qualification of a microbial inoculum for OECD Ready Biodegradability Tests (RBTs)
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The OECD has published guidelines for screening chemicals for biodegradability, such as the OECD 301 ready biodegradability tests (RBTs), in which a test substance is exposed to a microbial population, and the transformation or mineralization of the test substance is measured. While the simple nature of RBTs allows quick screening of chemicals, the outcomes are variable with a high rate of false negatives, *i.e.*, biodegradable substances that fail the RBT. One option to reduce the variability of the outcomes is to reduce the variability within the test method itself, with a focus on the microbial inoculum. Current test protocols recommend using domestic sewage, activated sludge or secondary effluent as the inoculum source, with an assumed microbial load (or cell density) and biodiversity. Several studies have demonstrated that the biodiversity, which is partly controlled by the microbial load, will determine whether a substance passes

an RBT. This renders the RBT more a test of the microbial inoculum's capability than the amenability of the substance to biodegradation. The health of the microbial inoculum, or the microbial activity, in a RBT is potentially verified by the inclusion of a reference compound, such as aniline, sodium acetate, or sodium benzoate. More consistent and accurate results in RBTs can be achieved by ensuring adequate microbial load to capture existing biodiversity and metabolic capability in an environmental system. Recently, the Shell Risk Science Team participated in the 'Life Sciences with Industry Workshop', coordinated by the University of Leiden Lorentz Centre, which brought together academics and industry to address a scientific problem. Our problem statement, 'What metrics can better define microbial quality' was addressed by a team of seven graduate students and early career researchers. In this poster, we present some outcomes of this workshop and propose criteria for microbial load, biodiversity and metabolic capability to qualify a microbial inoculum for use in a RBT. These criteria can be applied beyond RBTs, especially as DNA sequencing and rapid assessment technologies facilitate characterization of the microbial inoculum that has previously been a black box.

6.06P.4

Screening test for biodegradation in seawater (OECD306): how aging water affects the microbial community and biodegradation rates

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The first step in evaluation of a chemical's fate in the marine enviroment is often the OECD 306 test for biodegradation in seawater. However, there has been some concern about the reproducibility of this test partly because of variable results obtained by different laboratories. The OECD 306 test has often lower concentration of bacteria than the OECD 301 freshwater test series, and studies have shown that marine bacteria often show longer lag-phases than freshwater bacteria. Changes in test set-up that are accepted within the OECD 306 guideline which can influence the lag-phase include acclimatizing (or aging) of the test inoculum up to 7 days. The hypothesis tested in this study is that aging the seawater before use, especially in the cold season, will aid acclimating the microbial community and the reproducibility of the test. If acclimation of the seawater is performed in storage vessels, the microbial diversity should be higher and more uniform than if the acclimation is carried out in small test bottles, and then the lag-phase of the test will be shorter. Seawater from surface or deep water (60m) was collected in November, February and April to be used as test medium after 0 to 7 days of aging in a closed bottle test for biodegradation of the reference compound aniline with an incubation time of 7 days. Biodegradation was calculated as decrease in dissolved oxygen compared to theoretical oxygen demand. Changes in the microbial community during seawater aging was measured with flowcytometer followed by a microbial fingerprint analysis. The fingerprints and the biodegradation data were analysed by using Redundancy analysis and Monte Carlo permutations tests. In general, most of the variation in the microbial community composition are related to aging time and to some extent storage vessel. There seem to be a weak tendency towards faster biodegradation and more reproducible results with aging seawater, however, there were not statistically significant ($p>0.05$) trends. Biodegradation was better in deep water compared to surface water in April, while there was little difference in November, and surface water resulted in highest biodegradation rates in February. These results illustrates the variability in biodegradation rates that is experienced with this test set-up, and highlites the importance of doing repeated experiments with different seawater communities when trying to improve this test.

6.06P.6

Consequences of the "Isomer Guidance" on study design and workload in soil degradation study

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In this work, we will present the full data of an OECD 307 soil metabolism guideline study of a diastereoisomeric mixture herbicide under development, which was performed in accordance to the new EFSA "isomer guidance" from July 2019. The ^{14}C-radiolabeled test item is a mixture (1:1) of two diastereoisomers of eight possible isomers (three chiral centers). The aerobic degradation in soil was monitored and analyzed on eleven sampling points in four different EU soils with two replicates and one concentration. Full material balances were established (CO_2, NER, soil extracts) as well as extract analysis by radio-HPLC. On each sampling point for both replicates, fractions of the parent as well as of metabolites have been collected from an initial non-chiral first RP-HPLC separation and further analyzed with a chiral methods. Emphasis of the presented work is to show the monitoring of the diasteriomeric ratio of the parent substance and the metabolites during degradation and formation.First, we will discuss possible soil degradation study designs to address the new regulatory requirements with their benefits and drawbacks as well as the results we generated during the GLP OECD 307 aerobic soil degradation study of the investigated diastereoisomeric herbicide mixture with our used study design in accordance with the new guidance. Secondly, as a main result, we will show the isomeric monitoring of the parent degradation and the formation and later degradation of the isomeric metabolites in soil. One of the parent isomers is degrading faster than the other one, leading to different DT_{50}'s in soil. In the same time, the corresponding metabolite is formed quicker. Thus, we will show the difficulties and limitations of the monitoring of isomeric substances for the persistance assessment in laboratory environment. Further, considerations about the correct and useful way of isomer ratio data presentation and interpretation will be discussed. At last, we will give emphasis on the challenges within the study on fractionation and chiral HPLC analysis, together with newly formed problems in the sample handling, storage stability, limit of detection/quantification, reproducability, chiral HPLC method development and validation. The presented work will be a starting point for further discussion, future data interpretation and possible improvements for the regulatory framework in the persistance assessment.

6.06P.7

BROMINATED FLAME RETARDANTS AND PERFLUOROALKYL SUBSTANCES IN IRISH WASTE

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The presence of persistent organic pollutants (POPs) in the environment is an area of growing concern, with new compounds being listed in the Stockholm Convention yearly and ongoing research showing high prevalence in various microenvironments. Ongoing research between the National University of Ireland Galway and the University of Birmingham is investigating the presence of certain POPs – perfluoroalkyl substances (PFASs) and brominated flame retardants (BFRs) – in waste streams in Ireland. These chemicals are extensively used in a range of consumer items and have been shown to result in significant human exposure in indoor environments. Large-scale sampling at recycling sites around the country revealed that several hazardous BFRs including PBDEs, HBCDD, and TBBP-A are highly prominent in various end-of-life materials, including WEEE, furniture, vehicular textiles, and insulation foams. Many of these items are conventionally recycled, resulting in re-circulation of BFR-treated goods in the consumer market and possible re-use in goods not originally intended treatment. Non-recyclable goods such as furniture and textiles are instead largely landfilled. Results from our research has shown that the above BFRs as well as certain PFASs (including PFOS, PFOA, and PFHxS) to be present at high concentrations in landfill leachate as well as in the soil and groundwater surrounding these sites. A screening methods of BFR-treated goods using portable x-ray fluorescence (XRF) was developed as part of this project, which has shown an efficacy of > 95 % in correctly identifying BFR-containing materials. Such a screening method is vital in order to comply with EU regulations and remove these goods from circulation to prevent their recirculation and subsequent environmental impacts.

6.06P.8

Fluoropolymer Stability: How Can You Prove a Negative (Absence of Leachable Substances of Concern Over Time)?

T. Ruwona, B. Henry, W.L. Gore & Associates, Inc.; N. Timmer, T. Noij, Charles River Laboratories / Environmental Sciences

Certain per- and polyfluoroalkyl substances (PFAS) have been detected in environmental compartments and biota. Globally, regulators are struggling how to manage the grouping and regulation of the 4,730 PFAS (including polymers) identified by OECD. Polytetrafluoroethylene (PTFE), a member of the fluoropolymer class of the polymer category of PFAS, represents 58% of the global fluoropolymer use, as of 2016. PTFE meets the OECD Polymer of Low Concern (PLC) classification criteria. As such PTFE is not toxic or bioaccumulative. PTFE is stable and does not degrade, breakdown, or form toxic transformation products.The stability of PTFE means that it possesses a long environmental half-life, which translates into a classification of "persistent" under common regulatory and hazard assessment frameworks. Persistence alone cannot, by definition, present a risk to biota or the environment *in the absence of* inherent hazard, either from the fluoropolymer itself or from leachable substances of concern from the fluoropolymer. How then can the absence of leachable substances of concern from PTFE over time be proven? We propose a testing scheme, based on our knowledge of PTFE manufacturing processes, fluoropolymer chemical, physical and thermal properties, and existing OECD and OPPTS test guidelines for environmental fate, to determine what, if anything, may leach out from PTFE over time. Input from poster session participants as to the merits, challenges or shortcomings of the proposal is highly encouraged. One objective of the proposed testing framework is to provide consistent evidence that persistence of a substance does not necessarily imply toxicity or bioaccumulation, nor does it imply future degradation, release, or transformation into a continuous source of substances of concern.

6.06P.9

Endocrine Disruptors and REACH Authorisation - what is a sustainable

solution?

T. Griffiths, ERM / PSRA

Octylphenol Ethoxylates (OPnEO) and Nonylphenol Ethoxylates (NPnEO) were included in the latest prioritisation of substances onto the Authorisation (Annex XIV) list of substances of very high concern (SVHC) within the EU. OPnEO and NPnEO were included due to the endocrine disrupting properties of their breakdown products, namely Octyl Phenol (OP) and Nonyl Phenol (NP). OPnEOs, such as Triton X-100, are widely used in the European Union within the pharmaceutical and medical device industry. Without a valid authorisation these substances cannot be used in the European Union past the sunset date of 4 January 2021. None of the substances listed above were deemed by the European Chemicals Agency (ECHA) or the European Commission to be a threshold substance. Industries such as Pharmaceuticals and Medical Devices have strict control measures in place to limit emissions to the environment, however, without a threshold in place any discharge of OPnEO / NPnEO or the breakdown products of OP / NP is deemed to have an impact on the environment. Based on the above and the need within the authorisation process to show control of emissions the “simplest” measure could be seen as zero discharge policy, often achieved via incineration of waste. This waste stream has the potential to have very, very low levels of the ethoxylate present due to existing control measures. Based on the above background what is the most sustainable solution for treatment of waste containing endocrine disruptors at very low levels?

6.06P.10

Prediction of chemical transformation products using in silico tools and its application in chemical alternatives assessment: The case of decabromodiphenyl ether (decaBDE) alternatives

Z. Zheng, Umea University / Department of Chemistry; G.M. Peters, Chalmers University of Technology / Division of Environmental Systems Analysis; H. Arp, NGI / Environmental Technology; P. Andersson, Umea University / Department of Chemistry

Alternatives assessment is applied for minimising the risk of replacing a hazardous chemical with another problematic, hazardous chemical. The importance of considering chemical transformation products has been pointed out by many famous alternatives assessment frameworks, while the problem has rarely been properly taken care of in case studies due to the complexicity of transformation pathways To address this problem, this study explored the use of five widely-used *in silico* tools to predict transformation products for alternatives assessment, including mammalian and microbial metabolites as well as abiotic degradation products. Six case chemicals were taken from a previous study including the widely used flame retardant - decabromodiphenyl ether (decaBDE) – and its five alternatives. The *in silico* tools were able to identify a large number of transformation products for all case chemicals, and therefore a strategy for selecting the most important transformation products was established with the support of experimental evidence from literatures of two case chemicals (decaBDE and melamine), and about ten chemicals were selected out as the most important transformation products for each case chemical. The selected transformation products were then considered together with their mother compounds to assess the hazards. The hazard endpoints include persistence (P), bioaccumulation potential (B), toxicity (T) and the mobility in water (M) and the hazard data were calculated by open-source *in silico* tools as performed in a previous study. Prediction results indicates that many of the transformation products are less hazardous for some endpoints (typically P and B) while more hazardous for others (typically T and M) compared to their mother compound, and multi-criteria decision analysis (MCDA) tools were used to score and highlight the most hazardous transformation products. MCDA tools were then also used for the comparison of different alternatives with the consideration of their most hazardous transformation products, and were able to identify that some of the case chemical might be consider as less hazardous if only consider the mother compound, while the hazardous might be much higher for the transformation products, thus it might be a regrettable alternative. This study also pointed out the needs for more in silico tools for the prediction of transformation products, especially for abiotic transformation pathways.

6.06P.11

What do the experts say? - Identify and assess factors influencing the impacts of PBT and vPvB substances based on the Delphi method

K. Thiele, WUR; S. Gabbert, Wageningen University / Social Sciences; H. Tobi, Wageningen University WUR

PBT/vPvB substances remain in the environment over long time periods and accumulate in biota, which may lead to severe and irreversible effects in the long-run. It is not possible to reliably predict these effects based on current standard practices in chemicals risk assessment. Due to the lack of information on risks or impacts, a fundamental challenge for effective risk management of PBT/vPvB substances is to prioritise those PBT/vPvB substances for regulatory action, which are of highest concern in terms of the potential impacts on the environment and on human health. So far, there is no comprehensive and systematic approach defining (i) which information, apart from the P, B and T criteria, on PBT/vPvB substances is relevant and (ii) how to use this information in order to prioritise substances for risk management. There have been proposals for PBT rankings in the context of impact assessment and socio-economic analysis, however it has not been systematically investigated yet which factors of PBT/vPvB substances need to be considered and how in order to specify the concern to risk managers and policy makers in terms of potential environmental and human health impacts. Given the obstacles to assess these factors on the basis of scientific findings, expert knowledge is an important source of information to be considered in the evaluation of PBT/vPvB substances. Against this background, it is our purpose to explore experts’ opinion based on the Delphi method on what factors of PBT/vPvB substances are important to take into account in order to prioritise PBT/vPvB substances for risk management based on their concern and thus to support risk management decisions. The results of this study will contribute to the development of concern-based risk management strategies for PBT/vPvB substances to support regulators. In this presentation we would like to present first results of the study.

Science and Risk Communication in an Ever-changing World: How Could It Be Done to Build Trust? (P)

6.07P.1

The Bambi syndrome - The naïve positive opinion about natural fragrance compounds

U. Klaschka, University of Applied Sciences

A representative survey in the German population revealed that a fifth of the general population report health problems with subsequent societal impacts, when exposed to fragranced products. In this same study, every second person found products with “natural" fragrance ingredients healthier than products with synthetic fragrance ingredients, although emissions of hazardous air pollutants from these products are not significantly different from regular fragranced products. This uncritical positive opinion about natural substances mirrors the widespread naïve view on nature in today’s society, called “Bambi syndrome”. There were one out of seven people who found products with natural fragrance ingredients healthier, although these persons were aware that these so-called natural products typically emit hazardous air pollutants. This is an example for the fact that some consumers seem to have convictions which are in contrast to their knowledge. Reducing exposures such as through fragrance-free policies could mitigate health problems due to fragranced products. However, people who have the assumption that natural fragrances are healthier will not show a significant avoidance behavior. It is a difficult, but very urgent task for risk communication to fight against this widespread misconception in consumers.

6.07P.3

Effective Risk Communication: Preparing Hillsborough County for 2050 and 2100

M. Bourgeois, USF COPH / EOH; B. Cook, T. Sabia, University of South Florida, School of Architecture and Community Design / Florida Center, School of Architecture and Community Design; J. Bohn, College of Public Health, University of South Florida / Public Health Interdisciplinary Research & Education; E. Dunn, College of Public Health, University of South Florida / Global Disaster Management, Humanitarian Relief, and Homeland Security

Communicating risk to stakeholders can be challenging. One key reason for this difficulty is the lack of a common vocabulary. This ‘language barrier’ can be overcome with some creativity, common terminology and persistence. This difficulty is not restricted to scientists communicating risk to lay stakeholders; difficulties from lack of fluency can also occur between different disciplines. In 2018, an interdisciplinary team of landscape architecture, public health and urban design researchers formed a partnership with hazard and resilience professionals in county government to identify vulnerabilities in the local ecology and built environment called the Community Vulnerability Study (CVS). A product of that collaboration is the Community Vulnerability Handbook (CVH), a compendium of planning frameworks, case studies, local and regional policies and vulnerability assessments. Although its impetus was Senate Bill (SB) 1094, which requires all coastal municipalities in Florida to address the Perils of Flood within their Comprehensive Plans, the CVS can be used for other emergency management planning, urban planning and utility management, and will be used to guide Hillsborough County’s next iteration of their Local Mitigation Strategy (LMS). The LMS is a document that identifies potential hazards that may strike Hillsborough County and the nearby cities of Tampa, Temple Terrace, and Plant City. The LMS includes a hazard assessment and identifies potential mitigation strategies for the near and distant future. Public input is a key part of the process. Residents and community stakeholders are invited to attend LMS Working Group meetings. This involvement is facilitated by outreach. Getting beyond jargon with lay stakeholders can be accomplished using something as simple as a charrette. One major success was the workshop, where each discipline addressed the vulnerabilities of flood from the perspective of its associations to the built environment, public health, ecology and governance. Our risk communication utilized multiple strategies: a) regular meetings with interested parties, academic, governmental and nongovernmental; b) printed material/notes; c) routine updates via email; d) contact with team members by appointment; e) graduate level courses designed to work within and alongside the project; and f)contact with team via graduate students on demand. The Community Vulnerability Study demonstrates that it is possible to create a rigorous and nuanced document that delineates vulnerability assessments and mitigation strategies and effectively communicates that risk to stakeholders in various fields.

6.07P.4

Outreach and communication activites conducted by SETAC members - A survey of the state and room for improvement

T. Seiler, RWTH Aachen University / Ecosystem Analysis; A. Leopold, Calidris Environment BV / Calidris Environment BV; H. Hollert, Goethe-Universität Frankfurt am Main / Institute of Ecology, Evolution and Diversity; L. Mueller, RWTH Aachen University / Institute for Environmental Research; N. Oguguah, nigerian institute for oceanography and marine research / Fisheries Biology; E. Brockmeier, University of Pennsylvania, Philadelphia, USA; R. Ottermanns, RWTH Aachen University / Institute for Environmental Research

For the SETAC Europe annual meeting 2017 in Brussels, Belgium, the SETAC Europe Science and Risk Communication Interest Group – SCIRIC had set up an online survey asking SETAC members about their outreach and communication activities.We were interested in learning about the current state of outreach and communication from the SETAC community, with the goal to help improving the quality and success of such activities. This survey should help SCIRIC defining the direction of the future work, building a network of communication "experts" within SETAC, and developing tailored concepts and tools to support SETAC members in their outreach.The survey was announced at the conference using a SCIRIC IG poster with a sticky dot survey asking two questions: (1) How often are you communicating science and risk? (2) Does your institution regularly engage with the public?This was meant to get a first glimpse at two central questions and to make SETAC members aware of the online survey. A link and a QR code on the poster pointed to the full online survey. People could put a sticky dot, colour-coded for their sector, in a field beneath the respective question. Position of the dot in the field indicated the answer.Both surveys yielded close to 100 answers. On the poster we will present a full analysis of both datasets and furthermore share our experience with interactive elements on posters. Briefly, while the responses were skewed by academics being the largest group, both surveys found that a significant number of institutions do not regularly communicate science and/or reach out to other audiences. However, quite a large portion of the respondents claimed to be independently active in this regards and also receive encouragement and support from their organisations. Those who were not involved in communication seemed to be held back by a lack of knowledge and resources. Consequently, developing training, contacts to experts, guidance, examples and information on funding was deemed the most effective support SCIRIC could provide to foster outreach and science communication among SETAC membership.

6.07P.5

Effectively Presenting Science Information and Results without Advocating Policy or Preference.

W.L. Goodfellow, Exponent / Ecological and Biological Services Practice; T. Canfield, U.S. Environmental Protection Agency / Center for Environmental Solutions and Emergency Response; P.D. Guiney, University of Wisconsin-Madison / Molecular and Environmental Toxicology Center

Presenting technical information and one's position is seemingly under increasing pressure to make sweeping claims, maximize impacts or simply enhance being noticed. Environmental management and policy is only as good as the science that informs the discussions and ultimate decisions. Without a sound understanding of how environmental systems work and how stressors on these systems can affect ecosystems, programs intended to manage these systems will continually be off target and policies developed to protect and enhance these systems may be inadequate due to this overall lack of scientific understanding by decision makers and the general public. An important aspect of the science that forms the foundational information needed to make correct decisions for managing these systems and developing policy is that the science and how it was obtained have to be transparent, well documented and impartial to the potential regulatory decisions that are under consideration. This talk will set the stage for the second session "Advocating Science: Practical Approaches to Presenting Science Information and Results without Advocating Policy or Preference (Part 2)" by providing additional examples and experiences of the authors. The first session was presented at the SETAC Toronto meeting last November. Using case examples, the authors will explore issues related to proper science integrity and how the general misuse of science most particularly in ecotoxicology and chemistry applications can be improved. These examples will help to provide context as to how we can move forward to produce science that is impartial, fact-based, testable, does not presuppose a policy preference, and is a sound empirical set of information that provides the necessary data and weight-of-evidence foundation that all good decisions must rely upon.

6.07P.6

SETAC members' perception of the role of science in decision making

G. Oberg, University of British Columbia / IRES; A. Leopold, Calidris Environment BV / Calidris Environment BV; B. McIlroy-Young, University of British Columbia / Resources, Environment and Sustainability; T. Seiler, RWTH Aachen University / Ecosystem Analysis; R. Ottermanns, RWTH Aachen University / Institute for Environmental Research; D. Steel, University of British Columbia; B. De Marchi, University of Bergen / Centre for the Study of the Sciences and the Humanities

Many decisions in today's society rely heavily on science advice. However, providing advice to decision-makers on topics in environmental toxicology and chemistry is not a trivial task as there are many contested issues. To investigate how to best support its members to that end, SETAC invited its membership to participate in a survey about their views of the role of science in policy-making and how they might handle scientific controversies. The survey, which was created by a task force within SETAC Europe, in close cooperation with the Science and Risk Communication IG and the Egesta Lab at the University of British Columbia (UBC), Canada, was launched on 8 May 2019, and remained open until 31 July 2019. The results will be used to inform the design of workshops, courses and other educational initiatives related to science-based risk communication for the benefit of the SETAC community. The broader aim is to contribute to the SETAC Europe strategic goal of supporting science-based risk communication. Upon closing, 656 individuals had initiated the survey, but only 444 completed the survey (32% loss). Ahead of further investigations into this high loss, feedback suggests that it might be, in part, because people had not before thought about the situations described in the survey. The survey consisted of two sections where respondents were asked if they strongly agreed, somewhat agreed, somewhat disagreed or strongly disagreed with a series of questions, with the possibility of providing an open-ended comment at the end of each set of questions. We here report results from the first part of the survey, where respondents were asked about their views of the roles that scientists should play when they advise decision-makers. A notable deviation from earlier surveys of scientists' perception of the role of science in policy is that a considerably larger portion strongly agreed (31%) or somewhat agreed (47%) with the statement "Scientists with expert knowledge on the topic should *advocate* for what they believe is the best decision". Among these positive responses, almost half agreed (32%) or somewhat agreed (11%) with the statement "Scientists with expert knowledge on the topic should be *making decisions*". On our poster we will present a multivariate analysis of the responses to separate demographic groups more likely to either agree or disagree with these two statements, and also to identify factors impacting this opinion.

6.07P.7

Micro(nano)plastics and Society: How is scientific knowledge being transmitted to the public?

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Nowadays, plastic pollution is recognized as a major threat to oceans sustainability. Scientific studies have shown that the danger of plastic pollution does not disappear with the degradation of plastics down to the micro and nano sizes, emphasizing the relevance of a proper use and management of plastics. In this perspective, the role of society is determinant. Considering that people's behavior is highly influenced by knowledge, level of concern or by interaction with technologies, the present work aimed to study how scientific information is being transmitted to the general public, the concerns of scientists to make information available and easy to understand, and the current understanding of the public. For that, a stepwise approach was carried. First, a literature analysis was performed focused on papers that had as objective communication/education of the public, funded projects with that goal, and social media. Second, to understand the public's perception on the plastics problematic, different age groups were interviewed aiming to analyze their understanding, behavior related with this subject, and how much value is attributed to this problematic. Third, it was intended to educate citizens by using simple eco(toxico)logical tools that demonstrate how plastics can affect biota in general and how it can be translated to the human side. Overall, data show an increasing concern of scientists to the perception of the public to this topic. An increasing number of projects aiming to educate the public are being funded and society is also mobilizing with citizen science projects such as beach clean-up initiatives. Community programs for citizen's education are considered important tools for knowledge dissemination.

6.07P.8

Foundations of social-ecological systems pertinent to addressing challenges of climate change.

L. Kapustka, LK Consultancy

Systems are modulated through nested feedback loops that operate on varied spatial and temporal scales. Five attributes of ecological systems were described in Matthews, et al. 1996 Environ Toxicol Chem 15:597-603), namely that systems are complex, multidimensional, and dynamic; equilibrium is never attained; historical events determine current and future structures; past conditions cannot be repeated; and forecasting future state of systems is tenuous. Biggs et al. (2015) in Principles for Building Resilience: Sustaining Ecosystem Services in Social-Ecological Systems argued that maintaining diversity, redundancy, and connectivity across landscapes and managing slow-acting variables are vital in efforts to pursue sustainability. They also emphasize on the social side that understanding that social-ecological systems are complex, adaptive systems; that experimentation and learning about these systems should feed into broad-based societal participation in polycentric governance practices are critical to advance sustainability goals. The SETAC World Council in 2016 in adopting the Berlin Declaration on Sustainability acknowledged the "the growing recognition of the complexity of environmental issues … require[s] a shift in the ecological sciences to a transdisciplinary approach including social, economic, and behavioural sciences, and more direct collaboration with the traditional biophysical sciences." This talk will set the stage for the other talks in this session that will detail complex challenges of formulating sustainability goals and shaping scientific exploration of management options in the face of impending climate change. The overarching ambition of this talk and the entire session is to avoid making Type III errors, namely developing "correct answers, but having posed the wrong questions" – that is questions that do not take into account the reality of the complexity of social-ecological systems for which relevant models for outcomes are lacking.

6.07P.9

Green chemistry as the basis of the ethics and moral responsibility of an engineer of the future

A. Govorin, Irkutsk national research technical University.

Progress brings good and comfort to a person's life, however, along with new discoveries, environmental problems appear that, at the time of introducing innovative products into the world economy, could not be predicted. Thus, in the present tense, we have the negative consequences of technological progress in the form of global pollution. The main polluting element is plastic. The article discusses the processes of the appearance of plastic in the world, from the point of view of a consumer who was not aware of the potential harm of plastic products and could not imagine what its uncontrolled use could lead to. The key role in global pollution by plastic is played by the mentality, traditions and habits of people who do not keep up with modern discoveries. As an example, the article presents the environmental disaster currently occurring in large cities in India.Traditionally, in India, the country's population used disposable "dishes" from leaves. The utensils used for their intended purpose were not disposed of by any approved developed methods, were not collected centrally, but were simply thrown out by people under their feet, and because of the peculiarities of the tropical climate they rotted very quickly. But with the advent of plastic goods, the environmental situation deteriorated sharply. Life has undergone changes, and the mentality, traditions and education of people have remained unchanged. Used dishes, as before, were simply thrown away, but unlike leaves, plastic does not disappear, but accumulates, gets into rivers and from there into the world ocean.For the new scientific field of "green chemistry," Paul Anastos and J. S. Warner formulated 12 principles, the first of which is: "It is better to prevent the formation of emissions and by-products than to engage in their disposal, purification or destruction." That is, returning, for example, to plastic in Asia, the world is now struggling with the consequences of pollution, and in the ideal case, according to the first principle of green chemistry, we had to think about reducing the "life" of plastic to its worldwide distribution.To prevent such problematic situations, both globally and locally, future engineers must understand and be able to calculate the consequences of their actions. Green chemistry can be an excellent tool for predicting and preventing the causes of environmental problems, and should form the basis of the ethics and moral responsibility of future engineers.

The Role of Science in Derivation of Site-specific Environmental Quality Benchmarks and Management of Water Quality (P)

6.08P.1

SIMONI 2.0: future developments in smart integrated monitoring of the water quality

R. van der Oost, Waternet / Onderzoek en Advies; G. Sileno, Waternet / Research and Development; L. Moria, Waternet / Water Systems; T. Nguyen, Waterproef Laboratory, Research and Validation

Scientific research over the last decades demonstrated that water quality assessment with only chemical analyses is not reliable. Therefore, a paradigm shift from 'substances' to 'effects' must be encouraged in order to implement more holistic approaches in regular monitoring. An alternative Smart Integrated Monitoring (SIMONI) strategy has been developed by Dutch water research institutes. Key factors for generating this model were the selection of the most relevant bioassays and the design of effect-based trigger values (EBT). The SIMONI strategy applies chemical, biological and toxicological methods to determine whether the total mix of chemical pollutants may cause adverse effects to the aquatic ecosystem. Based upon the results obtained thus far, the SIMONI strategy provides an accurate picture of the potential ecological risks of a wide array of organic micropollutants. It appears that highest ecological risks generally occurred at agricultural sites, and waters receiving wwtp effluents, sewage overflows and landfill runoff. Many potential improvements of the SIMONI model are being investigated. The initial selection of toxicological endpoints was directed towards potential hazards of a broad spectrum of chemical micropollutants to aquatic organisms. The bioassay selection is continuously evaluated and updated. Different EBT algorithms were compared and evaluated. The SIMONI approach, using HC5 and background bioanalytical equivalents (BEQ) appeared to be a promising method.The impact of different techniques for micropollutants concentration on toxicity profiles is determined by comparing passive sampling with large-volume solid phase extraction (LVSPE). As the extraction affects the background BEQ of certain bioassays, extraction-specific EBT should be developed.The zebrafish embryo test (ZET) with qPCR biomarkers is applied in tier 2 SIMONI research. Results can be quantified with gene and pathway scores.If chemicals causing the environmental risks the increased risks cannot be identified by target analyses, non-target screening and HT-EDA are applied in tier 2. A novel risk classification will be developed, using the five classes that are also used to classify the ecological status of sites: high (blue), good (green), moderate (yellow), poor (orange) and bad (red) quality. *Key words: micropollutants, effect monitoring, bioassays, passive sampling, toxicity profiling, risk assessment*

6.08P.2

Sediment flushing in North Italian reservoirs: matching research, governance and management

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Reservoirs represent a strategic resource which requires careful management of hydraulic networks and dams for the water-energy nexus without neglecting the protection of water quality and ecosystems. Reservoirs are typical sites where sediment accumulation is favored and desiltation is needed to maintain water storage capacity, as well as proper functioning. Accumulated sediments are frequently removed by flushing, causing physical-mechanical impacts on aquatic communities in the downstream rivers due to increased turbidity and fine-grained sediment deposition. Moreover, ammonia, organic compounds and metals adsorbed on sediments were proved to be released during flushing, potentially inducing toxic effects. In Lombardy Region (Central Alps, North Italy) more than 600 dams are present, mainly used for hydropower generation. A strong As and a moderate Cd, Hg and Pb enrichment in reservoir sediments was emphasized, with potential ecotoxicological risk according to PEC (Probable Effect Concentrations) quotients. In this framework, a technical board composed by researchers and environmental managers developed "PrATo". PrATo is a protocol designed for assessing the toxic potential of sediments released by flushing from reservoirs and includes methods for sampling, chemical analysis and ecotoxicological evaluation of sediments, as well as criteria for risk assessment, based on cross-interpretation of results deriving from chemical, ecotoxicological and ecological analyses. In particular, a proper sediment:water dilution coefficient to be applied during flushing can be derived from analyses, in order to prevent adverse effects on aquatic organisms. The trial of the PrATo protocol in some reservoirs helped disentangling some crucial issues. For example, the definition of threshold concentrations may be biased by high natural background values (particularly for trace elements; e.g. As), by different on-site bioavailability and by synergistic effects of pollutants. Toxicity bioassays were proved to be essential to define the toxic potential of sediments. PrATo is an example of analytical approach derived from science and transferred into management in order to be a cost-effective tool for decision making. The bases of this protocol have been recently included into the "Technical directives for the preparation, approval and implementation of Management Plans for reservoirs" by Lombardy Region.

6.08P.3

Verifying the tiered approach proposed in the EFSA scientific opinion for sediment organisms; a case study with the fungicide fludioxonil

I. Roessink, T.C. Brock, Wageningen Environmental Research / Environmental Risk Assessment

28-Day sediment-spiked laboratory toxicity tests with eight benthic macroinvertebrates and the lipophilic fungicide fludioxonil were conducted to verify the tiered sediment effect assessment procedure currently used, and that is proposed for the future, by the European Food Safety Authority (EFSA). The test species were the oligochaetes *Lumbriculus variegatus* and *Tubifex tubifex*, the insects *Chironomus riparius* and *Caenis horaria*, the crustaceans *Hyalella azteca* and *Asellus aquaticus*, and the bivalves *Corbicula fluminalis* and *Pisidium amnicum*. Toxicity estimates were expressed in terms of total concentration and in organic carbon (OC) fraction of dry sediment as well as in pore water concentration. *L. variegatus* was more sensitive (28d-EC_{10} of 0.367 mg a.s./g OC) than *C. riparius* (28d-NOEC of 1.6 mg a.s./g OC) when artificial sediment was used. In tests with field-collected sediment, 28d-EC_{10} values for *L. variegatus* (0.952 mg a.s./g OC) and *C. riparius* (0.955 mg a.s./g OC) were very similar. Applying an assessment factor of 10 to these toxicity values gives estimates of the Tier-1 Regulatory Acceptable Concentration in sediment (Tier-1 RAC_{sed}) that are lower than the Tier-3 RAC_{sed} (0.37 mg/g OC) derived from the sediment-spiked microcosm experiment. The laboratory toxicity estimates for the eight benthic species tested on field-collected sediment allowed the application of the species sensitivity distribution (SSD) approach (Tier-2 RAC_{sed}). In this approach the Tier-2 RAC_{sed} is derived by applying an assessment factor of 3 to the Hazardous Concentration to 5% of the species. If in the SSD approach the 95% confidence bounds of the EC_{10} values are used as input, the Tier-2 RAC_{sed} estimate (0.193 mg a.s./g OC) is a factor of 2 lower than the Tier-3 RAC_{sed} estimate, and a factor of 4 higher than the Tier-1 RAC_{sed} estimate.

6.08P.4

USEPA Research on Mussels and Magna: Developing Toxicity Testing Methods and Guidance for New Species for Effluent and Ambient Waters

T.J. Norberg-King, U.S. Environmental Protection Agency / Great Lakes Toxicology and Ecology Division; J.M. Lazorchak, U.S. Environmental Protection Agency / ORD/CEMM/WECD/WMD

In the USA, the Clean Water Act (CWA) gives the Environmental Protection Agency (EPA) the authority to implement pollution control programs such as setting wastewater standards for industry and water quality standards for contaminants in surface waters. However, applying water quality criteria to control the release of chemicals into water bodies is insufficient to manage all potentially toxic pollutants in the effluent, as well as mixtures, and EPA has applied aquatic toxicity testing analyses to wastewater discharges. Further, the CWA stipulates that it is unlawful to discharge any pollutant from a point source into navigable waters unless a permit is obtained through the National Pollutant Discharge Elimination System permit program. Toxicity data is used to assess and control the discharge of toxic substances to surface waters under EPAs integrated approach to water-quality-based toxics control which compliments the aquatic-life-chemical-specific criteria and biological criteria. EPA has toxicity testing methods for effluent and ambient testing promulgated through the CWA Part 136, and toxicity test methods required by EPA are then codified in the regulations. The codified freshwater methods include four fish species (fathead minnow (*Pimephales promelas*), bannerfish shiner (*Cyprinella leedsi)*, rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salvelinus fontinalis*), three species of cladocerans (*Ceriodaphnia dubia*, *Daphnia magna*, *D. pulex*), and an alga (*Raphidocelis subcapitata*). In 2019, EPA began focusing on additional species and/or test methods. In the first stage, EPA will be developing protocols for the short-term chronic test using the cladoceran, *D. magna*, and acute and short-term chronic test protocols using freshwater mussel (*Lampsilis siliquoidea*). We have begun developing test protocols and performing test refinements in the EPA laboratories. These procedures will aid in the detection of both known and unknown chemical and biological contaminants in wastewater and ambient waters, and the short-term assays help prioritize problem contaminants and mixtures. EPA plans to develop test protocols for additional species, such as the acute and short-term methods for mayflies, amphipods, midge, trout, and a plant. In this presentation, we will outline the general study plans for each species for the first phase and the progress to date. *Disclaimer: This presentation does not necessarily reflect the views or the policies of the USEPA.*

6.08P.5

Contaminated sediments in Northern France: a risk of non-achievement for the good ecological and chemical status of the Water Framework Directive?

E. Prygiel, C. Hébrard-Labit, E. Chermette, Cerema

Sediment quality has been a long-term concern for most river managers but today the Water Framework Directive (WFD) raises new questions towards water quality issues. The aim of our study is to assess the various risks induced by sediments on surface water quality in relation to the good ecological and chemical status achievement for Northern France rivers. At the international scale, few studies are focused on *in situ* sediment resuspension impacts, but their results pinpoint the punctual exceeding of environmental thresholds and chronic effects on biota. In Northern France especially, rivers managers deal with locally heavy contaminated sediments because of former mining, textile and industrial activities. For example, a sediment monitoring conducted since the 90s has shown high trace metal concentrations: maximal values of Cd (136 $mg.kg^{-1}$), Pb (1700 $mg.kg^{-1}$) or Hg (8 $mg.kg^{-1}$) were still recorded in the 5 last years. Moreover for many rivers, trace metal content do not decrease with time in sediments, threatening the biota and the water quality. Northern France rivers are also intensively channelized to allow the boat traffic, that accounts for 10 % of the national waterways. Furthermore, navigated channels often reveal the most contaminated sediments and are more likely to reintroduce contaminants in the water column during boat traffic. The expected growth of navigation activity in the next decade with the opening of the Seine-North Europe Canal in Northern France region makes it a first range issue to improve our assessment and comprehension of sediment resuspension effect on ecological and chemical water quality. We assume that in the future, changes such as climate change impacts (with an increase of sediment runoff), the improvement of analytical methods, or the addition of new contaminants in the water regulatory monitoring are likely to become new factors limiting the achievement of good ecological and chemical status. Sediment quality, and sediment resuspension, could also hamper the WFD quality objectives in Northern France rivers, requiring more tools to asses the sediment quality and the risks of contaminants transfer to the water column and the biota.

6.08P.6

Effects of high temperature and a PSII herbicide (Irgarol 1051) on hermatypic coral Acropora tenuis evaluated using a compact ecotoxicity experiment system

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The recent global decline in tropical and subtropical hermatypic corals urgently requires research to determine the levels at which different factors influence coral bleaching. In addition to increased seawater temperatures due to climate change, coral reefs face several integrated threats, including agricultural runoff, pollutants derived from shipping activity, and overfishing. Bleaching of *Acropora* spp., the dominant hermatypic genus in southern Japan, reportedly occurs at approximately 30 °C. In order to conduct ecotoxicity experiments promptly to elucidate the factors driving coral bleaching, we developed a compact rearing and experiment system for hermatypic corals using a small glass aquarium, an LED light, and artificial seawater in the laboratory. In this symposium, we report on the ecotoxicity experiments which we carried out to determine the combined effects of high seawater temperature and Irgarol 1051 on the hermatypic coral *Acropora tenuis.* Irgarol 1051 is one of the dominant Photosystem II, or PSII, herbicides broadly used in both terrestrial and marine environments. We investigated the combined effects of high temperature (30 ºC) and Irgarol (1 μg/L) on *A. tenuis*. Samples of *A. tenuis* were exposed to four regimes (27.5 ºC, 27.5 ºC with Irgarol, 30.0 ºC, and 30.0 ºC with Irgarol), and the colour change and maximum effective quantum yield ($\Delta F/F_m$') were measured daily for 7 days. The coral colour was converted to RGB values. Each R, G, and B value ranges from 0 (darkest) to 255 (brightest). The RGB values of coral in both regimes without Irgarol decreased slightly (becoming darker) whereas the RGB values of corals in the two regimes with Irgarol increased (becoming lighter) after day 3. The $\Delta F/F_m$' of corals in the two regimes without Irgarol was slightly increased, that of corals under the two regimes with Irgarol decreased sharply by < 20 % on day 1. No significant differences in colour or $\Delta F/F_m$' were recognized between regimes of different temperatures. Thus, the present study indicates that *A. tenuis* could survive at seawater temperatures of 30 ºC, if the additional anthropogenic effects driving bleaching were limited.

6.08P.7

The development of a national water quality database to assess shifting baselines in a changing climate

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The quality of water in watersheds is expected to be altered due to climate change. However, there is insufficient scientific knowledge to predict how these changes are likely to be manifested. The assessment of long-term trends in historical data over time and space (*e.g.* watershed and regional variability) may offer insight into the potential impacts of future climate change scenarios on water quality. The compilation of 3 to 4 decades of national, provincial, and territorial water quality monitoring data across Canada was initiated in 2018. Using this data, this project aims to answer the following questions: (1) Are there specific metals or water quality parameters that show consistent responses to climate change that could be used as 'indicators' for future projections? (2) Are there specific areas in Canada that are more sensitive to changes in climate (i.e. hot spots)? (3) Does underlying geology influence the extent and magnitude of climate-driven changes in water quality? This project provides an opportunity to establish a national database to track and monitor water quality in a changing climate which would be of use to research organizations, governments as well as offer-ing potential for citizen science applications.

Current Developments in the Regulatory Assessment of Biocides in General and Chemicals in the Sediment Compartment in Particular (P)

6.09P.1

Spatio-temporal exposure of Plant Protection Products in OECD 218/219 sediment test systems - Modelling of experiments with natural sediments

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RWTH Aachen University / Institute of Environmental Research; P. Dalkmann, Bayer AG Crop Science Division / Environmental Safety; D. Faber, Bayer AG, Crop Science Division / BAG-CS-RD-ENVS-EFF-AQ; E. Bruns, Bayer AG, Division Bayer CropScience / Ecotoxicology

Sediment toxicity testing is currently revised under the premise to improve quality and consistency of regulatory risk assessments. In 2015, EFSA has published a scientific opinion on risk assessment for sediment organisms in which sediment-spiked (OECD 218) and water-spiked test systems (OECD 219) are considered to study chronic effects on sediment organisms (typically *Chironomus riparius*). Concentrations in overlying water and mean concentrations over the whole sediment depth are commonly used to derive effect endpoints. However, due to the design of these studies large concentration gradients occur between overlying water and sediment. Therefore, substantial temporal and spatial dynamics of local concentrations have to be expected, especially in the vicinity of the interface beween water and sediment where the Chironomids larvae are supposed to live (observations made in several internal studies). Following up previous work (Hammel et al, 2018, 2019) with artificial sediment acc. to OECD 218/219 we simulated here transport and redistribution of three compounds with the mechanistic model TOXSWA for experiments (see contribution submitted by Dorn et al. and Faber et al.) with natural sediments of different texture, OC and pH (acc. to OECD 308). Variable mobility of the active ingredients, mode of spiking and sediment properties allowed a comprehensive analysis how compound properties and test conditions influence local concentrations and their temporal behaviour. Presuming that chironomids live in the upper millimetres of the sediment their exposure can be very different from the average sediment concentrations (total and pore water) depending on substance mobility, test design and sediment properties. The numerical simulation allows to identify the ecotoxicologically relevant exposure which should be considered for the derivation of effect endpoints of chironomids. The successful application to natural sediments adds further evidence on the robustness of the modelling approach. Hammel K., Dorn A., Dalkmann P., Bruns E. and Faber D (2018). Spatio-temporal exposure of Plant Protection Products in OECD 219 sediment test systems - Comparison of model results with measurements. Platform presentation SETAC Europe 2018 conference Hammel K., Dorn A., Dalkmann P., Bruns E. and Faber D (2019). Spatio-temporal exposure of Plant Protection Products in OECD 218/219 sediment test systems – Modelling and measurements. Poster SETAC Europe 2019 conference

6.09P.2

New insights on relevant ecotoxicological exposure pathways for Chironomus riparius in OECD 218/219 tests using natural sediments

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The European Food Saftey Authority (EFSA) published in 2015 a scientific opinion on environmental risk assessment for sediment organisms. This includes a discussion on how regulatory acceptable concentrations for sediment organisms could be derived and what is the relevant exposure pathway for test organisms such as *Chironomus riparius*. To investigate the spatio-temporal behaviour of three different compounds and their bioavailability in water/sediment systems we conducted over the past three years several studies in-line with OECD 218/219. The compounds differed in their physico-chemical characteristics like the logKow. Following up this previous work using artificial sediment (Faber et al. & Hammel et al 2019), we observed, that *C. riparius* larvae live in the upper first mm of the sediment. Therefore, the larvae are predominantly exposed to the concentration in the overlying water and the concentrations on and in the upper sediment layer. The analytically measured concentrations show that the compound mobility and the mode of spiking have a major impact on the local concentrations in the system, which can be simulated by the TOXSWA model. In addition to the existing experiments two different natural sediments (acc. to OECD 308) were tested. These new studies were performed to investigate the impact of natural sediments on substance bioavailability and if the chironomid larvae live in the same sediment layer as compared to artificial sediment. The results from the new experiments were used as well for a robustness check of the model approach (TOXSWA). For that purpose the modelled concentrations were compared to analytically measured concentrations from the different sediment layers (see contributions submitted by Dorn et al. and Hammel et al.). Based on all results we are able to consider the distribution of the substance and the location of the test organism *C. riparius* in water/sediment test systems to better predict the relevant ecotoxicological exposure. These information should further support the set-up of reasonable sediment risk assessment approaches. Faber, D. et al. (2019): New insights of the relevant ecotoxicological exposure pathway of *Chironomus riparius* in OECD 218/219 tests. Poster SETAC Europe 2019 Hammel K. et al. (2019): Spatio-temporal exposure of Plant Protection Products in OECD 218/219 sediment test systems – Modelling and measurements. Poster SETAC Europe 2019

6.09P.4

Preliminary ecological risk assessment of DCOIT for Brazilian coastal areas

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Ecological Risk Assessment (ERA) is performed to assess how likely adverse ecological impacts may occur as a result of exposure to one or more environmental stressors. Risk Quotient (RQ) approach is widely used to do a risk characterization, providing important information for interpreting the risk results, as it can categorize degrees of concern for harmful environmental effects. DCOIT (isothiazolone class) is a co-biocide used as antifouling agent for preventing the growth and settlement of organisms on submerged surfaces. Although is expected to degrade rapidly, DCOIT has been detected in different matrices worldwide. Thus, the present study performed a preliminary ERA of DCOIT for coastal areas of Brazil under influence of maritime activities. Superficial sediments were collected in ports and marine areas along nine states of Brazil. Measured environment concentration (MEC) of DCOIT was obtained by ultrasound-assisted extraction of sediments and clean-up by solid phase extraction (C18 cartridge). Quantification was done by liquid chromatography with tandem mass spectrometry detection. Chronic toxicity values were obtained from a literature review and their reliability was checked using the Criteria for Reporting and Revaluating Ecotoxicity Data (CRED). The deterministic method was used to calculate the Predicted No Effect Concentration (PNEC) using 50 as assessment factor on the lowest NOEC value (most sensitive species). Finally, RQ were calculated according to the equation: RQ = MEC / PNEC. DCOIT was detected in 68% of sediments along all states of Brazil and levels ranged from < 0.2 to 227 ng g^{-1}. The lowest CENO was 9.7 ng g^{-1} resulting in a PNEC value of 0.19 ng g^{-1}. RQ < 1, indicating low to moderate risk, were found in 35 sites, while RQ > 1, indicating a high risk and that adverse effects are likely to occur, were found in 77 sites. Sites with higher risk were under direct influence of boatyards (pleasure and fishing boats). In addition, the presence of antifouling paint particles was also detected in sites with high levels of DCOIT, which may contribute to increase the environment risk of DCOIT. In conclusion, this preliminary ERA shows that coastal areas of Brazil are likely threaten by the presence of DCOIT in sediments, which highlight the need for adoption of environment policies by national authorities to protect the Brazilian coastal species, as there is no regulation on the use of DCOIT and other antifouling co-biocides in the country.

6.09P.5

The New Echa/Efsa Guidance on Endocrine Disruptors in the Context of the BPR - Requirements, First Experiences and Challenges

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In order to enable authorities and applicants to implement the new criteria for the identification of Endocrine Disruptors (ED), Echa and Efsa recently published the corresponding guidance document. It is intended for the assessment of active substances used in plant protection and biocidal products (BPs) under Regulations (EC) No. 1107/2009 and (EU) No. 528/2012, respectively. For the assessment of ED properties of substances, the guidance provides a five step approach: I. Gather all relevant information relevant for the ED assessment, II. Assemble, assess and integrate the lines of evidence, III. Initial analysis of the evidence, IV. Mode of action analysis, V. Conclusion. Due to the focus of the guidance, in most cases an in-depth re-evaluation of all available relevant (eco)tox information, including studies as well as literature data is required. In this context, 'Table Appendix E' is the basic tool for data gathering and subsequent assessment of potential ED properties. Experience has shown that existing evaluations and summaries of good quality guideline studies can be transferred into this table only to a limited extent and a great deal of manual work is still required. Besides this, the integration and assessment of the lines of evidence and a potentially subsequent substantial mode-of-action analysis (which, depending on the effects observed, may or may not be required) are core elements in the guidance and represent additional big challenges. Specifically for non-active substances/co-formulants in BPs, recently a more pragmatic, decision tree based approach was proposed, considering inter alia previous EU decisions or various database screenings. Co-formulants are mainly regulated under REACh, so this interface might cause difficulties for an ED assessment due to a possible lack of information and unclear or shared responsibilities. As BPs usually contain quite a number of co-formulants, an ED assessment, even if only required on the screening level, requires considerable efforts and capacities. Overall, comprehensive testing to have potential ED properties “sufficiently investigated”, which is foreseen by the Guidance Document and might be inevitable, will conflict with regulatory and ethical goals to minimise animal testing. With respect to data generation, it is important to discuss and align the testing strategy with the competent authority in case further data is required and in order to draw a conclusion on potential ED properties.

6.09P.6

Endocrine disruptor screening assessment of co-formulants in biocidal products - first experiences and development of an IT tool

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In 2018 the scientific criteria for determining endocrine-disrupting (ED) properties under the Biocidal Products Regulation 528/2012 and the Plant Protection

Products Regulation (EC) 1107/2009 entered into force. In order to assist applicants and assessors with identifying EDs in accordance with these criteria, the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA) developed a joint guidance document (ECHA/EFSA, 2018). This guidance document describes how all available scientific information should be gathered and evaluated for use in the ED hazard assessment. The guidance is focused on the assessment strategy for active substances in biocidal products (BPs) and plant protection products (PPPs), which are generally data rich. However, for biocides the assessment is not limited to active substance(s) and ED properties of co-formulants should also be assessed. According to the EU Commission's guidance note 'CA-March18-Doc.7.3.b-final' it is up to the evaluating bodies to decide, based on existing knowledge and scientific information, whether there is a need to evaluate a specific non-active substance in detail. In order to facilitate this process, a stepwise approach for a targeted determination of ED indications or properties of co-formulants in BPs was developed by the competent authorities (CG-34-2019-02 AP 16.5 e-consultation ED potential of co-formulants_final). This screening approach explains different steps for gathering existing information from other EU programs and is fitted to the often more data scarce co-formulants. The burden of gathering available scientific data for ED screening of co-formulants is placed on the applicants, which can very labor intensive, especially for biocidal product families (BPFs) with sometimes more than 50-100 co-formulants. In order to streamline this process, ARCHE Consulting and Chemycal have jointly developed an ED screening tool for co-formulants, which implements advanced data scraping technologies to automate a large part of this process by checking existing EU programs and websites with information on ED properties of chemicals. This tool can help applicants structuring and simplifying the tasks associated with ED assessment of co-formulants in a continuously developing regulatory framework, by receiving timely notifications when new scientific data emerges. In this poster, first experiences with the process and IT tool will be shared.

6.09P.7

Co-formulants of biocidal products under the biocide regulation: challenges an perspectives

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This Regulation provides for the authorization of products under conditions of sufficient effectiveness and no unacceptable effect on the environment and human health. Risk assessment is an instrument implemented to define the conditions of use of biocidal products without unacceptable effects on the environment and human health. These evaluations relate primarily to the active substance; additional assessments are carried out for the substances of concern (excluding the active substance) among the co-formulants contained in the biocidal products. An evaluation of the co-formulants is to be carried out beforehand for their intrinsic properties and their content in the biocidal product. Since 2014, criteria have been used to designate substances of concern (SoC, "substance of concern") in biocidal products. More recently (2018), the entry into force of the criteria for the determination of endocrine disrupting properties introduces new criteria to be considered for the evaluation of co-formulants. The result of applying the criteria may confirm the use of a co-formulant or consider re-formulating the biocidal product to suppress or to replace a co-formulant. It is necessary to have data on the co-formulants of the biocidal products in order to assess the SoC criteria for this ingredient. If identified as SoC then it will be necessary to carry out the risk assessment for the environment and for the human health in order to demonstrate a safe use. How much do we know about the properties of co-formulants contained in biocidal products? The present work is an approach to cover the requirements under the biocide regulation by: · collecting identification and hazard data on co-formulants · assessing the missing data to accurately qualify or not these co-formulants as SoCs · assessing of endocrine disrupting properties The objective is the development of a database of accurate, reliable and harmonised data for co-formulants used in biocidal products.

Refinement of the Risk to Birds and Mammals from Plant Protection Products: Higher Tier Studies and Approaches (P)

6.10P.1

Options for data interpretation and analyses of avian reproduction endpoints

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Avian reproduction studies (OECD 206; OCSPP 850.2300) are required to assess the risks to wild birds from potential exposure to plant protection products in many regions (e.g. EU, USA). Response variables are analysed to determine the no observed effect concentrations (NOECs) amongst treatment groups. Due to the complexity of these studies and the low statistical power of a number of variables assessed, careful interpretation is required. Statistical differences between the concurrent control and treated groups can arise due to random sampling and can appear to be treatment-related. Recent scientific work (SETAC 2019) indicated, case by case, how a toolbox based on different approaches (statistics, biological relevance, BMD, historical control data) can be used to improve the interpretation of standard avian reproduction studies by providing further context in the data assessment, with the goal of avoiding unnecessary vertebrate testing. Various categories have been identified for further analysis. The aim of this research is to present this analysis and demonstrate how we can use the toolbox more efficiently in order to set a chronic avian endpoint and facilitate a scientifically sound assessment of the data. (1) A clear protocol should be provided that indicates the trend test to use, the direction for the trend (increasing or decreasing), and a test procedure for determining whether the data are consistent with dose-response monotonicity. (2) When monotonicity is unrealized, protocols should provide correct multiple-comparison tests to use. (3) Wherever possible, the magnitude of effect in each type of response considered to be biologically relevant should be provided. (4) Where a historical control database exists, statistically significant differences between the current control and treatment mean responses should be compared to the range of mean control responses. (5) Proper protocols are needed to define when a regression model can be fit to the data, proper assessment of goodness-of-fit and the width of confidence intervals for EC10/20/30s. Wide confidence intervals or poor fit suggest unreliability of the regression result and should be dismissed. Overall a well-defined statistical protocol that prioritizes trend-based methods and allows for biological input and reference to an historical control database for each response will be summarised.

6.10P.2

Deriving ecologically relevant endpoints for wild mammal risk assessments: 5 years on, what is the state of play?

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The risks to wild mammals from potential exposure to plant protection products must be assessed in accordance with Regulation (EC) 1107/2009. The risk assessment is currently performed according to the bird and mammal guidance document (EFSA Journal 2009; 7(12): 1438). Under this guidance, the actual protection goal is to clearly establish no visible mortality and no long term repercussions for abundance and diversity, with the surrogate protection goal being to make mortality or reproductive effects unlikely. In the Tier I risk assessment for wild mammals, the lowest available no observed adverse effect level (NOAEL) from the developmental study or 2-generation rat study should be used. However, these endpoints may not be of ecological relevance for wild mammal populations in terms of addressing the protection goals. This is briefly discussed in Section 4.4 of EFSA (2009), in terms of which *types* of effects are considered relevant for wild mammal populations. However, little guidance is available on how to determine whether the effect is of ecological relevance; instead, a weight of evidence and expert judgement is required. In 2015 it was recommended by Member State ecotoxicology experts (EFSA supporting publication 2015: EN-924. 62 pp.) that ecologically relevant reproductive endpoints should be assessed and agreed during active substance evaluations at EU level rather than during national registrations. The aim of this poster is to review EU active substance evaluations that have occurred since 2015 in terms of how many have attempted to use a refined toxicity endpoint in the wild mammal risk assessment, what approaches were used, and the regulatory acceptance of those approaches. Recommendations for improvements to the future bird and mammal guidance document will be made where relevant.

6.10P.3

The use of video recording in order to collect data for higher-tier wildlife risk assessments under realistic conditions in the field

O. Fuelling, Tier3 Solutions GmbH / Field study; C. Cervencl, B. Giessing, C. Wolf, Tier3 Solutions GmbH; S. Norman, RidgewayEco

Providing data in order to assess essential parameters required for a refined exposure assessment can be challenging when studying wild animals. Fixed-position automated video recording can provide unique opportunities for observations of free-ranging animals and for gaining biological knowledge. The use of video cameras during field studies has many advantages. First of all animals can be studied under the most realistic conditions in the field where they are likely to perform natural behaviour. Secondly, video recording allows identification of diurnal, nocturnal and shy species that might not be seen by direct observation. And, thirdly, because the cameras are automatic, several fixed-position cameras can be set-up at the same location at the same time, all gathering information simultaneously. Furthermore, with the recent advancements in technology and the improvement of the image quality also very small objects can be observed, which facilitates detailed observations of foraging animals and their selected food items. We present two different camera systems with their specific advantages and limitations. We also show some examples of field studies (focal species studies, studies on breeding success and dietary studies) in which these systems have successfully been used either instead of or as an addition to conventional methods.

6.10P.4

Worth the risk? Food preferences of wood mice on newly drilled fields

O. Fuelling, Tier3 Solutions GmbH / Field study; I. Hotopp, Tier3 Solutions / Regulatory Science; M. Foudoulakis, Corteva agriscience / The Agriculture Division of DowDuPont

In accordance to EU regulations the risk to small mammals from applications of plant protection products should be assessed. If a potential risk is indicated in lower tier studies, more realistic field data can support a refined assessment following the EFSA GD on Risk Assessment for Birds and Mammals. Refinement steps include information on the use, i.e. on the attractiveness of a specific crop for a specific species. A habitat with its potential food source may be perceived as dangerous and, therefore, less attractive for mice like *Apodemus* spp. Published data show that wood mice occasionally enter freshly drilled fields. However, as wood mice are omnivorous, the reason to enter bare soil fields might be the search for animal prey. We conducted 2 studies to investigate the attractiveness of different seeds in and off-crop as well as opportunistic feeding on crop seeds or invertebrate food items in a potentially dangerous habitat. The 1st study followed the Giving-Up-Density (GUD) approach. Small mammals, usually wood mice, showed a clear preference for feeding under the cover of hedges and bushes. On average only 2.9 % of all seed types were consumed in the open, freshly drilled fields while 78.9 % of all seeds were consumed in the sheltering off-crop. In-crop and off-crop there was a preference for larger seeds like sunflower or maize. The small rape seeds were least attractive even under the cover of hedges and bushes. The results of the present GUD study indicate that small mammals perceive open bare-soil fields just after drilling as a dangerous environment where they spend little time in search for crop seeds. Other food items (e.g. earthworms or ground dwelling arthropods) available on such fields might be more attractive. This was investigated in the 2nd study approach. In the woodland egdes (off-crop) next to all study fields the occurrence of wood mice was confirmed by motion-triggered cameras pervious to the trial. On 4 out of 6 different locations the observations of wood mice entering the fields were very rare (0 to 4 filmed visits per field). Only 2 fields had a number of visits. During the first phases, when a total of 6000 mealworms and 6000 seeds of OSR were offered side by side, 71 mealworms (1.18 %) and 13 rape seeds (0.22%) were consumed by wood mice. During the second phase, when only OSR was offered, 14 rape seeds out of 6000 (0.23 %) were consumed. Further information will be provided about their prefence of the in field area.

6.10P.6

Small Seed Quantification in Wood Mice Feces (Apodemus sylvaticus) by Quantitative DNA Analysis

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As part of the authorization process for plant protection products, it is required to determine possible risk of products to birds and mammals. The wood mouse (*Apodemus sylvaticus*) is used as focal species for seed treatments because its biological and ecological characteristics make it particularly sensitive as a seed-eating small mammal. In models that are used for risk assessment, exposure is a key factor. However, estimates of intake of contaminated food sources in nature are difficult to obtain. To improve these intake estimates and parametrize models, data from focal species are needed based on realistic scenarios of pesticide application. This study aims to genetically quantify the content of small vegetable seeds in the diet of wood mice by analyzing droppings. This approach could yield more accurate results than stomach content analyses through visual estimation and does not require killing animals. In a laboratory feeding trial, twenty wood mice were fed 0, 1, 3, 5, 10, 15, 20 carrot and onion seeds and droppings were collected for 8 hours after seeds were completely consumed. Droplet digital PCR was used, which is especially accurate in quantifying low DNA concentrations with low variation, to measure target DNA concentration in the wood mice feces. The effects of DNA extraction method, sex, age, diet composition of the individual, and DNA marker used for target DNA quantification were also assessed. Target DNA was detectable in wood mouse feces even if fed with only one seed per plant species. A significant difference in target DNA concentration was found in droppings of mice fed with 1, 5 or 10 seeds per plant species. Target DNA concentration of mice fed with more than 10 seeds per plant species resulted in large variation among samples, which did not allow reliable quantification. The type of DNA extraction method and the DNA marker influenced quantification success but sex, age and diet composition did not. This study concludes that quantifying small seed intake in small omnivorous mammals is possible for up to 10 seeds in increments of 5 seeds in a laboratory setting. The next step is using these calibration data to quantify seed consumption in a realistic field scenario of seed treatment application. Ultimately, this could improve pesticide risk assessment models by more accurately assessing potential risk to non-target species, in turn ensuring a high level of protection for wild animals.

6.10P.7

Bird and mammal higher tier risk assessments: The appropriate use the 'PT consumer only approach'

A. Blakey, Syngenta / Environmental Safety; S. Kragten, R. Murfitt, Syngenta Ltd / Environmental Safety

The EFSA Guidance Document (GD) on Risk Assessment for Birds and Mammals (EFSA 2009) defines PT as the proportion of an animal's daily diet obtained in habitat treated with a plant protection product (PPP). As a worst-case Tier I risk assessment, it is assumed that individuals find all their food in the treated area and that PT = 1. Following identification of the focal species, the EFSA GD (2009) provides the following guidance on the selection of individuals to radio-track in order to derive a PT value for use in a higher tier risk assessment: *"It is recommended that for focal species caught within the crop, PT should be estimated from all individuals – whether they used the crop of concern or not. For the focal species caught in the general farmland, PT should be estimated from only those individuals proved by radio-tracking to be consumers i.e. PT > 0."* Despite the above guidance, regulators now often use only the "Consumer" PT approach for calculating a refined PT value, even when individuals were caught in or in close proximity to the crop. The 90%tile consumer PT value is used in the risk assessment only if >10 consumers are identified. For situations, where less than < 10 consumers are identified, the maximum PT value observed in the radio-tracking study is used. Here we review the appropriateness of the "Consumer" approach and explain why focusing on individual effects rather than population-level effects, is not in-line with the EFSA GD (2009) protection goals: *"The actual protection goal is to provide a high certainty that no visible mortality and no long-term repercussions on abundance and diversity will occur. "The surrogate protection goal is to make any mortality or reproductive effects unlikely."* In addition, hypothetical data sets based on typical wood mice radio-tracking data, are presented to explain why the "Consumer" PT approach is not appropriate for unattractive crop scenarios. Instead, we recommend calculating a 90% tile PT value based on all mammals i.e. individuals caught in or in close proximity to the crop, regardless of whether they used the crop or not.

6.10P.9

Long-term effects of spinosad on the Common vole (Microtus arvalis) on grassland as surrogate crop

C. Miersch, Tier3 Solutions; O. Fuelling, Tier3 Solutions GmbH / Field study; I. Hotopp, Tier3 Solutions / Regulatory Science; M. Foudoulakis, Corteva agriscience / The Agriculture Division of DowDuPont; A. Rossbach, Tier3 Solutions GmbH / Field team

Spinosad is used as an insecticidal spray in a number of crops in Europe with up to three applications per year. Based on the lowest endpoint for the wildlife risk assessment, a risk for small herbivorous mammals (Common vole) has been identified for various crops. A field effects study in grassland was conducted as a 'risk envelope study' to address the potential long-term risk to small herbivorous mammals in all uses concerned. Grassland is the primary habitat of voles and thus serves as the relevant surrogate crop. The species is abundant and permanently resident in the treated area and is feeding exclusively on treated food items (grasses and herbs). Thus, it is an ideal model organism in order to cover all potential impacts of the test item. The study period covered the entire reproductive period from April until November with applications conducted in June. A state-of-the-art design with 7 treatment and 7 control fields was chosen, using constant live trapping with the capture-mark-recapture (CMR) method. The following parameters were determined: **Population size and development, body weight, sex ratio, proportion of juveniles, reproductively active animals and the length of presence of individuals in the field.** Captured animals were marked with a passive integrated transponder (PIT) to distinguish individuals. This study design provides a solid data set which allows detecting potential differences in population development between treatment and control fields, using statistical mixed models. Differences **in** the duration of presence of individuals in the field are analyzed with the Cox-Hazard ratio.

6.10P.10

Long term effects of an herbicide on wild European rabbits - new methods for lagomorph field studies

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After spray application of a plant protection product (PPP), food sources of wild mammals (e.g. lagomorphs) may become contaminated. Therefore, EU registration demands a risk assessment for PPPs and its active substances. The initial risk assessment is based on worst case assumptions which, in many cases, lead to an overestimation of the actual risk. For a more realistic risk assessment, a field effect study in a relevant worst case exposure scenario may be conducted to assess effects on European rabbits (*Oryctolagus cuniculus*). The study presented here is to determine possible long-term effects on European rabbits exposed to the herbicide propyzamide under field realistic conditions. The study was conducted in commercially used orchards with grass covered ground. Orchards are one of the many crops where propyzamide can be applied but can be considered as a model crop as in contrast to arable crops with regular tilting, crop changes and other major disturbances, orchards are a more stable habitat with short grass which is the preferred food source of rabbits. The study therefore covers all crops which can be treated with the test item as a risk envelope study. Rabbit warrens are usually located at the edges of the orchards, where the individuals usually stay close to, feeding within approximately 200 meter radius. The study was conducted on 12 different orchards, comparing rabbit populations in six treated and six untreated (control) orchards. The test item was applied two times in autumn/winter, once in Oct 2019 and once in Jan 2020 with the maximum intended application rate according to the product label. All rabbits captured were individually ear-marked with passive integrated transponders (PITs), sexed, weighed and their reproductive state recorded. In order to monitor the ear-marked individuals, automatic PIT readers were installed. In addition, some individuals were equipped with GPS radio collars to follow their movement, especially around the periods of application. To assess possible effects on the reproductive performance of exposed rabbits the numbers of juvenile rabbits in treated versus untreated orchards and before and after the applications were compared with adequate statistical methods.

6.10P.11

Do not fly blindly into the night - How landscape parameters and species specific traits can be used for exposure scenarios in the risk assessment for European bat species

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Bats compose the second largest group of mammals (after Rodentia) and provide worldwide important ecosystems services from pollination to seed dispersion, whereas in Europe they play a vital role in the control of agricultural pests. Their importance is also reflected in the fact that all bats species occurring in Europe are protected by law (Annex I to the Agreement on the Conservation of Populations of European Bats, EUROBATS, 1991) under 'The Convention on Migratory Species (UNEP/CMS)'). However, bats are not specifically mentioned in the current risk assessment for plant protection products (PPPs), although their habitat preferences and hunting behaviour may lead to the exposure to PPPs. Recently, EFSA has published a scientific statement 'on the coverage of bats by the current pesticide risk assessment for birds and mammals', which concludes that bats are not sufficiently covered by the current risk assessment due to large variations in toxicological sensitivity and a lack of consideration of bat specific traits. Bat species vary greatly in their occurrence, mode of living, and their feeding behaviour. Therefore, their exposure to PPPs cannot be calculated in a generic approach, but has to be adapted to their species-specific characteristics. Furthermore, landscape parameters determine the likelihood of an area to be used by bats as habitat or hunting area, and thus play a key role in the determination of exposure. In consideration of these factors exposure scenarios for bats should be based on: Regional distribution across Europe. Habitat preferences consisting of regional characteristics such as altitudes and climatic conditions, and landscape structures such as open or small-scale landscapes.Feeding and hunting behaviour. Feeding guilds can be separated roughly into bats with aerial, ground-feeding (gleaning), or intermediate feeding behaviour. Diet requirements for specialists may have to be considered.This poster presentation shows how these three factors can be used to form a baseline exposure scenario, where knowledge gaps are, and provides an outlook on a theoretical framework for a scenario based risk assessment.

6.10P.12

Residues on flying insects for bat risk assessment

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Next The EFSA Guidance Document (GD) on Birds and Mammals (2009) is the key regulatory document on wildlife risk assessments for Plant Protection Products (PPPs) in the European Union (EU). An EFSA Working Group is currently preparing a revision of that EFSA GD (2009), and the new version is likely to include specific risk assessment scenarios for bats. The EFSA PPR Panel (EFSA 2019a) has published a scientific statement on bat risk assessment with a discussion of potential bat-specific exposure scenarios, including dietary exposure to spray application residues on flying insects. For dietary exposure estimation for insectivorous birds and mammals, the EFSA GD (2009) employs "RUD"-values (RUD = residue-per-unit dose; RUD x application rate = residue concentration in mg/kg) to predict the initial residues on ground dwelling invertebrates or foliage dwelling arthropods. A RUD value for flying insects is not included in EFSA GD (2009). However, residues in flying insects have been determined in a number a field studies that are evaluated in a review by Lahr et al (2018), who summarised these results in a spreadsheet that is available for download (also including RUDs for other bird and mammal food items). These results for flying insects have been taken up by EFSA 2019a which employed the maximum RUD value from these studies in their example for a dietary risk assessment on bats. We critically evaluated this data collection of RUD values on flying insects (Lahr 2018), and found that some of these RUDs appear incorrect or not compliant with the recommendations in the last EFSA document on recurring issues in ecotoxicology (EFSA 2019b). We also provide additional RUD data from new insect residue studies. Options for further research are discussed. EFSA (2009) Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. doi:10.2903/j.efsa.2009.1438 EFSA (2019a) Scientific statement on the coverage of bats by the current pesticide risk assessment for birds and mammals. doi:10.2903/j.efsa.2019.5758 EFSA (2019b) Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. doi:10.2903/sp.efsa.2019.EN-1673 Lahr et al (2018) Data collection for the estimation of ecological data (specific focal species, time spent in treated areas collecting food, composition of diet), residue level and residue decline on food items to be used in the risk assessment for birds and mammals. doi:10.2903/sp.efsa.2018.EN-1513

Challenges in the Future of Higher Tier Aquatic Studies in Regulatory Risk Assessment (P)

6.11P.1

Combined effects of psychoactive drug, fluoxetine hydrochloride and microplastics on the aquatic ecosystems over a chronic exposure: A limnotron study

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Pharmaceuticals are a major class of micropollutants ubiquitous at concentrations ranging between ng/L to fewer µg/L in surface freshwater systems. In environment, pharmaceuticals co-exist with multiple global change stressors. Hence it is critical to evaluate the combined effect and the stress these contaminants pose to aquatic ecosystems. In this study, the effect of pharmaceuticals along with microplastics, another ubiquitously distributed contaminant on the food webs are being studied. We hypothesized that sorption of fluoxetine to surface of microplastic can potentially cause alterations to food webs in limnotrons upon a chronic exposure. The experiments were performed in 9 indoor mesocosms called limnotrons with 845L of water and 40L of sediment. Microplastics (diameter:10.23 micron) were spiked into the sediment and water so as the number of beads were 529 spheres/Kg dry weight and 19 spheres/L respectively and the microplastic concentration in both phases were kept constant throughout. The pharmaceutical chosen for the study was fluoxetine hydrochloride -a psychoactive drugconsidering its prevalence in surface water systems. The experimental design was such that the concentration of pharmaceutical in water phase increased from 10 to 500 ng/L. Limnotrons were maintained at 20°C with 16:8 light:dark settings for 100 days.10L of water from every system was replaced with 10L of aerated groundwater with fluoxetine for 5 days/week. All the 9 limnotrons were monitored for phytoplankton composition, dissolved and particulate nutrients (C, N and P), Fluoxetine hydrochloride along with the abiotic parameters (pH, electrical conductivity in µS/cm and dissolved oxygen in mg/L). Comparative analysis of the differences between the development in these parameters in the limnotrons will assist in understanding the role of microplastics in risks posed by fluoxetine hydrochloride to aquatic ecosystems.

6.11P.2

A mesocosm approach to quantifying the impact of plastic degradation on freshwater ecosystems

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Plastic debris was first reported in the early 70s with little interest to the scientific community. Currently, with an estimated amount of 8 million tonnes reaching the oceans each year, is a global concern. The ecological consequences of these larger plastic litters are evident. Ingestion of plastics by birds and turtles as well as entanglement of marine mammals is widely described. A particular concern is the occurrence of smaller particles, termed microplastics (< 5 mm), which has attracted researchers' attention in the last decade. Microplastics in aquatic environment are formed by biodegradation, photodegradation, thermooxidative degradation of meso- and macro-plastics debris. Experimental studies have demonstrated that microplastics can cause growth inhibition, block digestive tracts, reduce feeding rates, diminish mobility and even cause mortality of aquatic organisms. Most research up to date focusses on small-scale lab experiments. The process of plastic weathering and fragmentation on more realistic ecosystem-scale is practically unknown. Using a mesocosm approach, we studied the degradation of plastics in the freshwater ecosystem, the mechanisms by which microplastics are derived from plastic debris, and the ecological impacts of this process. To this end, we exposed nine 988 L indoor mesocosms referred as Limnotrons (1.37 m depth, 0.97 m diameter,) filled with water from a mesotrophic shallow pond in Wageningen, The Netherlands, to different plastic and plastic alternative types, ranging from cups, lids, bags to straws. Temperature and light were adapted to mimic typical Dutch seasonality from late summer to autumn. We assessed phytoplankton biomass, C:N:P stoichiometry, dissolved nutrient availabilities, zooplankton abundance, plastic fouling ,weathering and breakdown. Our preliminary results indicate rapid fouling processes, with major changes in dissolved oxygen and primary production. Mesocosms are a useful approach to study plastic degradation, microplastics formation and their impacts under environmentally relevant conditions, and thus provide a vital step in ensuring effective mitigation and management strategies.

6.11P.3

New approaches to testing acute toxic effects on natural plankton and soil communities

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ERA of chemicals aims at the protection of the environment and its biodiversity. Currently, only single species tests with a few representatives were performed in the lower tier which cannot assess effects on biodiversity. More complex community approaches such as aquatic outdoor mesocosms and terrestrial TMEs or field studies are so far only used in higher-tier risk assessment of plant protection products. They are aimed to investigate the impact of anthropogenic stressors on natural communities including species interactions over a longer time period and under outdoor conditions. Such studies allow screening of non-standard organisms in natural communities for their sensitivity to a toxicant. However, the particular community composition in these model ecosystems may not always be sufficiently adjustable, and is strongly influenced by the test design of the model systems itself. We present a new approach of testing typical and well established field communities over short periods of only several days to a few weeks and is therefore designed for detecting acute effects on non-standard species and communities. These studies can be conducted under outdoor or laboratory conditions. Originally intended as toxicity screening tests for the more elaborate long-term mesocosm or TME studies, these short-term community studies also offer their own advantages: with these systems we can investigate natural communities and derive community endpoints such as biodiversity indices beside single species sensitivity for non-standard species. Furthermore, different natural communities derived from field sampling can be tested, which often cannot be established in semi-field studies, but inhabit relevant organisms (e.g. planktonic organisms from larger ponds and lakes). Small test units allow the use of a higher replicate number as well as the simultaneous testing of several communities from different locations. In addition, these systems can be used to investigate typical natural summer as well as winter communities (e.g. phytoplankton communities). We will present exemplary test designs that cover aquatic plankton communities (zooplankton and phytoplankton) as well as terrestrial soil communities (e.g. oribatid mites, collembolans).

6.11P.4

Lack of Latent Effects to Daphnia magna From Exposure to Chlorantraniliprole

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Latency is defined as delayed effects of exposure to a molecule after the exposure period is completed or the molecule is no longer detected in the field/area of interest (i.e., water, soil, sediment, etc.). It is the responsibility of the registrant to provide evidence of a lack of latency. The EFSA Guidance on tiered risk assessments for plant protection products for aquatic organisms in edge-of-field surface waters (2013) allows registrants to use a species sensitivity distribution (SSD) HC_5 endpoint (based on acute EC_{50} data) for long-term risk assessments if effects are rapid (i.e. first 96 hours of the chronic test) and if latency is not a factor. Because *Daphnia magna* is the most sensitive of the aquatic organisms exposed to chlorantraniliprole in laboratory studies, the determination of latent effects is based on studies conducted to this test organism. For chlorantraniliprole, both the acute (EC_{50}) and chronic (NOEC) *Daphnia magna* endpoints are driven by the rapid onset of immobility observed in the daphnid acute toxicity test and in the first 96 hours of the daphnid chronic test. Therefore, the short-term and long-term aquatic invertebrate risk assessments are based on the acute endpoint derived from an acute species sensitivity distribution (SSD) with the assertion that there was a lack of latency from exposure to chlorantraniliprole. To determine if latency is a real concern, a study was conducted with *Daphnia magna*. The study consisted of a short-term (48-hr) exposure to the aquatic invertebrate *Daphnia magna* followed by a subsequent 19-day test material-free period. The 48-hr exposure time is consistent with the regulatory *Daphnia magna* acute toxicity guideline and 21-days total time is consistent with the regulatory *Daphnia magna* chronic toxicity test guideline. The acute 48-hour EC50 from the guideline test and the latency test considered equivalent. The NOEC values from the guideline chronic daphnid test and the latency test are also considered equivalent. It can be concluded that the results from this higher tier test support the lack of any latent effects to *Daphnia magna* exposed to chlorantraniliprole. Therefore, it is acceptable to use the acute endpoint for the long-term aquatic risk assessment.

Evidence-Informed Policymaking and Stakeholder Engagement on Environmental Management in Africa (P)

6.12P.1

Investigating the Capacity for Science Advice and Utilisation of Scientific Evidence for Environmental Policymaking In Nigeria: A Case Study

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The development of policies to promote sustainable development of nations and continents is imperative for decision-makers. In Nigeria, the lack of capacity to utilise research evidence to inform decision making by policymakers (evidence users) is a major challenge. The aim of this study was to investigate the capacity for science advice and utilization of scientific evidence for environmental policymaking at a representative government policymaking institution in Africa. The methods used included the administration of a capacity building and needs assessment questionnaire to Scientific Officers across all departments at the Federal Ministry of Science and Technology, Abuja, Nigeria using SurveyMonkey. Also, oral interviews and horizon scanning sessions were conducted for directors of the ministries' departments as well as scientific officers of the Department of Planning Research and Policy Analysis. Finally, the project objectives and outcomes were communicated at project inception and end of project seminars to scientific officers and directors of the ministry and its MDAs. Seven (7) capacity building needs were identified from the survey and seminars were subsequently conducted to address these needs. On the basis of the capacity building seminars, three (3) concept notes were developed in concert with the scientific officers including the inauguration of a Sustainability Science Dialogue. Further, a project proposal to establish a Researchers-Policymakers forum for environmental policymaking in Africa was submitted for external funding. The study revealed the importance of capacity building in engendering evidence-informed decision making by policymakers. We recommend the facilitation of sabbaticals by policymakers to universities and researchers to government policymaking institutions to bridge the knowledge gap and promote the capacity for evidence utilisation in policymaking.

6.12P.2

Demystifying Indigenous Knowledge for Freshwater Governance Policy

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Freshwater policy has a diverse range of impacts on freshwater resources' physical and socio-economic systems. True and up to date information on areas affected by freshwater policy is critical to better understanding the drivers of resource degradation activities, as well as their relevance to biological, chemical water quality and sedimentation, in aid of freshwater conservation management. Planning policy data was traditionally executed from the government institution's offices, using secondary data. With the use of knowledge from the context of the environment or ecosystem, indigenous knowledge (IK) becomes a more practical alternative for integration into other existing freshwater policies as they provide a timely local, regional and global source of information. The study reviews the most recent approaches to water governance and evaluates the current approach in policies both at the global and regional level in IK. Using the mixed method approach this paper explores the moral and practical bases of IK from the local and formal institutional context through examination, observations, and understanding of IK implications to freshwater conservation. Respondents numbering 111 were used for the in-depth interview, 108 for the Focus Group Discussion and 354 for the Questionnaire administration. The study identified challenges encountered by stakeholders in policy formulation and implementation. The paper describes the historical trends of using IK and the scientific system in freshwater conservation. Finally, the results identified possible future opportunities to further reduce degradation of water resources using IK as part of the freshwater policy and developed a model for water policy formulation.

Addressing the Challenges of Applying New Approaches Supporting Chemical Safety Assessment and Regulatory Decision Making (P)

7.01P.1

Recent updates to the MechoA scheme, a tool to support mechanistically-driven read-across approaches.

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Mechanisms of Action (MechoA) scheme is a tool that has been developed to group chemicals based on their initial interactions with biological matter. To build a high quality QSAR or Read-Across, only chemicals with the same MechoA should be used. Indeed, two substances with a different MechoA but identical physico-chemical parameters would have different toxicity values (e.g. fish 96h-LC50) because some MechoAs are more potent than others and require different quantities of chemicals to reach the toxicity threshold, i.e. the amount necessary to produce toxic effects. MechoA scheme v1.0 was published in 2018 by Bauer et al. and achieved 92.2% correct classifications based on a validation set of nearly 500 molecules, while the Verhaar scheme, as modified in 2008 (Verhaar et al., 1992, Enoch et al., 2008) and the MOA by OASIS classification, an OECD QSAR Toolbox profiler mainly based on (Russom et al., 1997), obtained 54.0% and 82.5% correct classifications, respectively. In the field of toxicology, no general scheme for the classification of substances according to their mode or mechanism of action has been located. So, the new method developed in this work provided more accurate predictions than existing schemes in the field of ecotoxicology, while being the first classification scheme of its kind applicable also in the field of toxicology. Since then, the scheme has been updated to version 2.0, enabling better predictions for substances displaying several MechoAs and also extending the applicability domain with more direct docking disruption mechanisms. Additionally, we created the MechoA subgroup 6.8 "endocrine disruption", and a specific structural alerts scheme to predict this class is under development. The model has now been implemented in several software tools, to facilitate the use of this method in risk assessment strategies, with a SMILES code as the sole input: - iSafeRat® Online and iSafeRat® Desktop, 2 tools developed by KREATiS, which are respectively web-based and standalone tool, where MechoAs are connected to (eco)toxicity QSAR models, - the SAMSON platform, which includes various molecular modelling tools, - we are currently in the process of integrating it in the OECD QSAR Toolbox where it will be more easily coupled with the analogue search and category formation features included in this tool. All these tools are freely accessible except iSafeRat® Desktop.

7.01P.2

The development of a Molecular Initiating Event (MIE) based profiler for chemical classification and read-across

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Mode of Action (MoA) determination is an important part of understanding toxicity to aquatic organisms in the context of risk assessment and as part of regulatory schemes to enable read-across, chemical categorisation and prioritisation. With a lack of harmonisation of current chemical fragment based approaches to be able to adequately classify the full commercial chemical space, coupled with a shift towards mechanistic-based approaches, there is a need to better define classification approaches. The Adverse Outcome Pathway (AOP) concept which links a Molecular Initiating Event (MIE) and biochemical interactions to individual or population responses, has gained significant traction as a means to interpret apical toxicity endpoints mechanistically. Aligning with the AOP concept the aim of this study was to develop a scheme for classifying chemicals into MIEs relevant to aquatic toxicity supporting the grouping of chemicals for the purposes of endpoint prediction and read-across. The approach brings together existing and 'state of the art' knowledge as well as developing improved mechanistic based thinking into chemical grouping decision making. Structural alerts for the classification of the environmental effects of compounds across multiple species were collated from the literature and aligned to established MIEs for known toxicological mechanisms of action. These have been coded as SMARTS strings and implemented in a KNIME workflow for ease of use. In total over 390 MIE-derived alerts were established. The performance of the new environmental profiler Workflow to categorise a large database of molecular structures was also assessed

7.01P.3

Application of a Chemical Similarity Methodology to Identify and Assess Groups of Substances of Potential Very High Concern (SVHC)

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From a regulatory perspective, we aim to minimize exposure and emission of chemicals with serious and irreversible effects on human health or the environment as much as possible. This particularly includes substances that are carcinogenic, mutagenic or reprotoxic (CMR); persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB); or substances with an equivalent level of concern, like endocrine disruption (ED). Substances that meet these criteria can be identified as Substances of Very High Concern (SVHC) within the REACH regulation. The ultimate aim is to substitute these substances with safer alternatives. To facilitate the identification of potential SVHC substances and prevent "regrettable substitution", we recently developed a chemical similarity methodology that assesses whether a new chemical is structurally similar to a known SVHC [Wassenaar et al. 2019]. A high resemblance in structure might be an indication of comparable effects. The developed methodology showed a high internal performance on a dataset with non-SVHC substances (balanced accuracies > 80 % for the different SVHC-subgroups). However, the application performance of the method on large external datasets and its potential to identify groups of chemicals of potential concern was not evaluated. Within this study we applied the chemical similarity model on two datasets to investigate the application performance. The selected datasets include a list of chemicals without specific knowledge on potential concerns (i.e. all REACH registered substances) and a list of (potential) CMR and PBT/vPvB substances that are not yet identified as SVHC, like substances classified as CMR category 2. In addition, several case studies were analysed, like bisphenol A and perfluorinated chemicals. Furthermore, we evaluated the predicted chemical similarity of the model with an expert judgement approach. This study/poster shows the potential of the methodology to be applied in screening programs on single substances, but also on the identification of groups of chemicals of concern. Furthermore, we show how this method could be used to generate hypotheses on potential effects and how it could be applied within safe-by-design processes. In addition, some examples of follow-up assessment and evaluation are provided.

7.01P.4

Training a molecular dissimilarity measure for target specific activities

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p margin-bottom: 0.1in; direction: ltr; color: rgb(0, 0, 0); line-height: 115%; text-align: left; p.western p.cjk p.ctl Graph edit distance is a methodology used to compare graph structures such as molecules. With this methodology we can estimate the dissimilarity between two molecules by computing the cost of editions needed to transform one molecule into the other. These editions are known as edit operations and six of them are used in the graph edit distance computation: insertion, deletion and substitution of atoms and bonds. Each edit operation has an associated edit cost, which has to be determined depending on how different the attributes in the atoms and bonds are. In this study we use machine learning techniques to learn these edit costs when comparing molecules by means of the graph edit distance methodology. The graphs used in this study correspond to reduced structural representations of molecules, built according to their pharmacophore-type node descriptions. This representation is called extended reduced graph. In the experiments, graph edit distance using learned costs is applied to ligand-based virtual screening applications, which estimate the bioactivity of a new compound according to the bioactivity of similar compounds. Graph edit distance using learned costs performed better than graph edit distance using predefined costs. These experiments were performed with the publicly available dataset DUD-E. Overall, this work shows that graph edit distance along with learned edit costs can be used to deduce bioactivity similarities from a structurally diverse group of compounds. Furthermore, by optimizing the target-specific edit costs, it is possible to obtain useful structure-activity information to be used as a base for drug design studies.

Exploring the Potential of Wastewater-based Epidemiology to Monitor Human Exposure to Pollutants and Public Health (P)

7.02P.2

ESAR-Net: a collaborative effort to expand the application of wastewater epidemiology in Spain

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Data obtained from wastewater analysis can provide rapid and complementary insights in illict drug consumption at community level. Drug use has been assessed through wastewater analysis at national level in, for example, Australia, Belgium, Finland and South Korea and has also provided annually a one week snapshot of illicit drug volumes consumed in European cities (http://www.emcdda.europa.eu/topics/ pods/waste-water-analysis). However, a wastewater monitoring program did not exist in Spain, but leading experts have formed a network (https://www.esarnet.es/) to promote wastewater-based epidemiology at national level and communicate their findings to authorities and policymakers. Within Europe, Spain is an important country of transit of both cocaine and cannabis, due to its cultural, linguistic and colonial ties to Latin America and its proximity to Morocco. The quantity of seized cocaine and cannabis and prevalence of use, locates Spain at the top of Europe. In this work, a national wastewater campaign has been performed to get more insight on the consumption of illicit drugs and NPS within Spain for the first time. Wastewater results from 14 Spanish cities were compared with previously reported data and other national indicators. The cities, located in 7 of the 17 autonomous communities, cover approximately 6 million inhabitants (12.8 of the Spanish population). Untreated wastewater samples were analyzed for urinary biomarkers of amphetamine, methamphetamine, MDMA, cocaine and cannabis. In addition to these conventional drugs, weekend samples were monitored for several new psychoactive substances (NPS) (i.e. phenethylamines and cathinones). The selected NPS are known as possible replacement of these conventional drugs or among those previously reported. Finally, enantiomeric profiling of amphetamine was performed for one city in order to assure the results were due to consumption and not illegal dumping of production residues. This demonstrates another application of wastewater-based epidemiology, which allows to identify the origin of drugs in wastewater. *Acknowledgments:* This work has been supported by the Spanish State Research Agency (Agencia Estatal de Investigación, AEI) through the "Redes de Excelencia" programme, ESAR-Net, ref. CTM2016-81935-REDT

7.02P.3

Biomonitoring of human exposure to pesticides through wastewater analysis

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Humans are continuously exposed to pollutants by different routes, and human bio-monitoring is the tool commonly used to assess exposure to chemicals by measuring parent substances or metabolites in human body fluids, hair or nails. Wastewater-based epidemiology (WBE) is a complementary approach to human bio-monitoring that overcomes some of the existing limitations such as sampling biases, long realization time, high costs and ethical issues. Recently, alarming news stories in Dutch (press) media reported high exposure to pesticides of the population living near flower bulb fields. The WBE approach has until now been mainly applied and focussed on illicit drugs, but also has potential to estimate human exposure to pesticides. Some promising results have been published [1, 2], which allowed the evaluation of potential health risks by comparing the WBE data on pesticides with the acceptable daily intake [2]. In the present study the potential of WBE is explored for exposure assessment of pesticides, and to assess the actual human exposure in areas with relatively high use of pesticides. Several pesticide biomarkers have been identified and reference standards purchased (including isotopically labelled internal standards) for the development of a target analytical methodologies based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), with triple quadrupole mass analysers. Method development consisted of several optimization and validation steps related to sample preparation (including evaluation of matrix effects), LC and MS/MS. The areas selected for wastewater collection were near bulb fields (the Netherlands) and near citrus orchards (Spain), and also urban or industrial areas where pesticides are applied less frequently or not at all. The latter were selected as "control" areas. Finally, the objective is to perform a comprehensive risk assessment based on existing data from exposure models as used in authorization procedures and the data obtained in this study. References [1] Rousis NI, Zuccato E, Castiglioni S (2017) Wastewater-based epidemiology to assess human exposure to pyrethroid pesticides. Environ Int 99:213–220 [2] Rousis NI et al. (2017) Wastewater-based epidemiology to assess pan-European pesticide exposure. Water Res 121:270–279

7.02P.4

Chemical fingerprint of alcohol and nicotine consumption in Spanish wastewaters

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Wastewater provides a fingerprint of a specific population lifestyle. Tracing the right chemical markers in a wastewater sample allows back-calculating the amount of alcohol and tobacco consumed by the people contributing to that sample. This approach, also known as wastewater-based epidemiology (WBE), provides information on substance abuse in a rapid, anonymous and objective way, complementing the information obtained by traditional methods (surveys, medical and criminal indices, etc). WBE has been used in Spain to estimate the consumption of legal and illicit psychoactive substances. While 4 Spanish cities systematically apply the WBE on an annual basis to report the use of illicit drugs to the European Monitoring Centre for Drugs and Drugs Addiction, the WBE has been only occasionally applied in few cities to estimate alcohol and tobacco use. Based on this, the present work aimed at extending the application of the WBE approach in the Spanish territory (14 cities, 17 WWTPs, 13% of the Spanish population) to obtain a more reliable picture on alcohol and tobacco consumption in the country. This work also aimed at evaluating spatial and weekly consumption trends of these legal drugs and compare WBE-derived data with official consumption figures. For this, 24-h composite wastewater samples were collected at the inlet of 17 wastewater treatment plants (WWTPs) during one week in spring 2018, covering approximately 13% of the Spanish population, and urban areas of different size. The samples collected were analysed for urinary biomarkers of alcohol and nicotine (main psychoactive substance of tobacco). Results showed the presence of the three metabolites in all samples analyzed. Spatial variations in alcohol and nicotine consumption were observed among the investigated cities, and in the case of alcohol, also different consumption patterns were observed during the week. Extrapolation of WBE-derived consumption data at national level showed an annual consumption of alcohol lower than that reported by the World Health Organization (WHO). However, in the case of nicotine, consumption obtained by WBE approach was very similar to tobacco sales*Acknowledgments:* This work has been supported by the Spanish State Research Agency (Agencia Estatal de Investigación, AEI) through the "Redes de Excelencia" programme, ESAR-Net, ref. CTM2016-81935-REDT.

7.02P.5

Monitoring Infectious Diseases in Near-Real-Time for Rapid Outbreak Response

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As the arms race continues against infectious diseases, community disease surveillance and management is increasingly important. Infectious disease outbreaks have been a major cause of suffering and death throughout human history. In light of the recent swine flu, Zika and Ebola outbreaks the UK government added emerging infectious disease to the National Risk Register of Civil Emergencies. Furthermore, recent trends in population growth, urbanisation and rapid mass movement have made the human species intrinsically more vulnerable to infectious disease. These threats, along with antimicrobial resistance, vaccine refusal and climate change present a formidable challenge for public health management. [1] This study aims to assess the strengths of wastewater-based epidemiology as a tool for community infectious disease monitoring. Wastewater-based epidemiology promises to provide near-real-time and quantitative methods for the monitoring of infectious disease to enable outbreak control. These systems should ideally be capable of providing community-based data to decision makers in near-real-time on an international scale. The use of reverse transcription digital polymerase chain reaction for detection of pathogenic RNA in wastewater will likely form an essential part of this approach. A reverse transcription digital polymerase chain reaction methodology was developed for the routine monitoring of community disease outbreaks, using norovirus as a test-case pathogen. A target RNA sequence was identified, by which norovirus was detected in influent wastewater at a concentration of 51 aM (30 gene copies μL^{-1}). Method development work was carried out towards the identification of a stable sample storage approach, as well as towards the identification and mitigation of the causes of variability within the methodology. Initial method validation work

was also carried out, whereby the recovery of a target DNA sequence was used to assess the dynamic range of the assay. 1. 1. J. Lederberg, *Emerg. Infect. Dis.*, 1997, **3**, 417-23.

7.02P.6

Wastewater fingerprinting for public health assessment: Proteomic approach

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Wastewater-based epidemiology (WBE) has become a useful tool for measuring the chemical substances in the wastewater including a wide range of human related products to verify public health status. This approach has been successfully validated and applied to monitor substances of abuse in large scale studies across the globe (e.g. for illicit drugs, for tobacco, and for alcohol consumption) and to measure the concentrations of several pharmaceuticals, metabolites, and personal care products. Recent studies recommend yet further opportunity to evaluate the state of public health by adapting this approach for the analysis of a spectrum of endogenous biomarkers reflecting physiological states of stasis, stress, and disease (e.g., proteins, sugars, lipids, and nucleosides). However, the challenges of analysing these endogenous biomarkers from wastewater influent include the dilute nature of the material and the diverse complexity of the organic matter in the effluent that can interfere with the methods. One of these endogenous biomarkers, C-reactive protein (CRP), a positive acute-phase protein, which is generated in reaction to physiological stress such as inflammation, infection, or physical trauma in wastewater has been extensively studied in different populations as a marker of cardiovascular and renal function abnormalities, using clinical proteomics settings. As a result, it is an ideal marker to estimate community-wide health. Recently, hyphenated mass spectrometry (MS) successfully demonstrated accurate, reproducible quantification of some of these endogenous biomarkers in plasma samples, by the analysis of peptides produced via enzymatic digestion of protein targets, which is known as bottom-up (shotgun) proteomics. By combining the techniques of WBE, clinical proteomics and MS there exists the possibility for the monitoring of endogenous biomarkers in the wastewater. The aim of this work is the selection and identification and the detection of CRP peptides using a hyphenated triple quadrupole mass spectrometry within wastewater. **Acknowledgement:** This work is a part of the Wastewater Fingerprinting for Public Health Assessment (ENTRUST) project funded by Wessex Water and EPSRC IAA (grant no. EP/R51164X/1).

7.02P.8

Strengths of correlation between aquatic predictors and disinfection byproducts formation in chlorinated water

B. M, Agriculture Research Organization / Institute of Soil, Water and Environmental science; M. Borisover, Agricultural Research Organization / Institute of Soil, Water and Environmental science

The formation of chlorinated disinfection byproducts (cDBPs) in any source water, including drinking water affluent in organic matter (OM) precursors are remaining to be a great concern owing to its plausible genotoxic, and mutagenic effects. The absorbance (Abs_{254}, specific UV absorbance; SUVA (Abs_{254}/DOC)) and fluorescence (parallel factor analysis – PARAFAC, peak picking – PP, and regional integration – RI) based methods are utilized as aquatic predictors of DBPs formation in chlorinated water since the concentration of DBPs are often correlated with these aquatic predictors. The current study was intended to examine how the strengths of correlations between cDBP concentrations and their predictors are affected by source water types used for treatment, disinfection agents, DBP types formed, and by the type predictors used. Totally, 70 research studies cover the literature for the last two decades (1997-2019), reporting the correlations between cDBP concentrations and OM predictors were collected. The obtained data were grouped into four categorical variables that included the type of source water (six types), disinfection agent (three types), DBP class (four types) and the DBP predictors (three types; Abs – absorbance, Fluor – fluorescent, and organic carbon – OC). A set of 574 Pearson's (r) coefficients describing the strengths of correlations between DBP predictors and DBP concentrations ($p < 0.05$) of different combinations found were converted to Z score using Fisher transformation and analyzed by main effect ANOVA. The obtained results indicate that the source water and DBP predictors are the main significant parameters followed by disinfection agents, while DBPs type is a comparatively less critical parameter for DBP formation. OM rich wastewater statistically presented the strongest correlations of DBP predictors with DBP formation, among other water types, utilized. UV absorbance and OC concentrations over SUVA and fluorescence-based DBP predictors served as better predictors. The non-fluorescent OM present might have a high reactivity towards chlorination reactions. Considering different fluorescence-based techniques, RI and PARAFAC showed stronger correlations with DBP concentrations as compared to PP. Also, humic-like substances over proteinaceous substances of OM showed better correction between DBP concentration and its predictors. These findings emphatically prove the significance of non-fluorescent OM in assessing toxic DBPs formation upon chlorination.

7.02P.9

Wastewater-Based Epidemiology for Monitoring Community derived Antibiotics and Resistant Genes

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Antimicrobial resistance (AMR) has been hailed as one of the critical public health threats facing 21st century. The discovery and wide-spread availability of antibiotics since the 1970s revolutionized medicine, however over-prescribing has resulted in the increased development of multi-drug resistance in pathogens. Current AMR surveillance within populations relies heavily on clinical data. This however covers only a small proportion of the community as samples are from those who have seeked medical aid, hence might not be representative of the wider population. Wastewater-based epidemiology (WBE) is a promising approach of sampling influent wastewater for biomarkers in order to give public health information on the population that has contributed. The analysis of AMR related biomarkers in WBE could therefore provide key spatiotemporal and comprehensive information on resistance circulating in communities giving complimentary information to current AMR clinical data.This study presents results from a year-long study in two catchment areas in the South-West combining the analysis of antibiotics, metabolites and resistance genes in influent wastewater to investigate AMR within populations. Using advanced analytical tools, chemistry and biological techniques have been combined together to give insight to the antimicrobial patterns and resistance of two communities Over 200 influent wastewater samples have been collected from two different catchment areas, with up to ten 24-hr composite samples from each site per month. Over 60 different antibiotics and metabolites covering a range of different classes have been investigated. Next generation DNA sequencing along with dPCR on seasonally relevant samples have given insight to the diversity of resistant genes present in wastewater. Catchment prescription data of antibiotics demonstrates seasonal prescribing patterns which is reflected from wastewater analysis.

Future Risks of Chemicals (P)

7.03P.1

Modelling the interaction between climate change and chemical effects at different levels of biological organization - a PhD proposal

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Since the beginning of the Industrial Revolution, chemical pollution has been a major threat to freshwater ecosystems. However, there are not only many toxic substances, they also come in combination with other stressors. In the face of global climate change (GCC), temperature and CO2 levels are predicted to increase in future ecosystems. It is expected that the effects of chemicals will change due to these additional stressors, as multiple stressors might act antagonistically or synergistically. These combined effects are likely to have different mechanisms at different levels of biological organization (e.g., individual, population, and community). Thus, to manage ecological risks in the future, there is an urgent need to understand the effects caused by multiple stressors and how they can be transferred amongst these levels of biological organization. Based on mechanistic knowledge, in silico methods already enable to predict single stressor effects on of freshwater systems. This research project is part of the Marie Curie ECORISK250 ITN project (www.ecorisk2050.eu) and aims to determine the interactive effect mechanisms of chemicals and GCC and provide a more holistic description of ecosystem response to multiple-stressors. To active this, we will improve existing state-of-the-art modelling frameworks to enable prediction of multiple-stressor effects. In a bottom-up approach, the individual level effects, investigated through toxicokinetic-toxicodynamic and dynamic energy budget theory approaches, will be translated to the population and community level, using individual based models and food-web models. Finally, this research aims to support future ecological risk assessment with the developed and advanced modelling tools that enable realistic assessment of combined effects of chemicals and GCC on freshwater ecosystems in the future.

7.03P.2

Development of scenarios for future emissions of chemicals from agricultural, industrial and urban systems

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The extensive use of chemicals leads to concern for the environmental quality of air, soils and water. Chemicals are used in daily life in various ways. Both during their production and use phase as well as after use, (residues of) these chemicals can enter the environment and water systems. The use and production of chemicals are expected to grow rapidly driven by drivers such as population growth, urbanisation and economic growth. This could lead to further emissions of chemicals to water posing significant water quality concerns. Socio-economic scenario analysis is a useful tool to investigate the long-term consequences of future change and mitigation options. In order to better understand how the future emissions of chemicals will change, we will relate current (2000-2014) sectoral emissions of chemicals to the corresponding sectors in the Integrated Model to assess the Global environment (IMAGE3.0) model. We use the publicly available chemical emissions data from the E-PRTR database and Leclerc et al., (2019) to determine empirical relationships between socio-economic drivers (e.g. population growth, economic growth, agricultural productivity, change in land use etc.) with emissions of chemicals. The sectoral classification on E-PRTR widely vary from point sources (industrial activities and wastewater treatment plants) to diffuse sources (agricultural activity, atmospheric deposition, transportation etc.). We explicitly choose socio-economic drivers relating to emissions of individual chemicals (industrial chemicals and pesticides) based on major sectors and sub-activity contributing to the emissions as per the E-PRTR and population growth for pharmaceutical emissions. Subsequently, we will use Shared Socio-economic Pathways (SSP) from the IMAGE Integrated assessment model as input for our empirical emission models to develop emission scenarios for chemicals for 2050. We will include the three SSP scenarios: SSP1 ("Sustainability"), SSP2 ("Middle of the Road") and SSP3 ("Regional Rivalry"), including changes in animal husbandry and focus on three different regions (north, central, southern Europe). Furthermore, we will also develop scenarios describing mitigation efforts.

7.03P.3

Precision agriculture techniques: an opportunity to revolutionize the use of plant protection products

J. Davies, Syngenta / Environmental Safety

Ongoing revision of risk assessment schemes for the registration of plant protection products in the EU, continually raises new technical challenges and barriers to the development of efficacious crop protection products. For example, in the area of non-target terrestrial plant risk assessment, EFSA's Opinion published in 2014, redefined non-target plants as all plants growing outside fields and those growing within fields that are not the intended pesticide target. It also states that non-crop plants growing in in-field areas provide ecosystem services including food web support, aesthetic value, genetic resources and endangered species, which require protection from the adverse effects of plant protection products. Since 2014, options for protecting ecosystem services provided by non-crop plants have been discussed in various stakeholder workshops. However, the regulatory debates around these issues rarely consider advances in agricultural technology (i.e. precision agriculture techniques) that have the potential to modify the way in which herbicides are applied in the future and will minimize the risks perceived to be associated with their use. For example, increasing precision enablement of crop sprayers includes the use of GPS systems, boom section control and pressure variation, so enabling variable applications across fields. Meanwhile, increased understanding of the environmental factors driving target weed distributions, field scouting by drones and digital image analyses allows development of field-specific weed and treatment maps. Combination of these approaches will enable patch spraying whereby only the infested area of a field is treated. Estimates suggest that these approaches will permit a 6 to 81% reduction in the use of herbicide selective for broad-leaved weeds and a 20-79% reduction for products selective for grass weeds while use of automatic boom section control has been found to reduce use by 15.2 to 17.5% when compared with no boom section control. Moreover, technology is also available to enable traceability of products from manufacturing plant to field and limit their use to the label recommended rates, so providing regulatory assurance against mis-use. This presentation aims to raise awareness of the potential environmental benefits of the use of precision technologies for weed control and highlight the need for regulatory risk assessment processes that accommodate the use of these technologies.

7.03P.4

Freshwater rivers in the 21st century: an uncertain fate for chemical contaminants?

T. Lane, University of York / Environment Department; J.B. Sallach, University of York / Environment; A. Boxall, University of York / Department of Environment and Geography

Water security issues are becoming more relevant worldwide and specific concerns surrounding freshwater river quality have emerged as an important area of focus. Recent monitoring campaigns have identified chemical contaminants in river systems above regulatory guidelines across nearly all chemical classes and forecasts of future usage indicate that exposure to chemical contaminants is likely to increase. Concurrently, global changes are contributing to shifts in environmental conditions and water chemistry of riverine systems (e.g. temperature, dissolved organic carbon, nutrients, hydrology) which could alter the fugacity of chemicals, thereby reducing or increasing exposure potential for aquatic organisms. The objective of this study was to investigate how predicted changes in water quality parameters of freshwater rivers will influence the fate and uptake between three compartments – water, sediment and organism – for compounds representing a diverse range of physicochemical properties. Water chemistry profiles were manipulated across a range of temperatures (12 - 24°C) and concentrations of dissolved organic carbon, nutrients and salinity. Test organisms (*Daphnia magna*, *Lumbriculus variegatus*and *Hyalella azteca*) were selected to represent different species-specific traits. This work will help determine how different environmental conditions, physicochemical properties and species-specific traits might drive chemical fate and uptake in freshwater environments. Future work will focus on developing combined fate and uptake models for estimating the influence of environmental change on the uptake of chemicals into aquatic invertebrates.

7.03P.5

Predicting environmental risks of pharmaceuticals in Norwegian surface water

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Although a number of studies have shown that pharmaceuticals in the environment can impact ecosystems, our ability to predict and quantify the risks these pharmaceuticals pose is still limited. Although at present regulatory risk assessment in the EU relies on an estimate of the proportion of the population using a medication, and the largest typical dose at which it will be taken, an alternate approach has been developed and tested that uses the annual weight of sales together with estimations of wastewater processing removal rate of pollutants in water, population, wastewater produced per person per day, and the dilution ratio of wastewater entering surface waters. In Norway, a highly developed country with an aging population, records of pharmaceutical sales are maintained back to the year 1995, allowing for a prediction of past and present environmental risk. In this study, we applied a predictive model on a substance-by-substance basis to pharmaceutical sales data from 2018 to produce predicted environmental concentrations in surface waters. These predicted concentrations were then compared to predicted no-effect concentrations (where available) to calculate risk quotients or ratios. From this data we were able to compile a top 10 prioritisation list of the pharmaceutical substances predicted to pose the greatest risk to surface water ecosystems. Of the substances with available toxicity data, we found that hormonal contraceptives contributed a massive proportion of the overall risk burden - estradiol (RQ = 46), ethinylestradiol (32) and norethisterone (3.7), while over-the-counter painkillers were also well represented – ibuprofen (9.8), paracetamol (8.5) and naproxen (1.4). Also present were amoxicillin (17), a β-lactam antibiotic, abiraterone (15), an androgen/oestrogen agonist used to treat prostate cancer, mycophenolic acid (9.6), an immunosuppressant, and simvastatin (2.3), a statin. Once validated, this predictive framework will allow us to assess past risks in Norway and estimate future risks, allowing for pre-emptive mitigation measures to be tested and put in place before risks arise.

The Use of Citizen Science in Environmental Toxicology and Chemistry (P)

7.04P.1

The "Mass experiment" - A citizen science project regarding plastic pollution

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Plastic pollution is a major environmental challenge and a dominant part of global marine litter. Numerous clean up activities have been conducted across the globe, many of which engange the public in citizen science (CS) activities, based on scientific protocols such as the Marine LitterWatch protocol developed by the European Environmental Agency (EEA). Here we present data collected in the "Mass experiment" (ME) conducted in fall 2019. ME engaged school children and young people in primary or secondary education, in a collection of plastic litter nationwide in Denmark. The aim of the Mass experiment was to conduct the first scientific mapping of plastic litter at a national scale. All sampling was conducted following a protocol developed from the EEAs Marine LitterWatch protocol. This ensured scientific quality of the protocol and subsequently allowed for the data to be included in EEAs Marine LitterWatch (MLW) database. The Mass experiment protocol was adapted in two important ways. 1) the catagories were limited to 22 plastic categories, whereas the MLW protocol addresses all marine litter. 2) the Mass experiment collected plastic litter in 7 different nature types whereas MLW is focused solely on beaches. The seven nature types were: beaches, dunes, forest paths, ditches along larger roads, small rural roads, parks and streams/lakes. All samplings were conducted over a 100 meter transect with varible width. Each sample was registered with a single GPS coordinate in the middle of the transect. Approximately 57,000 people participated in the Mass experiment in a three week period, equal to almost 1% of the Danish population. Data from 3543 transcets were collected, covering 7 nature types. A total of 374,082 pieces of plastic litter were collected and categorised. Only 66 samples did not contained any plastic illustrating that plastic litter is present though out the country, despite a relatively effecting waste handling system. The study illustrated that plastic litter is present in all nature types and thus not just a marine challenge. The majority of plastic found stems from littering. Our results indicate that policy measures, such a deposit/return systems, have a positive influence on the amont of littering, as illustrated by the low number of plastic bottles found.

7.04P.2

Fishing for Litter While Sailing: Blackfish Citizen Science Samples Contribute to Plastic Pollution Inventory

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The accumulation of plastic debris in marine environments and their potential negative effects to marine organisms have become issues of high priority for environmental policy. Macro- and microplastic, large (over 5 mm) and small (5 mm - 1 µm) plastic particles and fibres, respectively, are amongst the most abundant pieces of litter found in the marine environment. Plastic litter can accumulate in the water column, on beaches, in sediments and in biota, posing a risk to marine organisms and having impact in the economy of local communities depending on tourism, aquaculture and fishing. Plastic pollution is persistent and widely outspread, and the collection of baseline data from surface seawater to correctly grasp the pollution problem is time-consuming and expensive. Marine Citizen Science, in particular the collaboration between research institutes and Ships of Opportunity (SOOP), can enhance data acquisition capabilities, simultaneously empowering citizens, improving environmental awareness and increasing Ocean Literacy. In this project, the Flanders Marine Institute (VLIZ) is partnering with the Blackfish ship team (blackfish.be) to collect data on marine plastic litter from seawater surface (www.youtube.com/watch?v=_4cFpDIs3Ok&feature=youtu.be). To date it is unclear whether the ratio between the numeric amount of macro- and microplastic is universal in all locations, or whether particles of different sizes show different transportation behaviour. This project will focus in acquiring data that will enable modelling the fate of plastic particles of different size ranges. Between 2019 and 2021 the Blackfish, a sustainably built sailing ship, will participate in the Transquadra (www.transquadra.com) sailing race, navigating between the Netherlands, Belgium, United Kingdom, France, Portugal, Spain, Mediterranean, Madeira (PT), and Martinique. During this journey the Blackfish sailing team will collect marine litter using a manta-trawl after having been trained by the research group from VLIZ on the sampling protocol. The boat will sail onto locations where conventional research vessels only occasionally navigate, proving a unique opportunity to collect samples suitable for a scientific output. Thanks to the collective effort of the Blackfish, VLIZ and the non-profit WWF, this sampling campaign will have a high impact in increasing public Science Literacy and awareness on plastic pollution.

7.04P.3

Plastic Pollution Perceptions and Behaviour - A study on Danish pupils before and after the "Mass Experiment"

N. Oturai, Roskilde University / Dept of Science & Environment; S. Pahl, University of Plymouth / School of Psychology; K. Syberg, Roskilde University / Dept of Science and Environment

A recent study shows that behaviour change programmes can be a faster and more cost-effective solution to plastic pollution than regulation, and may thus pose as a supplement in environmental decision-making (Benartzi et al., 2017; Dietz et al., 2009). 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 (European Commission, 2019). Beyond *knowledge*, key predictors of environmental behaviour count a range of factors, such as *identity*, *perceived control*, *values*, *social norms*, *nature-connectedness* and *self-efficacy* (Pahl & Wyles, 2017). Citizen Science (CS) has gained momentum in the field of environmental monitoring, and also as a scientifically acceptable method for studying prevalence of plastic (Hidalgo-Ruz & Thiel, 2015). In this context, our study seeks insights into whether and how a nation-wide CS intervention shifts young citizens' environmental perceptions and self-reported behaviour, hypothesising that the intervention will increase the school students' *risk perception*, *self-efficacy* and *empowerment*, as well as *self-reported actions* in the follow up-survey compared to the baseline. With more than 57.000 students signed up for the 'Mass Experiment' (ME), organised by ASTRA (National Center for Learning in Science, Technology and Health in Denmark) with MarinePlastic (Danish center for research in marine plastic pollution) this is to our knowledge the largest CS activity on plastic debris targeting young people. Two-day activities included lectures on plastics in the environment and had the students (aged 6-16 years) collect plastic litter from predefined nature-sites. Post collection the students reported numbers and types of items according to a modified protocol based on the European Environment Agency program 'Marine LitterWatch'. Twelve questions investigated perceptions and behaviour in an online survey distributed before (3-week time span) and after (4-week time span) the intervention. The students contributed to the study voluntarily and anonymously, however unique team-ID made comparisons by team possible. Analyses will use non-parametric Wilcoxon signed rank test for hypotheses testing regarding *self-reported behaviour*, *self-identification*, *empowerment*, *self-efficacy* and *social norms*. At the time of writing, the data collection before and after the ME is completed and has yielded 3747 (pre) and 2355 (post) responses.

Poster Corner Abstracts

Innovative Assessment Tools and Criteria for the Protection of Ecosystems and Human Health (PC)

1.14PC.1

Toxicologic effects in clams Ruditapes philippinarum exposed to remediated seawater by GO-PEI previously contaminated with Hg under warming scenario

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Nowadays several studies demonstrated that the increase of temperatures have a negative impact in the environment, especially on marine ecosystems. Consequently, the organisms subject to warming scenarios can show histological and physiological alterations on growth and reproduction, increase of oxidative stress, as well as decrease of metabolic rate and respiratory capacity. Moreover, marine organisms may not only affect to temperature increase but also, be exposed to metal(loid)s derived from natural and anthropogenic activities, which are correlated to world population growth. Studied demonstrated that higher concentrations of metal(loid)s (especially heavy metals) in bivalves induced histological, physiological and biochemical impairments. Furthermore, the interactions between stressors as pollutants (heavy metals) and climatic change (warming scenario), could cause additive and antagonistic effects as reported in several studies. For this, different approaches have been applied to remove metal(loid)s from aquatic ecosystems. In particular the mercury (Hg) is a widespread in marine environmental, which has already proven to induce toxic impacts in a diversity of species, like bivalves, especially at warming scenario. Therefore, our group developed a material based on GO functionalized with polyethyleneimine (PEI) that proved to be effective in the remediation of Hg contaminated seawater. Nevertheless, no studies have been made to evaluate the toxicity of the remediated seawater and the possible effects in aquatic organisms after the remediation treatment under high temperature. For this, the present study intends to assess the possibly toxicity of seawater, previously contaminated with Hg and remediated by GO-PEI, using the clams *Ruditapes philippinarum* under actual and predicted warming scenario. The results obtained demonstrated that seawater contaminated with Hg and/or Hg+GO-PEI induced higher toxicity in clams exposed to 17 and 22ºC compared to organisms exposed to remediated seawater at the same temperatures. Moreover, similar histological, physiological and biochemical alterations were observed between organisms exposed to control and remediated seawater, independently on the temperatures (17 and 21ºC).

1.14PC.2

Assess environmental toxicity risks in running waters by exploiting functional morphological markers in Hydra vulgaris Pallas, 1766

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The European Water Framework Directive (WFD) requires to assess the health status of freshwaters by the development and application of environmental monitoring tools. Thanks to its biological characteristics, Hydra contributes to assess superficial running water quality. Most of studies on Hydra are mainly focused on toxicological tests but, since last decade, scientific papers take into account more frequently the application of Hydra assays also for evaluating environmental toxicity status, in particular in freshwater habitats. In this work, Hydra vulgaris Pallas, 1766 is tested as early warning system for detecting teratogenic effects in running freshwaters by exploiting its fast regeneration ability. We are suggesting a modified version of Hydra assay based on morphological biomarkers for proposing a novel version of the Teratogenic Risk Index (TRI). The new TRI matrix shows 5 qualitative classes (from very high teratogenic risk=5, to no risk=1) and is applied for the first time for monitoring teratogenicity of Latium (Italy) running waters. Results point out a general low teratogenic risk, focusing also on some criticisms. In addition, the resulting TRI classes were compared to the outputs from biotic indices of the WFD, showing not correlating results. If compared to chemical analysis, TRI has two major advantages: it provides information on the chemical's cumulative effects on biota in the natural mixture (also if their concentration in waters are below the impacting threshold) and it is economical. Usually, the synergy among all chemicals in running freshwaters cannot be tested in laboratory by lack of time and resources. Accuracy of TRI results will be conducted by a comparison between H. vulgaris and zebrafish embryos, and it is proposed to timely identify teratogenic hot spots. This work emphasizes the role of Hydra as a good biological model in the water quality assessment activities. Moreover, the Hydra assays have rising potentials to keep the attention high on the impact of emergent contaminants on both habitat and human health.

1.14PC.3

• A new Decision Support Tool for managing the impacts of blue spaces on human health and well-being in the context of BlueHealth project

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BlueHealth EU project, funded by Horizon 2020, has the aim to understand links between exposure to blue space and health and well-being through a large-scale systematic programme of interdisciplinary research. During this project a Decision Support Tool (DST) has been developed for facilitating the evaluation of blue space effects on human wellbeing. The DST guides users through a series of steps to identify the key health risks and benefits of a given blue space project/activities. It is organized in consequential sections: Introduction, Choice of blue space typology (ie marine front, river, blue in green space), Selection of "targets" (Human health protection and minimize risks, Promote human health benefits, Promote ecosystem benefits), and Individuation of "subtargets". The output of DST is a report, containing a list of statements describing risk and benefits. DST was firstly applied in Appia Antica Park in Rome for a wild wetland requalification project through the creation of a small pond, pathways and benches. This project aimed to improve the access and use to citizens of this area aiming to enhance their mental and physical activities. "Targets" selected were, "Promote human health" and "Improve the Ecosystem services" with following subtargets "Nature experience", "Sociocultural interaction", "Noise reduction", "Shore activities, "Local climate regulation", "Habitat restoration". DST provided a total of 49 indications: for "Promote health" pertinent benefits for this project referred to the reduction of heat stress, thanks to the realization of seating or shelter in shaded areas, to the decrease of noise exposure's impact with natural water sounds, or to the promotion of social interaction with accessible open space. For "Improve ecosystems service" the more relevant indications have been focused on protection of wildlife, for instance limiting the access seasonal or permanent to some areas, identifying and managing invasive species. Furthermore, DST analysis focused on maintenance of infrastructures realized, and management of the blue and green space. This application showed how Bluehealth DST provided e a complete and exhaustive list of risk and benefits, to be considered for this project development.

1.14PC.4

Towards Assessing Tobacco and Cannabis Contaminants in Protected Areas

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Parks and nature preserves are protected areas used for recreation; they also provide ecosystem services, for example bee pollination, maintenance of food webs, and nutrient recycling for plant growth. Yet, depending on their location, protected areas can be contaminated by pollutants that negatively impact ecosystem services. Here, an environmental forensics approach is developed and used to assess tobacco product and *Cannabis* (marijuana) use and cultivation as contributors of related contaminants affecting protected areas. The research questions are: Where are do conventional cigarette butts and e-cigarette debris accumulate, and where is *Cannabis* cultivated and used, near protected areas? How does associated contamination—such as cigarette butts, e-cigarette debris, and dissolved chemical residues—relate to proximate urbanization? What chemicals (e.g. nicotine, cotinine, cellulose acetate fibers, and cannabidiols) evidence tobacco and *Cannabis* pollution entering protected areas via surface waters, drains, and nearby sewers? The approach starts by synthesizing published evidence for tobacco product and *Cannabis* contaminants affecting protected areas. Next, site-specific information is gathered for hypothesizing geospatial factors—including cigarette butt and e-cigarette debris counts, and *Cannabis* cultivation and dispensary locations—influencing tobacco and *Cannabis* contaminant sources. Field sites are University of California Natural Reserve System (NRS) protected areas ("Reserves") that might be contaminated, given surrounding land uses, businesses, infrastructure such as roadways and sewers,

population size and makeup, regional climate conditions, and aspects internal to the Reserves. Field sampling of water, soil, and sediments is conducted, and preliminary results are presented from analyzing samples for tobacco product or *Cannabis* (cultivation and use) pollutants. Results are compared to estimated contaminant sources based on geospatial factors, as a test of the overall approach. This research is a foundation for future studies of tobacco product and *Cannabis* (cultivation and use) effects on protected areas broadly, which can inform new policy arguments in tobacco control and *Cannabis* cultivation and use.

Chemical Safety Assessment of Surfactants: Current Challenges in Regulatory Science and Future Prospects (PC)

1.15PC.1
Bioconcentration of Anionic Surfactants in Fish
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Anionic surfactants are widely used in household, consumer and industrial products. Many of the uses are in down-the-drain products, which means that anionic surfactants are discharged in large quantities to wastewater from where they can reach the natural environment either via direct discharge or via WWTPs. Consequently, accumulation in fish is relevant. There are, however, few measurements of bioconcentration factors (BCFs) in fish. In this work we measured the BCF of 10 anionic surfactants in juvenile rainbow trout. The test chemicals included 5 sulfonates, 2 sulfates, 1 linear alkylbenzene sulfonate, 1 lauryl ether sulfate, and dioctyl sodium sulfosuccinate. They were combined in a mixture that the fish were then exposed to in a single experiment. The experiments were conducted according to OECD 305 with modifications. A pre-test was conducted to estimate the elimination half-life. Based on the results, the exposure phase was limited to 4 d, while the clearance phase was 7 d. Fish and water sampling were conducted hourly at the beginning of the exposure and clearance phases. Triplicate water samples and triplicate fish samples were analysed at each time point. No adverse effects of the chemicals on fish health were observed. The concentrations of the test chemicals in water were relatively constant during the exposure experiment, and they decreased at least 2 orders of magnitude lower than during the exposure phase. All of the test chemicals approached a steady state concentration in the fish during the exposure phase, with the exception of the chemical with the longest alkyl chain, C16SO3. Rapid first order elimination kinetics were observed for most of the test chemicals. BCFs for most test chemicals with the exception of C16SO3 were < 100 L/kg. This dataset will provide insight into the magnitude of anionic surfactant bioaccumulation, the underlying mechanisms controlling it, and how it varies with the structure of the surfactant.

1.15PC.2
Development of coarse-grained simulation methods to study membrane partitioning
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Within a risk assessment, the partitioning between n-octanol and water (log KOW) is commonly used as a surrogate for estimating passive partitioning through cell membranes and predicting chemical absorption, distribution and accumulation through biological and environmental systems such as skin, tissue-plasma, or aquatic organisms, as well as for toxicity prediction. Whilst the use of log KOW provides good estimates of membrane partitioning for small, neutral organics, the specific interactions of polar, charged, or amphiphilic compounds with the ordered three-dimensional structure of biomembranes cannot be adequately described by isotropic solvent. The situation becomes even more complex for surface-active compounds such as surfactants which, due to their amphiphilic properties, accumulate at interfaces making empirical measurement of log KOW difficult. There is considerable evidence in external literature to suggest that predictions of partitioning in biological systems for such chemicals can be improved by using membrane-water partitioning (log KMW) in place of log KOW. A number of theoretical approaches for calculating log KMW, such as COSMO-RS theory, used in commercially available software, have been developed as an alternative to experimental methods which are often expensive or time-consuming. While these theoretical methods work well for simpler molecules, they often perform less well in more complex cases, such as large or ionic molecules. We have therefore developed a molecular simulation-based method for calculating log KMW. Such an approach can be used to directly model the behaviour of a solute molecule within a lipid bilayer, and so is able to capture more of the effects which influence partitioning. Our approach uses coarse-grained simulations, which are far more computationally efficient than all-atom simulations, allowing for rapid screening of a large number of molecules. Within this presentation, the simulation method is described, and results are compared to those from other computational and experimental methods.

1.15PC.3
Surfactants: Substances of Concern? ECHA's current challenges in safety assessment
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Surfactants represent a group of high-impact chemicals due to their widespread professional and household uses, resulting in direct and indirect exposure to the environment. ECHA is facing reoccurring problems with REACH registration dossiers for surfactants:

- Physico-chemical data are often unreliable, e.g. water solubility is reported without taking into account the critical micelle concentration as the solubility limit, the values are approximated based on visual inspection only, or the surface tension is waived altogether.
- Biodegradation screening studies often suffer from deficient data on study details such as: insufficient information on the used inoculum (e.g. pre-adaptation), the test substance is toxic to the inoculum, or the substance adsorbs to test vessels and matrix.
- Experimental data from bioaccumulation studies is scarce since the study requirement often was waived with low Kow, in the meantime recognised as a poor predictor for the bioaccumulation potential of for surfactants and ionisable substances.
- Submitted data from aquatic toxicity studies may be unreliable due to sorption of the surfactants to test organisms, matrix and test apparatus. Also, some aquatic toxicity studies apply the 'bulk approach', using increased concentrations of dissolved and/or particulate organic matter, which in turn acts as sorption sink and therefore decreases the bioavailable fraction of sorptive test substances, resulting in underestimation of toxicity.

ECHA recognises that the assessment of surfactants is open to review. With recent scientific development, especially regarding the behaviour of surfactants in complex matrices, the P & B properties of selected surfactants may be re-assessed, which could potentially lead to the identification of some of them as substance of concern. We use illustrative examples from data dossier to highlight the dossier data deficiencies and to discuss ways to reduce them. Furthermore, we demonstrate, how ECHA's approach of grouping structurally similar substances can be applied to surfactants in order to a) spot substances of concern (SoC) in the chemical universe, (b) allocate them to the correct priority pool and c) to correctly decide on further actions such as data generation/assessment or regulatory risk management.

1.15PC.4
Using rainbow trout S9 clearance rates as first W-o-E for biotransformation potential for surfactants
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Surfactants are experimentally challenging chemicals to work with, as they may: accumulate at all kinds of interfaces, occur in complex mixtures, readily biodegrade, and fall outside the applicability domain of test guidelines and models. In particular, there are often no or highly uncertain octanol-water partition coefficients (Kow) for surfactants. In the CEFIC-ECO37 project we focused on series of individual surfactant structures and measure cell membrane-water partition coefficients (Kmw) to potentially replace Kow model parameters, and rainbow trout (RT)-S9 hepatic clearance rates to determine the potential impact of biotransformation on fish bioconcentration factors. Trends in Kmw and RT-S9 with surfactant structure have been identified that allow for extrapolations to analogue structures within various surfactant types. For RT-S9, rapid clearance was observed for C_9-C_{13} homologues for alkylsulfates, alkylsulfonates, alcohol ethoxylates, tertiary alkylamines and secondary alkylamines. For C_{14}-C_{18} homologues of these classes, however, strongly reduced or even insignificant RT-S9 clearance was measured. Of course, that is problematic because the longer chain homologues have the highest tissue binding affinities, and BCF values above the PBT thresholds could result in the absence of biotransformation. This absence of in vitro clearance in the RT-S9 assay is either due to enzymatic limitations, or due to systematically reduced accessibility in the cellular matrix, or a systematic artefact in the test conditions of the RT-S9 assay. We discuss each option with examples, and provide an outlook for Weight of Evidence (W-o-E) options that could be followed in risk assessment schemes. Remaining challenges of the approach with *in vitro* biotransformation assays, e.g. in terms of QIVIVE and surfactant chemical domains, will also be discussed.

Improving Exposure Characterization of In Vitro Testing to Support Quantitative Extrapolations to In Vivo Systems (PC)

1.16PC.1

Can TK-TD modelling bridge the gap between in vitro and in vivo mammalian toxicity data?

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Using toxicokinetic-toxicodynamic (TK-TD) modelling it is possible to estimate internal toxicant concentration over time and link this directly to observed effects on growth rate of laboratory rats. Repeated dietary dose testing is used to assess longer term toxicity of chemicals, such as pesticides, to mammals. However, the internal pesticide concentration varies significantly as feeding rate relative to body size fluctuates over time. We used TK-TD models to estimate the effects that would be caused by a constant internal concentration of a pesticide. This presents the possibility of comparison with data from *in vitro* experiments - in which only a constant exposure can be achieved - to investigate the potential for *in vitro* to *in vivo* extrapolation. While *in vivo* TK-TD models were used to identify relevant internal concentrations, *in vitro* TK models estimated the experimental conditions required to replicate them in cultured cells. Cell population growth could then be monitored with a view to extrapolating through time and comparing with *in vivo* predictions. A similar approach has proved successful with fish cells however, accounting for the fluctuating exposure in mammalian testing presents a major additional hurdle. In our study, observed cell proliferation did not show a significant pattern with the pesticide concentration for any of the five compounds included and so it was not possible to extrapolate to effects on growth rate *in vivo*. However, this study demonstrates how TK-TD modelling can play a vital role as a bridge between *in vitro* and *in vivo* data. We also highlight areas for future work toward the goal of *in vitro-in vivo* extrapolation of graded sublethal effects. In particular, changes to data collection in regulatory toxicokinetics studies may improve the accuracy of TK models and so greatly aid further progress.

1.16PC.2

Quantification of concentrations of organic ions in 96-well plates for improved exposure assessment in cell-based bioassays

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The freely dissolved concentration in the assay medium ($C_{free,medium}$) is considered a better metric of effective concentrations in cell-based *in vitro* bioassays than the nominal concentration (C_{nom}), because it corrects for binding to medium components and can directly be compared to freely dissolved plasma concentrations for quantitative *in vitro*-to-*in vivo* extrapolation. In this study, $C_{free,medium}$ was determined experimentally for 15 chemicals in two cell-based *in vitro* bioassays, the AREc32 assay that tests for oxidative stress response and the PPARγ assay that detects chemicals that activate the peroxisome proliferator-activated receptor gamma (PPARγ). Aliquots of bioassay medium were sampled at the time point of dosing and after 24 h of incubation and $C_{free,medium}$ was measured for ten chemicals using C18-coated solid-phase microextraction (SPME) fibers. All chemicals were found to be cytotoxic in the AREc32 assay and twelve of the test chemicals were cytotoxic in the PPARγ assay. Three chemicals activated the oxidative stress response (propranolol, genistein, labetalol) and nine chemicals activated PPARγ (caffeine, lamotrigine, diclofenac, 2,4-dichlorophenoxyacetic acid, naproxen, ibuprofen, torasemide, warfarin, telmisartan). For the hydrophilic chemicals caffeine and lamotrigine C_{nom} and $C_{free,medium}$ were similar in both assays and consequently the nominal and freely dissolved effect concentrations for cytotoxicity ($IC_{10,nom}$ and $IC_{10,free}$), and activation of PPARγ ($EC_{10,nom}$ and $EC_{10,free}$) were similar (within a factor of 1.7). For the acids naproxen, ibuprofen, warfarin and diclofenac $C_{free,medium}$ was close to C_{nom} at high concentrations, leading to similar $IC_{10,nom}$ and $IC_{10,free}$ (within a factor of 4). In contrast, $EC_{10,nom}$ and $EC_{10,free}$ of the organic acids differed by up to a factor of 11, because the free fraction was found to be lower at low concentrations. A mass-balance model was used to predict $C_{free,medium}$ and agreed well with the experimentally determined $C_{free,medium}$ for the hydrophilic chemicals and the acid genistein (RMSE< 0.3 log-units). Non-linear binding to assay medium was found for six of the 15 test chemicals in at least one assay. For these chemicals the mass-balance model underestimated $C_{free,medium}$ (RMSE up to 2.4). The results of this study emphasize the need for experimental exposure assessment as many processes such as saturation of specific protein binding sites cannot be covered by currently available prediction models.

1.16PC.3

Transfer of organic chemicals across the fish intestinal epithelium: an experimental and computational study

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We tested the transfer of eleven chemicals across the rainbow trout intestinal epithelium, using the RTgutGC cell line and a newly developed exposure chamber, TransFEr, specifically designed to work with hydrophobic and volatile chemicals. A combined experimental and modeling approach, which included MS-based quantification of the chemicals in the different compartments of our setup and ODE-based mathematical modeling of chemical transport across compartments, allowed us to determine the dominant routes of transfer for most chemicals, determine intestinal biotransformation rates and define the optimal time points for taking samples for chemical analysis. Against the current assumption in chemical uptake modeling, chemical transfer across the rainbow trout intestinal epithelium did not strictly depend on the logKOW but resulted from chemical-specific intracellular accumulation and biotransformation combined with paracellular and active transport. The test system can be transferred to other cell-based barrier systems, such as the fish gill or mammalian intestinal models and may improve in vitro–in vivo extrapolation and further refine the prediction of chemical bioaccumulation into organisms, taking into account for extra-hepatic biotransformation.

Advancing Use of Behavioural Studies in Regulatory Assessment of Contaminants (PC)

2.11PC.1

Behavioural effects of the pharmaceutical fluoxetine on freshwater invertebrates: a comparison of toxicity between the laboratory and the field.

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Fluoxetine is one of the worlds' most prescribed antidepressant and is frequently found in surface waters all over the world. However, little is known about the effects of fluoxetine on the behaviour of freshwater invertebrates, a functionally diverse and ecologically important group of aquatic organisms. Usually, lab studies are performed to assess the effects of widely spread and frequently used chemicals on organisms potentially exposed to it. However, conditions in the lab are strictly controlled, and are therefore hard to compare to field conditions. We hypothesise that the chemical response of organisms under laboratory conditions will differ from the response found under more-realistic semi-field conditions. We test this using the ecologically relevant freshwater invertebrate *Gammarus pulex* (Crustacea, Amphipoda). We compare the behavioural response of *G. pulex* after short- and long-term exposure to different concentrations of fluoxetine (0.2, 2, 20, 200 µg/L) under laboratory conditions, to similar experiments performed in in-situ cage experiments under semi-field conditions. Our main endpoint is swimming velocity, which represents behaviour with a potential impact on community structure through adjusted predation susceptibility. Additionally, we also study effects on growth and feeding rates. Under semi-field conditions, we found large differences in swimming velocities between the acute- and chronic exposure, where activity in the chronic exposure had reduced significantly. In the lab, differences between acute- and chronic exposure were less apparent. Differences between the lab and the field for the acute exposure were small. Significant effects of fluoxetine concentrations were only found under lab conditions, and solely for intermediate fluoxetine concentrations (2 and 20 µg/L). Overall, we conclude that differences between lab and semi-field exposure could primarily be attributed to differences in habitat structure provided in the experimental setup. This leads us to question whether standard toxicity tests stimulate baseline behaviour of the organisms under study, or that current test-designs actually cause a continuous stress response. Our results highlight the need for understanding baseline behaviour of test organisms better.

2.11PC.2

Impact of the two antidepressants citalopram and venlafaxine on brown trout (salmo trutta f. fario)

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In Germany, citalopram and venlafaxine are the most frequently prescribed antidepressants. They belong to the selective groups of antidepressants called

SSRI (selective serotonin reuptake inhibitors) and SSNRI (selective serotonin and noradrenalin reuptake inhibitors). Due to high consumption rates and poor elimination rates both antidepressants are frequently found in surface waters. Since, effects of these antidepressants on aquatic wildlife are not yet properly understood, we investigated the consequences of exposure to either venlafaxine or citalopram in brown trout at different life stages. For this, brown trout eggs in the eyed ova stage were exposed to 0, 1, 10, 100, 1000 µg/L venlafaxine and citalopram in a semi-static three block design at 7 and 11°C for 5 months. Juvenile brown trout were exposed in a similar design for 4 weeks at 7°C. Endpoints like mortality, weight, length and for brown trout larvae only, hatching rate and heart rate were assessed. Additionally, behaviour during the exposure and in a stressful artificial swimming measurement device was recorded. Furthermore, the biochemical markers superoxide dismutase, acetylcholinesterase, hsp70 level and cortisol content were examined as well as histopathology of the liver. Also water concentrations of both substances and tissue concentrations of citalopram were analysed. Nominal water concentrations complied with real concentrations and tissue accumulation of citalopram was much stronger in juvenile fish compared to larvae. It was shown that an exposure to either venlafaxine or citalopram has a strong impact on behaviour of brown trout larvae and juveniles, resulting in an increased swimming activity and reduced anxiety of the fish. Related to this, both antidepressant showed to reduce growth in brown trout larvae and citalopram even in juveniles. Both antidepressants neither had any influence on any biochemical marker nor on mortality, hatching rate and heart rate. The effects on liver histopathology are being assessed at the moment. In general, the results of this study make clear that both antidepressants have strong effect on behaviour and related traits in brown trout disregarded of the life stage of the fish. The present study is part of the project Effect-Net which is funded by the Ministry of Science, Research and the Arts of the Land of Baden-Württemberg within the framework of the Water Research Network Baden-Württemberg.

Impacts on Agroecosystems Resulting from Chemical Exposure in the Anthropocene (PC)

2.12PC.1

Investigation on the impacts of raw and bioremediated olive oil mill waste on agroecosystems

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Agricultural practices, such as olive growing and related olive oil extraction processes can impact agroecosystems due to the large amounts of olive mill waste (OMW) that are produced and spread on land. The main aim of our study was to investigate the effects of raw olive mill wastewater and olive mill semi-solid waste on different components of agroecosystems. OMW (olive mill semi-solid waste and olive mill wastewater) were collected at two different oil mills in Tuscany and subjected to a bioremediation process based on bioaugmentation. Fungi strains able to metabolize polyphenols were isolated from OMW, grown and added to olive semi-solid waste or olive mill wastewater, composted with chopped straw and olive leaves. Polyphenol concentrations and pH were detected in OMW before and during bioremediation process which last 6 months. Specimens of *Eisenia fetida* and *Gambusia affinis* were exposed to both kinds of OMW, before and after the bioremediation treatment. A set of biomarkers was applied to investigate eventual neurotoxic affects (AChE inhibition), oxidative stress (LPO, catalase), and genotoxicity (Comet and ENA assays) of raw and bioremediated OMW. Mortality was also measured. Maize seeds were differently treated by incubating seeds with either water and raw or bioremediated olive mill wastewater, at different times, Germination Index was computated. Tobacco and tomato plants were grown in pots in presence of raw or bioaugmented OMW. Photosynthesis-related molecules, such as content of RuBisCO (ribulose-1,5-bisphosphate carboxylase oxygenase) and of the photosynthetic pigments lutein, chlorophylls, and β-carotene were investigated in tobacco. Growth tests were carried out on tobacco and tomato plants. This study provides valuable information on the potential impact of OMW on agroecosystems. In particular, negative effects were found both on animals, such as mortality, neurotoxicity, oxidative stress and genotoxicity, and on plants such as inhibition of germination and root growth, alterations of photosynthetic parameters and plant growth. OMW is a complex mixture in which surely the high levels of polyphenols play a key role for toxicity, but the possible presence of other chemical compounds used in agriculture should not be overlooked. The bioaugmentation treatment in which the decrease in the levels of polyphenols and acidity was highlighted resulted in a net decrease in toxicity and the new byproducts obtained can be proposed as fertilizers.

2.12PC.2

Pharmacodynamics of Psychoactive Pharmaceuticals and Plants

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A wide range of pharmaceuticals detected in treated wastewater are introduced into the agricultural environment. Thus, their interaction and affect on plants are of increasing concern. The research aimed to examine the affect of psychoactive compounds on plant by measuring the translocation and accumulation of nutrients in tomato and cucumber plants. The study examined the pharmacodynamic relationship between the concentration of drug and the effect on nutrient homeostasis. Plants were grown in hydroponic solutions and exposed to Carbamazepine, Diazepam and Lamotrigine at concentrations of approximately 0.02, 0.2, 1 and 4 µM or to mixture of all three compounds at the different concentrations. Following exposure plants were decapitated and connected to a pressurized suction-based xylem sap collecting system. The xylem sap was analysed along with the nutrient solution to normalize results as the Transpiration Steam Concentration Factor (TSCF). The xylem sap, nutrient solution, leaves and roots of plants were all analysed for nutrient concentrations. The exposure to the psychoactive pharmaceutical exhibited a concentration dependent trend on the translocation of specific nutrients. In the cucumber plant iron, phosphorous and copper were all negatively affected by the exposure to all three compounds (Carbamazepine, Diazepam and Lamotrigine), with xylem sap concentrations reducing to 20% of the control. In tomato plants, similar trends were observed for iron, phosphorous and copper as well as other nutrients such as zinc, molybdenum and sulfur. Lamotrigine showed a greater effect on a range of different nutrients as compared to Carbamazepine and Diazepam. Tomato and cucumber plants were also exposed to a mixture of all three compounds at different concentrations. Results showed that mixtures have a greater affect in comparison to individual exposure and suggest a synergistic effect rather than simply being an additive effect. The reduced xylem sap concentrations of iron, phosphorous and copper was observed at environmental relevant concentrations. The results suggest possible interaction at the molecular level that may influence the homeostasis of copper and Iron and effect phosphate metabolism.

2.12PC.3

Comparative metabolomic analysis of plant responses to stress including drought and pharmaceutical exposure

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The agroecosystem is frequently subjected to biotic and abiotic stress events with increasing frequency and from multiple sources in the Anthropocene. Among these are increasing droughts brought upon by a changing climate. In response to drought conditions and limitations in freshwater resources, reuse of wastewater for crop irrigation has become increasingly necessary. However, wastewater from both domestic and agricultural sources is composed of medicinal residues, including antibiotics, excreted by humans and livestock and inefficiently removed in treatment processes. Increasing pharmaceutical exposure represents an emerging stressor that characterises the Anthropocene. Here we show how high-resolution mass spectrometric analysis of the extractable plant metabolome can be used to evaluate the response of crops to multiple agroecosystem stressors. First, we show the impact of drought stress on both drought tolerant (B-76) and susceptible (B-73) maize hybrids. Then we apply the approach to evaluate the impacts of environmentally relevant mixtures of 11 antibiotic compounds in irrigation water on barley grain. An untargeted study of the water-soluble metabolites was performed to identify features that undergo changes as due to drought stress. A dataset of full scan positive ion mode LC-MS data, collected using Bruker SolariX FTICR MS, was investigated using statistical techniques (PCA and LDA). The features causing variance between watering regimes will be investigated. Antibiotic exposure did not result in clear groupings based on exposure. Further analysis will reveal what, if any, changes in biochemical expression resulted from increasing antibiotic exposure. The agroecosystem faces increasing stressors both climatically and as a result of increased human interaction in the Anthropocene. Providing reliable and sustainable food production in the face of rapidly increasing global population remains one of the most serious challenges we face. Understanding the impacts that stressors have on plants is necessary to evaluate conventional and emerging farming practices. Metabolomic analysis provides a high throughput pool to provide insight into plant response to stress.

A Risky Life History: Contaminant Threats to Scavenging Wildlife (PC)

2.13PC.1

Anticoagulant rodenticides in predatory birds: probabilistic characterisation of toxic liver concentrations - Part II

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Anticoagulant rodenticides dominate pest rodent control globally. Second generation anticoagulants (SGARs) are persistent, bioaccumulative and toxic chemicals that have become widespread contaminants of terrestrial birds of prey. We present data on concentrations of anticoagulants and autopsy results for 608 raptorial birds found dead or brought into rehab, 1988-2018 in British Columbia. Larger generalist owls, e.g. barred owl (*Strix varia*) and great-horned owl (*Bubo virginianus*), had the greatest incidence of exposure; smaller owls e.g. barn owl (*Tyto alba*) had lower incidence , as did hawks, e.g. red-tailed hawk (*Buteo jamaicensis*) and Cooper's Hawk. Bird eating merlins (*Falco Columbarius*) had lowest incidence of exposure among diurnal raptors. This presentation focuses on updating our determination of the probability of lethal AR poisoning, which increased above 0.1 ug/g SGAR in liver, and rapidly above 0.5 ug/g. To determine those values, we modeled autopsy and liver residue data, which showed barn owls to be the most sensitive to SGAR poisoning, while great-horned owl least. Over the study period, the incidence of exposure to SGARs increased from 1988 onwards, remaining consistently high (above 75%) from 2003- 2009, then declining from 2009-2018, which coincides with introduction of regulatory measures in Canada to reduce usage of the most toxic SGARs. Vehicle collision was the most common cause of death for all raptors, followed by trauma, undetermined cause, emaciation, and AR poisoning, which was more common than window collision.

2.13PC.2

Monitoring NSAIDs in carrion and vultures after diclofenac registration for veterinary use in Spain

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The severe impact of diclofenac in Asian vultures due to its veterinary use has been one of the biggest ecological catastrophes of the last decades, pointing out the deficient environmental risk assessment of some of these compounds. In the case of diclofenac, there was an evident gap in the knowledge of the high toxicity that this non-steroidal anti-inflammatory drug (NSAID) has in Old-World vultures that can feed on treated livestock (LD50 in *Gyps bengalensis* of 98-225 µg/kg body mass). Despite this fact, diclofenac was registered for livestock treatment in Spain in 2013, a country that hosts the largest population of European vultures. In this study we have evaluated the risk of exposure to diclofenac and ten other NSAIDs in vultures after its commercialization in Spain. With this purpose we sampled 156 pig, 25 sheep and 2 bovine carrion intended for vulture consumption in feeding stations. In addition, we sampled 183 vulture carcasses regarding the four target species: Eurasian Griffon vulture (*Gyps fulvus*) (n = 160), Cinereous vulture (*Aegypius monachus*) (n = 8), Egyptian vulture (*Neophron percnopterus*) (n = 7) and Bearded vulture (*Gypaetus barbatus*) (n = 8). Carrion samples (muscle, liver and kidney) and vulture samples (liver and kidney) were analysed by liquid chromatography coupled to mass spectrometry (LC-MS-QTOF). Five of the pig carrions analysed (3.27%) had NSAID residues, specifically flunixin (n = 2, 1.31%), diclofenac, ketoprofen and meloxicam (n = 1, 0.65%, each). Six of the Eurasian griffon vultures analysed (3.72%) showed NSAID residues, specifically flunixin (n = 4, 2.48%) and meloxicam (n = 2, 1.24%). Two vultures with high levels of flunixin in kidney and liver had visceral gout lesions and another one presented signs of renal failure, suggesting an association with NSAID intoxication. Residue levels in carrion and scavengers indicate a limited risk of poisoning. However, veterinary use of NSAIDs can still be a threat for scavengers if veterinarians and farmers ignore the associated risk when treated livestock is used to feed vultures.

2.13PC.3

Barbiturate poisoning in scavengers in Spain

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Avian scavengers are exposed to a wide variety of contaminants through carrion consumption. In many cases, carrion comes from animals which died as consequence of an infectious diseases or physical injuries, but it is also common that scavengers feed on carcasses from previously poisoned animals. In this context, scavengers may suffer secondary poisonings that can have lethal effects. Moreover, veterinary drugs like anti-inflammatories or antibiotics used in livestock treatments are expected to reach scavengers through diet because they are commonly used in sick animals with high chances of dying during the treatment. This explains the huge impact of diclofenac on the Asian vultures by the end of the 20th century. Euthanizing drugs are another pharmaceutical group which can easily affect scavengers if euthanized animals are not properly disposed for carrion destruction. In both cases, carrions containing non-steroidal anti-inflammatory drugs (NSAIDs) and euthanizing drugs used in treated animals, should never be available to scavengers. Here we present the cases of barbiturate poisoning detected in wildlife in Spain between 2004 and 2019, which have been recorded within the monitoring performed by a laboratory of forensic wildlife toxicology for the diagnosis of intentional and accidental poisonings. In this study, we have analysed of 2849 suspected cases of wild and domestic animal poisonings in the natural environment (3846 animals and 925 baits). Analyses have been performed following a protocol that covers the detections of the different families of chemicals involved in animal poisonings by using GC-EI-MS, LC-ESI-MS, LC-QTOF-MS and GF-AAS. Poisoning was confirmed in 1107 cases (38.8%) by chemical analyses. Barbiturate poisoning was confirmed by GC-EI-MS in full-scan mode in 25 cases (2.3% of the confirmed poisonings) that affected 42 animals. These cases have been divided in three categories: intentional poisonings (n=6, 14.3%, including some baits), accidental poisonings (n=32, 76.2%) and euthanasia or veterinary treatments (n=4, 9.5%). It is remarkable the presence in the last decade of several cases affecting griffon and Egyptian vultures that can respond to the disposal of carcasses in the field or in garbage dumps of animals that have been euthanized with barbiturates, mostly with pentobarbital.

2.13PC.4

Causes of death in Red Kites (Milvus milvus) in the Pyreneans in France : impact of poisoning during a 13-year survey (2005-2018)

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Red kites are a unique European raptor species, listed in the IUCN Red list of species ("vulnerable"). As an opportunistic scavenger species, it may be exposed to many sources of chemicals, including illicit poisoning. A specific National Action Plan and a European program have been developed to help protect this species. In the Pyreneans, a network has been established in 2005 to collect all birds found dead. Each bird undergoes X-Ray analysis and a complete necropsy. Whenever possible, crop/gizzard contents, liver and kidney samples were collected for systematic toxicological investigation. The following chemicals were analysed : carbamate and organophosphate insecticides, organochlorine insecticides, anticoagulant rodenticides, Pb, Cd. Cu was surveyed between 2005 and 2012. Toxicant concentration was compared to published results for similar species to classify cases as potential poisoning or exposure cases. For each individual, all results (necropsy+tox analysis+circumstances) were considered before ascribing a definite cause of death.A total of 272 scavengers (vultures and Kites) have been collected, among which 81 Red kites. Poisoning was the primary cause of death in 17 birds and 32 birds had at least evidence of high exposure to one or more chemical. Median Cd concentrations in the liver/kidney appear high compared to many other raptor species. Statistical association of some causes of death appear strongly associated with the presence of some chemicals and would suggest more investigation (collision/electrocution and high lead exposure).

New Methods in Environmental Analytical Chemistry: From Interrogation of Complex Matrices to Innovative Tools for Monitoring (PC)

3.14PC.1

Characterization of the endocrine disruptors pollutant load according to the size of air particles

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Since the advent of industrial activity, emissions in the atmosphere of anthropogenic-induced molecules have diversified and intensified, leading to an increase in air pollution, to which humans are permanently exposed, especially in urban areas. The particulate phase is believed to be the main cause of health-related effects due to air pollution exposure. Thus, the conformation of particles influences the particles toxicity by their granulometry and composition; the finer particles are, the deeper they can penetrate into the respiratory tract. The diameter of Particulate Matter (PM) currently regulated is less than 10 µm (PM_{10}) and less than 2.5 µm ($PM_{2.5}$) (reaching the upper respiratory tract and pulmonary alveoli respectively). In addition, particles consist of an inorganic fraction and an organic fraction which can adsorb biological materials or semi-volatile organic compounds, several of them being suspected or known endocrine disruptors compounds (EDCs). This project aims to study the link between particle size and their composition as bioactive endocrine disruptors, during a cold season in Paris (urban areas), and, to characterize the potential health-effects resulting from the contamination by EDCs. Since January 2019, particles belonging to 3 size classes (Total Suspended Particles, PM_{10} and $PM_{2.5}$) have been collected at 3 sites. The collected PM was chemically analyzed for a wide range of endocrine disruptors (59 compounds) by gas chromatography and liquid chromatography associated to mass spectrometry. This characterization was made by particle type, depending on the size of particulate matter collected. To evaluate the potential biological effects of our samples, some tests were conduct to identify the bioactivity of the extracted particles, using various *in vitro* bioassays (transactivation tests for estrogenicity, anti-androgenicity or dioxin-like effect, genotoxic effect). Considering the distribution in endocrine disruptors, the first results we obtained suggest that, the finest fraction of the particles collected ($PM_{2.5}$) supports the highest concentrations of organic pollutants sought, with a predominance of phthalate compounds. And preliminary bioassay results shows that it is also this fraction that has the most pronounced endocrine disrupting effect. These results demonstrate that knowledge of the chemical composition and physical properties of particles is important in order to accurately determine their health impact.

3.14PC.2

Game of Unknowns: Non-Target Analysis strategies for identifying emerging PFAS in Water

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Per/Polyfluoroalkyl substances (PFAS), are compounds that have uniquely desirable properties for use in various industries. However, their wide-ranging use leads to emission into the environment, and as PFAS are persistent and bioaccumulate in the environment and wildlife, they are contaminants of concern. Monitoring PFAS precursors present in an environmental sample may impact decisions in treatment processes at remediation sites and help deduce possible degradation products that could exist in the environment. Consequently, scientists are contributing newly identified PFAS structures and spectra to various publicly available databases: growing the list of precursors and degradation by-products, some listing thousands of PFAS. Traditionally, methods such as USEPA 537 and ASTM 7979 are designed to monitor a small and discrete number of PFAS compounds, thought to be end-products of degradation processes occurring in environmental systems. LC-MS/MS technology is usually employed to quantify commonly monitored PFAS end-products. Without standards, adding target compounds to an LC-MS/MS method is restrictive and it would be logistically difficult to monitor all possible PFAS without knowing them and having standards. LC-QTOF technology allows the simultaneous quantification of commonly monitored PFAS whilst acquiring untargeted data that can be screened for suspected PFAS precursors. The non-target nature of the data acquired also allows for retrospective detection of new PFAS as the scientific community learns more about these emerging contaminants. The total fluorinated compounds in a sample may be underestimated by not monitoring the precursor compounds of which these compounds are formed from. Sample preparation techniques such as the Total Oxidizable Precursor (TOP) Assay attempt to measure the total fluorinated compounds by forcing degradation of precursors into measurable end-products. This technique is time consuming and may not degrade all precursors into measurable end-products. Additionally, some countries decided to phase out specific classes of PFAS manufacturing and use, which has led manufactures to find alternative classes of PFAS, leading to new precursors and degradation products being found in environmental samples. This study uses a simple dilution (with MeOH) and acidification extraction of non-potable water samples to quantify ~20 commonly monitored PFAS and determines the accuracy, recovery and estimated limits of detection on an LC-QTOF. Further, the ability to collect untargeted data allowed us to screen against a custom database of PFAS compounds to identify additional PFAS that were not in our original target list without the need for standards or re-injection of the sample.

3.14PC.3

Quantification of the anthropogenic Ce fraction in biosolids based on REE patterns and the oxidation state of Ce

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Engineered CeO_2-nanoparticles (NP) emitted to wastewater streams contain only negligible amounts of additional rare earth elements (REE) and Ce occurs in the tetravalent state. In natural environments, Ce predominantly occurs in accessory minerals in its trivalent state and is associated with other REEs. Thus, the oxidation state and the REE patterns may allow distinguishing between anthropogenic and geogenic Ce. In various soil samples, we found between 70 and 100% of Ce in the trivalent state and very comparable REE patterns. Assuming that engineered Ce is present in the tetravalent state and free of other REEs, we estimated the fraction of engineered Ce in bio-solids from several wastewater treatment plants. Consistent fractions of engineered Ce in sewage sludge were obtained based on the ratio of Ce to Dysprosium, Erbium and Europium. The engineered Ce fractions calculated based on the oxidation state of Ce were in general agreement with these results, but were associated with larger uncertainties, especially at low Ce concentrations. Electron microscopy analyses dominantly revealed Ce-particles in the nanosize range. Thus, engineered Ce fraction and associated masses derived from REE patterns maybe used to validate the results from mass flow models, estimating the loads of Ce-NP discharged to wastewater systems.

Non-Extractable Residue (NER) in Regulatory Testing of Chemicals (PC)

3.25PC.1

Strategy to elucidate if NER are a hidden hazard or a safe sink of an active ingredient in soil

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For decades, researchers have tried to elaborate the chemical structures of NER resulting during the degradation of active ingredients. In case radiotracer methods are used when performing soil metabolism studies of compounds the amounts of NER formed can be quantified very well. However, since structure elucidation fails for the resulting insoluble and complex polymeric matrices, reasonable regulatory concerns about the impact of NER appeared, i.e. if such NER is formed rather quickly after treatment of soil. Therefore, it is of increasing importance to clarify if such NER can be still regarded as a safe sink, thus do not contain a hidden hazard in the context of risk assessments. Key element for proposing further studies to decrease above-mentioned concerns is the assumption that a fast formed *^{14}C-NER* is regarded as a "*major soil metabolite*" of an active ingredient. In consequence, the following data for ^{14}C-NER are needed for a valid exposure and risk assessment: Formation fractions for ^{14}C-NER covering a range of soilsMetabolism data for ^{14}C-NER in at least three soils indicating which components can be released to which proportions into soil under standardized viable soil conditions (exceptionally under special soil conditions to simulate any later land-use change conditions, e.g. flooding, cropping)Sufficient DT_{50} values for ^{14}C-NER to estimate its accumulation potential Finally, NER is not any longer assessed as black box (including a potential hidden hazard), but as an immobile and well-understood metabolite in soil.

Environmental Risk Assessment of Pharmaceuticals: Connecting across Disciplines and Continents (PC)

4.09PC.1

Towards an ecologically relevant Environmental Quality Standard for diclofenac?

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Diclofenac was first highlighted as a candidate Water Framework Directive (WFD) Priority Substance (PS) in 2011 and an annual average (AA) freshwater EQS of 0.1 µg L^{-1} proposed. This draft EQS dossier has since been updated by the German Federal Environment Agency (UBA), most recently in 2018. The UBA proposed a reduction of the AA EQS to 0.05 µg L^{-1}. The driving data in EQS is histopathological responses in fish, in particular kidneys, liver and gills, rather than on generally accepted population-relevant ecotoxicity endpoints. This focus on histopathological responses in fish has potentially led to the wider, population-relevant, ecotoxicity dataset for diclofenac (i.e. that data generated for widely-accepted population-relevant endpoints across a range of taxonomic groups) holding less weight in the EQS derivation than would be typical for most substances for which EQS may be being proposed under WFD. The aims of this study are to: · Re-examine the chronic ecotoxicity dataset for diclofenac and specifically conduct an extensive search for published (freshwater and marine) chronic ecotoxicity data on diclofenac; · Compile an updated reliable and relevant dataset for diclofenac and identify data gaps; · Fill these data gaps by conducting new laboratory studies with diclofenac; · Examine the potential for a probabilistic (Species Sensitivity Distribution (SSD)) approach to EQS derivation for diclofenac; and, · Compare the outcomes of this approach with the previously proposed EQS.The updated chronic aquatic dataset for diclofenac covers adverse effects in a range of fish, marine and freshwater invertebrate and plant species (18 species across 8 taxonomic groups). New studies are being undertaken on echinoderms, freshwater molluscs, marine and freshwater polychaete worms, and insects as these represent potential taxonomic data gaps with respect to EQS derivation. These new studies are currently in progress, with results expected at the end of 2019. The full results of these studies and the implications of these results for the overall chronic population-relevant dataset for diclofenac and EQS derivation will be presented.The new data will be incorporated into the overall chronic dataset for diclofenac, and conclusions presented with respect to statistical differences between the freshwater and marine datasets, and the optimal approach to EQS derivation. Species Sensitivity Distributions (SSDs) will also be shown for the combined (freshwater and marine) dataset, and the freshwater dataset alone, and HC5 concentrations (with appropriate proposed Assessment Factors) compared to the currently proposed EQS for diclofenac.

4.09PC.2

Investigating the Impact and Scientific Justification Behind Changes to Groundwater Risk Assessment of Human Pharmaceuticals

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One aspect of the proposed revision to the European Medicines Agency (EMA) Environmental Risk Assessment (ERA) guidance is the inclusion of additional precautionary measures for the assessment of risk to groundwater organisms. Current guidance recommends calculating a predicted no effect concentration (PNEC) for groundwater based on the NOEC from the chronic study with *Daphnia magna*. Aquatic invertebrates are considered the most relevant test group for groundwater ecology, which have been demonstrated to be mainly composed of crustacea [Deharveng et al., 2009; Di Lorenzo et al., 2019]. The updated proposal, recommends the use of a more precautionary approach, using the overall $PNEC_{surfacewater}$ (based on the lowest of fish, *Daphnia* or algae data). In addition, a further assessment factor of 10 is applied to the $PNEC_{surfacewater}$ to account for the groundwater ecosystems being "*fundamentally different to surface water ecosystems*" and potentially being more "*vulnerable*" to perturbations. No changes are proposed to the initial assessment of groundwater exposure, where the predicted environmental concentration (PEC) in groundwater is assumed to be 25% that of the $PEC_{surfacewater}$. These changes to the ERA fundamentally alter the significance of the groundwater risk assessment in isolation, and in the context of the overall ERA. However, no scientific justification has been provided to justify such a precautionary and fundamental change.Ecotoxicology and exposure data on 131 human active pharmaceutical ingredients (APIs) were extracted from recently published analyses. Surface water and groundwater PNECs were derived following both the EMA 2006 & 2018 guidance. $PEC_{surfacewater}$ values, based on worst-case consumption data, were extracted and used to calculated $PEC_{groundwater}$ for each API. These data were then utilised to conduct groundwater risk assessments allowing a comparison of the Risk Quotients (RQs) across guidance documents and the relationship between surface water and groundwater risk assessments to be investigated. Here we will present the potential impact of these proposed revisions to the dynamics of the ERA, data generation, risk refinement, labelling, and potential for reduced access or choice for patients; as well as highlighting the potential knowledge gaps to help reduce uncertainty in this area.

4.09PC.4

Does treatment of cats and dogs with pharmaceuticals pose a risk for the environment?

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Cats and dogs are treated with extremely toxic substances against parasites like mites, fleas and ticks. This may cause emissions into the environment and may subsequently also be harmful for non-target organisms in these environments. Currently, the information on this issue is very limited. In the EU and other countries using the VICH guideline, the environmental risk assessment (ERA) for veterinary pharmaceutical products used for pets stops in phase I of the assessment. This is based on the generic assumption that use of these products will cause negligible environmental exposure and thus, no risks. This may, nevertheless not be true for extremely toxic active compounds. We will show that this assumption may not be correct, both based on calculations and measurements for fipronil. Fipronil is widely used against fleas and ticks in dogs and cats, usually by drops administered on the skin of the animal. Use data obtained from the Netherlands and Denmark show that if 0.3% of fipronil sold by veterinarians ends up in the water, the environmental risk limit will be exceeded. This is confirmed by measurements in the Netherlands, where fipronil is detected in 43% of all effluents. The limit of detection was 14-140 times higher than the risk limit. Thus, when fipronil is detected, the risk limit is exceeded by at least a factor of 14 but when fipronil is not detected, a risk may still occur. Although fipronil is also authorised for use as a biocide (e.g. gel against cockroaches), information combined shows that the use of fipronil as veterinary pharmaceutical may pose a risk to aquatic ecosystems.

Developments in the Ecological and Human Health Risk Assessment of Biopesticides: Microorganisms, Botanicals and Semiochemicals (PC)

4.16PC.1

Multicomponent biopesticide test items: Analytical challenges and opportunities

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Typical environmental protection products are composed of either a single active ingredient or a well defined mixture of active compounds. In contrast, many biopesticides are complex multicomponent mixtures composed of an undefined (or unknown) set of active ingredients. This leads to significant analytical challenges in environmental fate and exotoxicological testing of these products. Liquid chromatography coupled with mass spectrometry (LC/MS) is widely used for the quantitation of environmental protection products across a range of study types. Typical procedures rely on the selection of a limited number of well-defined components to monitor by multiple reaction monitoring (MRM) using triple quadrupole instruments. While this method offers excellent sensitivity and selectivity, it is poorly suited to the analysis of complex mixtures. Here we present an alternative analytical approach to the analysis of multicomponent test items by using high resolution-accurate mass spectrometry (HRAM) to first identify and then quantify active ingredients in biopesticide test items. The application of this method for the support of environmental fate and ecotoxicological studies is also discussed. The test item was a polysaccharide of algal origin made up of a mixture of multiple sugars and was analysed using a ThermoFisher Q-Exactive instrument coupled to a Dionex Ultimate 3000 UHPLC system. Multiple components corresponding to polysaccharides were detected as triply charged ions ($[M-3H]^{3-}$). In total, over 20 distinct polysaccharides were identified with a mixture of isotopomers detected for each. To quantify the test item, the five most intense peaks were selected as marker compounds. These components were used to measure the concentration of the test item in subsequent tests. Calibration curves were plotted between 0.1-50 µg/mL, with linearity >0.999 and the bias of each individual point < 10%. Due to the instability of the test item, modifications were made to the guidelines to successfully perform OECD 106 (adsorption/desorption), OECD 307 (transformation in soil), and OECD 221 (*Lemna* sp. growth inhibition) tests. The required values were obtained for all three test types.

4.16PC.2

Unique Challenges in Evaluating the Pathogenicity of Microbial Pesticides in Adult and Immature Honey Bees

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In order to establish the safety of microbial pesticides, pathogenic potential must be evaluated. The US EPA has an existing honey bee pathogenicity test guideline (885.4380) for adult honey bees. We explored challenges and possible improvements to 885.4380. Because there is no equivalent pathogenicity guideline for larval bees, we also evaluated the utility of the OECD Guidance Document No. 239 (Honey Bee Larval Toxicity following Repeated Exposure) for use with microbial pesticides. Challenges for the EPA adult honey bee pathogenicity test guideline include the following. · Unpalatable liquid sucrose diet due to high recommended test concentrations. · The particulate nature of the test material can result in: o clogging of the feeding tube restricting food access, and o difficulties in maintaining homogeneity of test material in sugar solutions. · Test duration of 30 days is difficult to achieve regarding honey bee survival and average lifespan. To address the problems with the treated liquid sucrose diet, we have used a pollen diet for dosing the bees. The bees were also fed untreated liquid sucrose. We are continuing to work on methods that will prolong the health and survival of the bees, trying to reliably extend the testing period to at least 20 days. OECD Guidance Document No. 239 is designed for chemical pesticides. An evaluation was conducted to determine if it could be adapted to microbial pesticides since some pathogens may only affect the larvae (e.g. *Paenibacillus larvae,* American foulbrood). Because royal jelly, a component of the larval bee test diet, is reported to have antimicrobial properties, we conducted a series of microbial inhibition tests to evaluate whether larval bee diet and royal jelly would inhibit microbial growth. We tested several strains of soil bacteria and bacterial and fungal bee pathogens. The fungal pathogen was not inhibited by the larval bee diet (or royal jelly), but the bacterial growth was inhibited by both substances for all bacteria tested, including *Paenibacillus larvae*. This finding may make the test unreliable for certain microbial pesticides. Currently, OECD is developing their own microbial pesticide testing guidelines, and the EPA has indicated they want to update their existing microbial pesticide test guidelines. However, additional research is needed to determine which testing options may be feasible and provide the best improvements.

4.16PC.3

Regulatory approach and developments in fate and behaviour for botanical pesticides

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Despite the growing interest in botanical pesticides, there is little specific guidance on the regulatory approach and the data requirements for these products in the EU. The regulatory framework is based on that for 'conventional' chemical plant protection products, with the same data requirements used to derive endpoints for exposure assessments. ERM has recently been involved in submissions of botanical plant protection substances in the EU. These were plant extracts, complex mixtures of substances including the biologically-active components and other materials of common plant origin such as carbohydrates, lignin, fibre and ash in variable proportions. These botanical pesticides can also be considered to be classified as UVCB substances (substances of Unknown or

Variable composition, Complex reaction products or Biological materials) in the EU. The submissions had to address demanding data requirements such as route and rate of degradation in soil, surface water and sediment with an exposure assessment for the soil, surface water and groundwater compartments. Challenges included the difficulty of generating data for use in the exposure assessment using conventional laboratory studies on soil degradation, soil photolysis and degradation in water/sediment studies. These studies require radiolabelled test items in order to follow degradation pathways. This is impractical with UVCBs as the components are numerous and generally far more structurally complex than conventional chemicals. Soil degradation and soil sorption endpoints are the most crucial parameters in the exposure assessment. Alternative methods to derive such parameters were necessary. This poster describes the methods used in order to be able to perform full exposure assessments for soil, surface water and groundwater. The exposure assessments showed an acceptable risk to groundwater and to soil and surface water-dwelling organisms. ERM has received positive feedback on the approach from the authority responsible for the evaluation of the first of these biopesticides as it progresses through the EU's regulatory system.

4.16PC.4

Regulatory risk assessment for environment and non-target species related to the use of microorganisms as plant protection products

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Microorganisms (i.e. bacteria, fungi, viruses) used in the composition of plant protection products (PPP) or pesticides are subject to a risk assessment at European level as active substances, and at national level for the placing on the market of PPP containing them. This risk assessment is regulated by the data requirements of Regulation (EC) No 1107/2009 [1]. It is mandatory before any possible placing on the market.For "microorganism" active substances and microorganism containing products, the risk assessments of environmental and non-target species is based partly on the current risk assessment methods for PPP containing chemical active substances. However, given the specificities relative to this type of active substance, needed information/data have to be adapted for regulatory risk assessment purpose. Knowledge on the identity of the microorganism, its biology, its mode of action (known or suspected), its conditions of growth in the environment as well as a precise definition of the conditions of use should be available to evaluate potential risks to the environment and non-target species. The characterisation of the microorganism should provide knowledge about its fate and dissemination in the environment (i.e. soil, water and air), to estimate its capacity to produce potential metabolites of concern, and to estimate short-term and long-term risks for non-target species. Risks related to pathogenicity and infectivity should also be assessed. A synthesis of the knowledge available in the scientific literature [2] at the level of the genus or species, and more rarely of the strain, can make it possible to inform these requirements and to define, if necessary, the additional data to be submitted for the strain under assessment.The risk assessment approach for microorganisms, its main steps and the related conclusions will be presented in terms of protection of the environment and non-target species.[1] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, http://data.europa.eu/eli/reg/2009/1107/oj[2] European Food Safety Authority; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1-50). EFSA Journal 2011;9(2):2092. [49 pp.]. doi:10.2903/j.efsa.2011. 2092

4.16PC.5

Characterization of botanical UVCBs for active substance registration under the plant protection product regulation: an opportunity for data miners

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Considering a steadily growing world food demand, loss of and competition for arable land, and a generally expected increase of pest pressure, crop protection will become ever more critical. In Europe, margins for intensification of conventional pesticide use are small. As such, farmers will for a larger part have to rely on green alternatives. Among these, botanical extracts hold a curious position; despite the fact that 400 million years of plant-pest co-evolution have left us with thousands of potential, 'nature-designed' active substances to choose from, only slightly less than twenty are currently approved for use under EU regulation (EC) No 1107/2009 (which regulates the plant protection product market). This paradox is mainly explained by the fact that there is no clear-cut regulatory context for the risk assessment of UVCBs (Unknown or Variable composition Complex reaction products or Biological materials), a group of largely undefined multi-component mixtures that includes nearly all botanicals. The main issue relates to pinpointing the individual components that are of concern for humans, animals, and/or the environment, which impacts the core of the risk assessment. The special Guidance document, SANCO/11470/2012, intended to facilitate the approval of botanical active substances does however not provide any starting point as to how adequate identification of just these components among the many is actually achieved. This is not surprising as, at the time the Guidance was being developed, the technology to distinguish between biologically active and inactive extract constituents was still only slightly beyond being fiction. In the meantime however, the technology, i.e., metabolomics coupled with biochemometric data analysis (M/BDA), has grown and may be mature enough to tip the scales in favour of a scientifically well-balanced risk assessment practice. In this first one of two presentations on this topic, (i) the potential of botanical active substances for plant protection in a global context will be discussed, (ii) the specific problems encountered in risk assessment of these complex mixtures are described, (iii) opportunities and caveats of implementation of M/BDA into the botanical active substance regulatory context are covered, and (iv) possible new routes enabled and/or encouraged by embracing the novel technology will be explored.

Dyes as Environmental Contaminants (PC)

4.17PC.1

BioColour project - Search for environmentally sound bio-based colourants and processes for textile, packaging and coating applications

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Bio-based materials and new technologies have gained growing interest in different applications, as companies want to enhance their products' sustainability and remove environmental and hazardous pollutants from the manufacturing process. Bio-degradable and recyclable textile and package innovations are increasing. However, currently used synthetic colourants are designed to be stable, which contradicts the interest in bio-degradability. BioColour is a six year research project aiming to develop new methods for large scale production of biocolourants, along with their characterisation and applications. A multidisciplinary team of experts are undertaking studies pertaining to biocolorant production methods, structure – property relationships, toxicology and dye-substrate interactions. Co-creation and investigation of cultural, societal and ethical aspects associated with producing and applying biocolourants are also investigated. Our studies are focused on three sources of colourants (agriculture, forest industry waste streams, and microbes) and three application areas (textiles, packaging and coatings). Firstly, the toxicity of the new biocolourants to human health and the environment is assessed. This will include the ability of their metabolites to cause harmful effects. Metabolic profiles of biocolourants in aqueous media will be characterized by non-targeted metabolomics. Also, the mutagenicity and toxicity on human cell lines will be evaluated. The waterless dyeing techniques such as the SC-CO_2 and atmospheric-plasma aided dyeing, mass colouration and reduced colourant concentrations in dyeing and printing will be examined. With the studies of interactions between biocolourants and substrates understanding for improved and sustainable colouration methods can be gained. Further, studies of colourants' UV-protective, anti-microbial, water repellent and electrical conductive properties will be performed. To launch biocolourants successfully, we aim to understand impacts of biocolour production at the society and stakeholder level, and how consumers value and negotiate the new qualities of products into their choices. Consumer awareness of environmental hazards and climate change is increasing, and educational attainment is a strong predictor of climate change awareness. Promoting environmental literacy through biocolours and sustainable-driven business is one solution to meet the challenges of the future.

4.17PC.2

Toxicological safety assessment of biocolourants developed in the BioColour - project

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Over 10,000 different dyes and pigments are used industrially and synthetic dyes are massively used in textile industry. During the dyeing process the losses of colourants to the environment can reach 10–50%. Also, workers in the textile industry may be exposed to dyes and some of the dyes are toxic, having carcinogenic and mutagenic potential. The aim of the Bio-based Dyes and Pigments for Colour Palette (BioColour) project is to develop new methods of biocolourant production, characterization and application, which enable the build-up of novel processes leading to a variety of sustainable items. Bio-based is not a synonym for a safe colourant and our objective is to assess the toxicity of novel natural dyes and pigments to human health and the environment. Potential new colourants are blue indigo from woad (*Isatis tinctoria*), dyes from bark, lignin and microbes (mushrooms, fungi, yeasts). Chemical characterization of dyes will be performed at the University of Helsinki and purity at the North Carolina State

University (NCSU). At NCSU researchers will perform a mutagenicity screening with the miniaturized version of the Ames test (Microplate format, MPA). Dyes, which are negative in the MPA test will be studied in human cell lines at the University of Eastern Finland. Cell viability, cytotoxicity, reactive oxygen species production as well as cytoplasmic and mitochondrional production of superoxide will be studied in THP-1 monocytes and HepG2 liver cells. Mitochondrial metabolism will also be assessed with Seahorse XF Analyzer (Agilent). Molecular targets of toxicity will be assessed in transcript and protein level. OECD Test No. 442D: In Vitro Skin Sensitisation with KeratinoSens^{TM} or LuSens^{TM} cell lines will be used to study skin sensitisation. The aquatic toxicity of the dyes will be performed using the Daphnia model at the University of Campinas. We already screened the mutagenicity of four anthraquinone dyes produced by fungi, using the MPA and found two promising dyes which provided negative results: a mixture of Dermorubin (98.2%) and 5-Cl-dermorubin (1.8%) and Dermocybin (99.7%). The 48h, EC50 were determined for *Daphnia similis*, and they are 7.7 and 0.99 mg/L respectively. At the end of the project we aim to provide a complete hazard assessment of biocolourants to be safely used in dyeing processes.

4.17PC.3

Strategy for the evaluation of mutagenicity of natural dyes: Application to the BioColour project

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Biodegradable and recyclable textile and package innovations are receiving great interest but they still use synthetic dyes as colorants. With this in mind, the heart of the new BioColour programme involves establishing substitutes for synthetic dyes among the families of natural dyes. Although this idea seem plausible, for safety reasons the identified natural dyes must be tested for their toxicological properties before use. Based on the results of a previous project on mutagenicity of dyes and a literature review, we decided to use the miniaturized version of the Salmonella/microsome, MPA in a tier approach. For this study, we selected and tested two promising anthraquinone natural dyes obtained by fungi and also included two natural dyes that had their mutagenicity documented in the literature. We used the MPA protocol with TA97a, TA98, TA100, YG1041 strains because they would detect a wide range of types of mutations, and also TA1537 which is sensitive to anthraquinones. Tests were performed in the absence and presence of the metabolic activation at the concentration of 5% and in some cases with 10% and 30% of S9 in the mix. We concluded that the MPA is adequate to evaluate mutagenicity of dyes with limited quantity availabe for testing and that the use of 10% of S9 can enhance the sensitivity of the test. Therefore our strategy to verify the mutagenic activity of natural anthraquinone dyes will be starting with TA1537, followed by the other four strains, using 10% of S9 in the mix, in a tier approach. If positive for the first strain, no further testing is required, and the dye will be considered as mutagenic for the Salmonella/microsome assay. And to consider a dye as not mutagenic, all strains need to provide negative results. We conclude that dyes C (dermorubyin) and D (dermocybin) are not mutagenic. Dye E (emodin) mutagenicity was confirmed and Dye F (emodin glucoside), although not mutagenic in the assay, can be easily converted to dye E.

4.17PC.4

Design and Toxicity Evaluation of Dyes for Waterless Dyeing of Textile Fibers

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On a worldwide basis, textile dyeing operations produce very large volumes of wastewater, which if discharged into the environment could have adverse effects on plant and animal life as well as drinking water. Thus, there is continuing interest in dyeing processes that circumvent wastewater production, making the development of waterless methods for dyeing textile materials a rational approach to accomplishing this goal. In view of this vision, the use of atmospheric plasma technology to apply dyes to textile fibers was examined, as no water is needed for the dyeing process. Step 1 of our approach involved the design of solvent soluble dyes suitable for this waterless process. This led to a family of new azo and anthraquinone dyes having one or two acrylate groups for bonding to textile fibers. Members of this group gave good covalent bonding to and good technical properties on natural and synthetic fibers following spray application and plasma treatment. Following proof of concept for our waterless dyeing method, the aquatic toxicity of 7 new dyes was tested at a maximum concentration of 1,000 µg/L, the limit of solubility of the dyes. Stock solutions were prepared in deionized water containing 1% of dimethyl sulfoxide (DMSO) to facilitate dissolution. Acute toxicity tests with *Daphnia similis* were performed according to OECD guideline 202. Negative controls and solvent controls were tested accordingly. Twenty neonates (< 24 h old) from 2-3 week-old mothers were placed in 4 replicates for each concentration (5 organisms/replicate). Tests were performed at 21 ± 1 °C under a photoperiod of 16 h light and 8 h darkness. After 48 h, the number of immobile organisms was recorded. Data were statistically analyzed using the Trimmed Spearman–Karber method to calculate the 50% effective concentrations (EC50) and the results were compared to Disperse Red 1 (DR1) prototype dye. With an EC50 = 100 µg/L, one dye was more toxic than DR1 (EC50 = 180 µg/L). Four dyes were less toxic and neither one was able to immobilize 100% of organisms at the highest concentration tested (1,000 µg/L). Two dyes showed no toxicity until 1,000 µg/L and were also non-mutagenic in the Salmonella/microsome mutagenicity. The latter 2 dyes also gave good results in our fiber dyeing studies. Dye structure-properties relationships will be covered in our presentation.

Environmental Risk Assessment of UV filters (PC)

4.18PC.1

UV filters and other personal care products: water-sediment distribution and bioaccumulation in fish in the Evrotas River

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Occurrence and distribution of chemical pollutants in water, sediment and biota is critical in defining the prevalence of pollutants in the aquatic ecosystem and its potential impact on environmental quality. UV filters (UVFs) are included in most personal care products (PCPs), but also found in other products (fabrics, plastics, paints.) to protect them from the deleterious effect of UV sunlight. PCPs are currently a matter of concern due to their extensive use, environmental ubiquity, pseudo-persistence and ecotoxicity in aquatic ecosystems. Monitoring programmes for pollutants assessment in Greece, do not include UV filtrers or any other PCP. In this context, the aim of this work was to provide occurrence data on selected PCPs in water, sediment and biota along the Evrotas river basin (Greece), in order to fill the gap of information at EU level. Overexploitation of groundwater and water abstraction from water surface led to a dramatic long-term flow reduction as well as desiccation of large parts of the Evrotas River. As a result of desiccation and contamination, massive fish deaths occur. Samples collection (water, sediment and fish) was conducted at four reaches of Evrotas River, in June 2014 and July 2015, targeting for different levels of water stress and quality. Eleven organic UV filters (benzophenones, camphor and PABA derivatives, crylenes, cinnamates), nine benzotriazole UV blockers, three paraben preservatives and three musk fragrances were investigated. The present study confirmed the ubiquity of PCPs in aquatic ecosystems The integral survey (water, sediment and biota) of the Evrotas), evidenced the dilution effects of the pollution at higher flow. More lipophilic PCPs tend to sorb onto sediments and/or to accumulate in fish tissues of the endemic chub *Squalius keadicus*. Among the 26 analysed PCPs, the endocrine disruptor UV filter BP3 in water, 4MBC in sediments and BP2 in fish where the most prevalent. The ecological risk posed by the target PCPs in Evrotas resulted in no hazard for the species tested with the exceptions of BP3 (high risk for *Daphnia magna*) and ODPABA (medium risk for *Raphidocelis subcapitata*). These findings suggest that both UVFs are a potential threat to the aquatic environment. *Acknowledgements* - This study has been partially supported by the EU project GLOBAQUA (FP7-ENV-2013, GA 603629) and by the Water JPI & Spanish Ministry of Science, Innovation and Universities through MARadentro project (PCI2019-103603).

4.18PC.2

Toxicity of UV filters on marine bacteria: combined effects with damaging solar radiation

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Authors: C. Lozano, J. Givens, P. Lebaron & S. Matallana-Surget Track and session: Environmental Risk Assessment of UV Filters (Carys Mitchelmore, Iain Davies) Keywords: UV filters, Microbiology, Ecotoxicology, Organic pollutants The use of personal care and sunscreen products is increasing worldwide, releasing up to 6000 tons per year of toxic chemicals in marine coastal ecosystems. Very recently, Hawaii was the first US state to ban oxybenzone and octinoxate harmful to marine environments. Most of UV-filters are hydrophobic organic compounds known to bioaccumulate easily and cause detrimental effects. The effects of UV-filters were assessed in vitro, in vivo or in situ, on several organisms ranging from bacteria, fishes, and algae to humans. These studies highlighted the toxicity of UV-filters through cell growth inhibition, endocrine disruption, and neurotoxicity. However, the effect of UV filters on marine bacteria remains poorly documented. We present here the first screening assessing the impact of UV-filters on marine backterioplankton. A total of 27 heterotrophic marine bacterial strains, isolated from the coastal Mediterranean Sea were subjected to different treatments with five commonly used UV filters:

oxybenzone, octinoxate, enzacamene, homosalate and octocrylene. Bacterial growth and survival were monitored for each strain in different physiological states (exponential or stationary phase). In addition, sensitive bacteria were used for the assessment of the combined effect of UV filter with solar radiation. Our results emphasized that bacteria present a wide variety of response when exposed to different concentrations of UV filters. The most resistant strains were found to tolerate up to 4 mg/L for every compound while the growth of sensitive strains was inhibited from 100 µg/L. The phototoxicity analysis revealed no photo-enhanced toxicity on bacteria. Physiological state was found to be a key parameter in bacterial susceptibility as stationary phase cells were resistant, regardless of the species and the compound studied.

4.18PC.3
Investigating the acute and chronic toxicity of the UV filter Benzophenone-3 (oxybenzone) on the coral Galaxea fascicularis
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Despite limited toxicological studies in hard coral species, sources of UV filters besides sunscreens, legislative activity in Hawaii and Key West has led to bans on the sale of sunscreens containing the specific active ingredient benzophenone-3 (BP-3 or oxybenzone). The handful of studies have shown highly variable toxicological responses in adult and larval forms of coral, with acute and chronic toxicity values in the parts per billion to million. Comparison between existing studies are limited due to differing exposure regimes, coral species, life stages as well as a lack of analytical verification. Given this limited amount of variable data on the toxicity of BP-3 to coral, additional toxicological assessments are necessary to determine the environmental risk of this UV filter. Furthermore, toxicity tests using corals should follow standard procedures similar to those conducted for other invertebrate test species to allow for better comparison across studies. Therefore, we conducted a number of acute (i.e. 96-hour) and chronic (i.e. 28 days) toxicity tests based on standard EPA and OECD guidelines in *Galaxea fascicularis*; a common shallow-water hard coral species. These experiments were coupled with chemical analysis to determine the actual aqueous concentrations at several time points during the exposure. Multiple endpoints pertinent to risk assessments (i.e. mortality and growth) were examined along with other biological endpoints such as bleaching, algal cell loss, photosynthetic efficiency and poly retraction. NOECs, LC_{50}s, and EC_{50}s were calculated in order to compare the results of this test to previous assessments of BP-3 toxicity in corals. We also used a number of common reference toxicants to compare the sensitivity of *Galaxea fascicularis* with other corals. Finally, a risk assessment of BP-3 on *Galaxea fascicularis* was conducted using these results coupled with global analytical values of BP-3 in seawater from a variety of coral reef locations.

4.18PC.4
Assessing the Environmental Risk of UV Filters to Coral
I. Davies, Personal Care Products Council / Science; E.E. Burns, PCPC (Personal Care Products Council) / Science
UV filters are active ingredients in sunscreen products that are designed to protect people against the harmful effects of solar radiation. In recent years, UV filters have been detected in coastal marine waters, which has led to concerns that these materials may be contributing to coral reef degradation. At a time when coral reefs are in overall decline, it is important to assess the risk that UV filters pose to coral reefs and use this information to guide environmental management and policy decisions. Sources of UV filters in the environment are wash-off of sunscreen ingredients from bathers' skin and wastewater treatment plant effluent. Other sources of UV filters include plastic, industrial products and naturally derived compounds. UV filters have been detected in the marine environment at ng/L concentrations. Meanwhile, a limited number of ecotoxicological investigations have looked at the effects of UV filters on various coral species. Most of these studies have investigated acute effects on coral, such as mortality and bleaching. The following presentation will summarize the state of science regarding exposure of the marine environment to UV filters and the hazard these materials pose to coral. Exposure and effects data can then be used to assess the environmental risk of UV filters to coral. However, regulatory guidance for assessing the risk posed by UV filters to coral is limited and there is a need for a coral-specific environmental risk assessment framework. This presentation will therefore propose an environmental risk assessment framework for coral based on guidance published by the European Chemicals Agency and The U.S. Environmental Protection Agency. It is also important to assess environmental risk using reliable exposure and effects data. The presentation will also provide an assessment of the reliability of existing data following published data reliability assessment guidance. Additionally, given the extensive data gaps that exist for UV filters, future research needs will be identified and an overview of how these data should be used for a coral-specific environmental risk assessment will be discussed.

4.18PC.5
Systematic Toxicity Tests with Corals of Different Age Classes - First results of Coral Larvae Tests
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Coral reefs are currently declining at a historically unprecedented pace: besides the main destructive factor of climate change, an array of other anthropogenic disturbances such as nutrients and pollutants may affect coral reefs as well. Standardized and internationally recognized toxicity tests on various freshwater and marine organisms have been established for decades and are the basis for hazard and risk assessments within legislations for use of chemicals around the world. However, for corals no such standardized tests exist although they are necessary to derive scientifically sound regulatory actions. Therefore, there is an urgent need for the development of validated and standardized test designs in order to evaluate the hazardous effects of various substances entering the marine environment, focusing on corals. Our study approach incorporates both acute and chronic toxicity assays using different life stages of corals such as planula larvae, juveniles and adult corals in order to compose suitable and representative protocols as a basis for internationally recognized standardized tests. We assess the applicability of exposure assays with a wide spectrum of chemical compounds using a range of lipophilic and hydrophilic substances with a focus on UV-filters. The first set of experiments focussed on acute coral larvae toxicity. We developed an assay that was based on coral larvae and includes survival and metamorphosis as suitable endpoints. A range of 13 hydrophilic and lipophilic substances (including 3 candidates for a positive control) were tested and the results allowed for a clear discrimination between mortality and metamorphosis effects in coral larvae. Typically, observed metamorphosis increased as mortality decreases. However, in one case a clear suppression in metamorphosis in absence of any mortality was observed, which indicates that the use of both endpoints is recommended in coral larval toxicity testing as they are relevant for coral larvae survival.

Environmental Risks of Neonicotinoid Insecticides: Are They the Outliers? (PC)

4.19PC.1
Risks of imidacloprid to aquatic ecosystems: are current risk assessments protective?
P. van den Brink, Wageningen Environmental Research / Department of Environmental Sciences
Imidacloprid is one of the major insecticides which is used worldwide and could affect non-targeted organisms through runoff, leaching and spray drift into water body after its use. It could harm aquatic organisms since imidacloprid is highly toxic for aquatic insects and several other aquatic invertebrates. In the Netherlands the measured concentrations of imidacloprid regularly exceed the Regulatory Acceptable Concentration (RAC=0.027 µg/L), although this has decreased since its use is restricted in 2013. In order to evaluate the ecological consequences of these RAC exceedances we evaluated the protectiveness of the chronic first tier risk assessment using the results of a literature review performed by Brock et al. (2016) and some toxicity experiments. Brock et al. (2016) evaluated the level of protection provided by the RAC as calculated by the chronic first tier of the EU risk assessment by comparing these values with the RACs based on the ecological threshold option (ETO-RACs) as derived from microcosm and mesocosm experiments. The chronic Tier-1 RACs calculated according to the EFSA approach resulted in an insufficient protection level for one neonicotinoid (multiple applications) and for several pyrethroids as they exceeded the ETO-RAC7d-TWA. The first tier RAC was just protective n case of a single application of a neonicotinoid pesticide. In a population experiment the smallest individuals of an insensitve crustancean species were less abundant after exposure to 0.003 µg/L of imidacloprid for four months. Other experiments indicate that imidacloprid and one of its toxic metabolites binds irreversable to the receptor, explaining the increase of the toxicity with time. Two microcosm experiments performed in Asia also show that tropical species might be more sensitive than temperate ones. In this talk I will also try to make a read across and evaluate whether the findings of imidacloprid are also applicable the other neonicotinoid insecticides.

4.19PC.2
The toxicokinetics of imidacloprid and imidacloprid-olefin in two aquatic nontargeted species
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1.Introduction Imidacloprid is one of the most used neonicotinoids, which raised a high environmental concern in the last decade, among others to the aquatic system. However, the exact mechanism leading to the effects of imidacloprid and its metabolites to aquatic invertebrates is still unknown, while particular concern

has been raised about its long-term toxicity. Earlier experiments showed that especially mayfly insects are particularly sensitive to imidacloprid, with effect sizes increasing with time, even beyond the duration of the normal chronic toxicity experiments. Similarly, it has been found that even quite insensitive crustacean species become sensitive when the exposure period is long (i.e. 3 months; Van den Brink, pers. comm.). In our study, we first studied the toxicity of imidacloprid (IMI) and its metabolites to a sensitive nontargeted mayfly species: Cloeon dipterum and a less sensitive one: Gammarus pulex. After this, toxicokinetic experiments were conducted with the metabolites to improve the understanding of the long-term toxicity of selected compounds. 2.Methodology Two types of experiments were performed. Firstly, the toxicity of each chemical was assessed by a 4 days standard toxicity experiment. Secondly, the toxicokinetics of IMI and IMI-ole, which proved to be the toxic metabolite, were studied in the same species in an 2 days uptake and 3 days elimination experiment. The toxicokinetic parameters were fitted using a published TK model with and without the inclusion of biotransformation. Internal and external concentration of parent compound and the metabolites were analysed by LC-MS/MS. 3. Results Our result found that the biotransformation product IMI-ole showed similar toxicity to the mother compound IMI and was also hard to eliminate from C. dipterum, while IMI is biotransformed to IMI-ole in C. dipterum. Hence IMI-ole may responsible for the increased toxicity of IMI in C. dipterum with increasing exposure time. Our results suggest that IMI-ole may bind irreversible to the target receptor and, herewith, may be important for the toxicity of IMI after long-term exposure. The sensitivity difference between invertebrates is not only depending on the bioaccumulation of the mother compound, but also on its biotransformation.

4.19PC.3

Short-term benefits and carry-over effects of early-life pesticide exposure

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Pesticide can impact top consumers by directly reducing key biodemographic traits or indirectly by reducing resource availability. Neonicotinoids, a family of pesticides known to have contributed to the decline of invertebrates density, are also suspected to disrupt vertebrate metabolism regulation. For farmland bird species, neonicotinoids potentially result in two stressors through reduction of food availability and alteration of metabolism, but very little is known regarding their effect on bird's growth rate. We performed a cross-design experiment in Zebra finches (Taeniopygia guttata), a granivorous species. Birds were exposed to very low dose of imidacloprid (0.205 mg/kg bw) and/or food intake rate reduction from day 5 to day 30 after hatching. Mass gain was greater in birds exposed to imidacloprid and the negative effect of food restriction during chick growth was compensated for pest-exposed individuals. This early-exposure to neonicotinoid was carry-over to the adult stage: two-years-old birds presented higher metabolism associated with greater lean mass. This study presents the first evidence that short and low-dose neonicotinoids exposure have the capacity to durably alter bird's phenotype.

Sustainability Dietary Patterns: Nutritional and Environmental Implications (PC)

5.08PC.1

A comprehensive sustainability evaluation of eating patterns for Americans

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Diet disparities in the US are well-documented, with gender, race, and socioeconomic level influencing the quantity and quality of foods consumed. Here we examine for the first time the sustainability potential of self-reported dietary patterns for American consumers by quantifying their nutritional health burden, environmental impacts, and affordability. Self-reported US dietary patterns were determined based on the reported daily intake of ~7,000 foods from a nationally representative sample of Americans (N=13,331) from the National Health and Nutrition Examination Survey (NHANES) 2008-2016 database. For each dietary pattern, we estimate health burden in minutes of healthy life lost or gained per person per day using the Health Nutritional Index (HENI). HENI is an epidemiology-based nutritional assessment tool that quantifies the health burden associated with dietary deviations the Global Burden of Disease (GBD) diet based on 16 dietary risk factors. Using life cycle assessment (LCA), we determined the climate change, land use, and health impacts from fine particulate matter (PM2.5) associated with these dietary patterns by utilizing cradle-to-farm gate or processing gate food-specific impact estimates for ~7,000 foods in NHANES that account for loss and waste. Dietary pattern affordability was evaluated based on daily cost that was calculated using national food prices adjusted for inflation. The HENI score for the average US diet was estimated at 22 minutes gained per person per day, which is five times lower than the GBD diet (124 minutes gained/pers-d). The corresponding environmental impacts were estimated at 5.3 kg CO2 eq/pers-d for climate change, 4.4 ha/pers-d for land occupation, and 1.2 minutes lost/pers-d from air pollution. The daily cost of the average US diet was $6.2 per person. Race, household income, and physical activity influence both the sustainability potential of dietary patterns for all indicators. Overall, dietary patterns with higher intakes of fruits, vegetables, and whole grains and lower beef, pork, and processed meat intake yielded higher HENI scores and lower environmental impacts, however, they were more expensive. Our findings highlight the nutrition, environmental, and economic trade-offs of diets and could inform evidence-based guidelines that promote human and environmental health and are affordable for all.

5.08PC.2

The global carbon and resource footprint of Denmark's food consumption 1995-2011

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Abstract Many studies have highlighted the fast-growing and harmful effects of the increased global food trade and consumption on the environment, such as the rise and displacement of greenhouse gas (GHG) emissions, natural resource use and biodiversity losses from major consuming nations to major food-exporting/producing countries. The current Danish environmental policy lays down ambitious targets aimed at tackling the domestic environmental externalities of the country's agri-food production, especially those related to climate change and water pollution. This research aims to provide information on the environmental impacts associated with Danish food consumption with regards to greenhouse gases, land and water use beyond Denmark's borders. We carry out a consumption-based accounting of Denmark's food-related environmental impacts from 1995 to 2011 using an environmentally extended multi-regional input-output (EE-MRIO) model and the latest version of the EXIOBASE database which provides detailed food product groups and extensive coverage of countries including Denmark's major food trade partners. We track the GHG emissions, water and land use embodied in plant-based and animal-based food products consumed in Denmark from the first stage of supply chains to final consumption. We find that the carbon footprint of food consumption by Danish households has decreased by 22% at the expense of an increase in water and land footprint between 1995 and 2011. This study seeks to provide longitudinal data on Denmark's food consumption-based environmental impact accounts and policy-relevant information for raising awareness on consumer responsibility for the sustainability of the global food system through sustainable food choices and consumption. Lastly, to track Denmark's progress towards not only a climate-neutral and environmentally friendly local but also global food industry that enhances welfare.

5.08PC.3

Carbon foot-printing the dietary habits of the Spanish climatic zones at household level

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There is an urgent need to change food consumption habits towards more sustainable ones, since it is one of the most effective measures at the individual level to mitigate greenhouse gas (GHG) emissions associated with the food system, which is one of the main responsible for climate change derived from human activities. Consumption habits can be very different worldwide, and even within the same country, depending on many factors, such as culture or climate; accordingly, measures that need to be taken to improve these eating habits can also be specific. Therefore, taking Spain as an example, these variations can be expected to be remarkable, mainly due to the great cultural and climate diversity throughout the Spanish territory. Consequently, taking into account the five predominant climate zones that can be identified in the country, the main goal of this study is to detect fluctuations between different food consumption patterns in terms of carbon footprint. To do so, the carbon footprint has been estimated using a life cycle assessment approach considering a cradle to gate perspective, i.e. taking into account the production stage of the foodstuffs included in the food basket of each climatic zone. Based on the results, the carbon footprint considerably varies throughout the territory (171 ± 110 kg CO2 eq·inhabitant-1·year-1). The northern regions, where the coldest climates are located, are associated with higher carbon footprints. The rationale behind these results are higher consumption rates of livestock-based products, which account for about 50% of GHG emissions and are primarily responsible for fluctuations in the total carbon footprint of each zone. Among these foods, it is worth to highlight the beef meat whose daily consumption is around 80% higher in colder regions than in warmer ones. Consequently, it would be advisable to intensify social campaigns aimed at the adoption of food patterns that prioritize the consumption of fruits and vegetables. This is why it is important to develop regional food recommendation policies based on the agricultural production of fresh and local products.

5.08PC.4
Nutritionally and environmentally optimized agri-food production systems for sustainable diets
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Alleviating environmental and health challenges will require a major reformation of our food system. Interventions, in this area, have been inadequate because they focus on issues of yields and calories while ignoring those of nutrition. Addressing these sustainability challenges will require the co-implementation of production-side interventions along with consumption-side interventions that seek to increase the supply of nutritious- and environmentally- friendly foods via improved agricultural and food processing practices, in addition to methods that generate more robust data on current and potential states of the dual nutritional and environmental sustainability of agri-food production systems. Doing this will promote an enhanced enabling environment from which diets can be optimized. To this end, we seek to better incorporate aspects of nutrition into environmental sustainability analyses, within the Life Cycle Assessment (LCA) framework. Our end aim is to better account for nutrition directly in the LCA functional unit unlike in other sustainability analyses that mainly include nutrition as a separate indicator. For our study, we use metrics reflective of nutrient diversity (e.g., Rao's Quadratic Entropy) and nutrient quantity (e.g., Nutritional Yield). To test our approach, we conduct a global dual environmental and nutritional assessment at the regional and national levels by combining six regionally and nationally differentiated datasets related to food production, trade, nutrition, and environmental impacts. Overall, we expect the tradeoffs between food groups to change when nutrition is accounted for. For example, the differences between animal-sourced meat and plant-based meat can decrease, when measured on a nutritional basis, particularly within populations that do not currently have an adequate supply of protein. Finally, we present a robust uncertainty and sensitivity analysis. Optimizing agri-food production systems on both nutritional and environmental bases will lead to enhanced sustainable diets because it can support a supply of nutritious food and alleviate consumer-choice pressure. Alternatively, it can encourage more nutritionally-diverse forms of production that can, in turn, increase environmental resilience and sustainability. Ultimately, it can help drive the creation of a more equitable food system in which all communities, not just those with financial means, have access to sustainable, healthy, and safe foods.

5.08PC.5
A novel Functional Unit to compare diet LCAs
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To overcome the global challenge of designing food systems that can satisfy the food demand of a growing global population, without threating the environment, has usually focused on the food production side, for example, through intensification (Garnett et al., 2013). However, food choices play also a significant role. Bajželj et al. (2014) show that changes in dietary patterns towards a "healthy diet", as well as the reduction of food waste, are the best strategies to ensure food security by 2050 while reducing current GHG emissions by 45%. Facing this significant role of food choices and eating patterns, more research analysing the impacts of diets with a life cycle assessment (LCA) approach has been published in the last decade (Hällstrom et al., 2015). However, methodological issues remain a challenge, especially in defining diets' function, and subsequently on selecting the functional unit (FU) of the system under study. While most studies use a mass- or energy-based FU, this study proposes a new approach to account for the energy and nutrient content within the FU of a diet. This approach allows to compare the environmental impacts of diets independently of their caloric and nutritional content, and it is applied to the Spanish context, as a case study.

Current Developments in the Regulatory Assessment of Biocides in General and Chemicals in the Sediment Compartment in Particular (PC)

6.09PC.1
Over-conservative environmental risk assessment for biocides
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A prospective and comprehensive environmental risk assessment (ERA) must be performed on the active substance for the market authorisation of biocidal products. Additional ERA may be conducted for non-active substance (co-formulants identified as Substances of concern) in a similar manner. The hazard data required for the ERA include the determination of a set of properties (physical-chemical, fate properties, short-term and long-term ecotoxicity) for the substance to be assessed. Besides, an exposure assessment has to be conducted for the normal intended use of the biocidal product with using a realistic worst-case scenario in order to estimate the concentrations to which the environmental compartments may be exposed to. For any endpoint/input parameter to be defined in the course of hazard assessment and exposure assessment, a strict application of the precautionary principle leads to the selection of the worst-case value. On the overall, the accumulation of worst-case values leads to an overconservative risk assessment. The purpose of this presentation is to highlight on some aspects of environmental risk assessment that need more realism and pragmatism for a comprehensive environmental risk assessment for biocides. The beyond realistic based on a few examples. · PNEC determination. The use of the EC10 endpoint instead of a NOEC. · Use of DT50. The initial approach for a prospective environmental risk assessment will proceed with a Tier 1 calculation, which assumes 100% of the applied chemical will be released and ignores the formation of degradation by-products neither biodegradation of the active substance, or consumption of active substance due to its biocide activity. These initial assumptions may lead to an overestimation of the environmental exposure and risk for the active substance. The initial environmental risk assessment can be refined with supporting data *e.g.* on the degradation and/or dissipation time of the active substance. · Default input values from available emission scenario documents and possible refinements of these values. Outlook: Revision of ESDs/guidance in order to allow a more realistic worst case approach for environmental risk assessments of Biocides

6.09PC.2
The argument for Specific Protection Goals in Biocidal Products risk assessments - case studies
F. Ericher, CEA
Biocidal products are assessed within the Biocidal Product Regulation (BPR). Under the BPR, generic protection goals are specified as "no unacceptable environmental effects, including impacts on biodiversity and the ecosystem", while specific protection goals (SPG) have not been derived. EFSA has further described the purpose of SPG, which is to clarify "entities that need to be protected, the attributes and/or functions of those entities, as well as the magnitude, temporal and spatial scales of effects on those attributes and/or functions that can be tolerated, without impacting the general protection goal, and the required degree of certainty with which the protection goal defined should be achieved" (EFSA 2010). During the 2015 ECHA soil workshop, the outcome of the discussions stated that, under REACH and BPR : *"Most participants thought setting specific Protection Goals (SPG) within the approach of Environmental Services was relevant – within the limits of policy set general PG's" "We as Risk Assessors should seize the opportunity to develop SPG's based on our 'science' and what we can actually measure"* "*Development of SPGs is needed across the industries (e.g., biocides, pesticides, industrial chemicals, veterinary medicines, fertilizers) and regions (EU/NA) should include clear definition of: - Land/soil use - Product use - Exposure scenario and time scale*" Current BPR risk assessment approaches (PEC strictly lesser than PNEC) are based on the assumption of exposure of a significantly large spatial and temporal scale, affecting an ecologically viable environmental compartment. This risk assessment approach may, however, be less appropriate in cases where there is temporal exceedance, where the size of the zone impacted is limited, where land use already does not permit ecological viability and/or when human health is to be protected. In those cases, decision on the acceptability of the product may be determined on a case-by-case basis. This method, however, results in a large level of uncertainty as to the acceptability of the dossier, which makes it difficult for applicants to invest and generate relevant data (e.g. by designing bespoke protocols for laboratory studies). This is where SPG may be most helpful, by allowing the definition of exposure assessment goals and hence a framework where products can be assessed according to relevant standards and that provides some foundation for investment. The poster aim to present case studies of biocidal scenarios where SPG could support the risk assessment process.

6.09PC.3
Increasing the relevance of the emission scenario of Cu used as wood preservative with TICKET-UWM
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Copper compounds are used as wood preservatives to protect wooden structures placed in water bodies or adjacent to riverbanks. As copper will be released to the water column during its use, its potential impact to the aquatic compartment needs to be evaluated. The methodology on how to assess the potential impact to the aquatic compartment pertaining to the use of wood preservatives according the Biocides Product Regulation is described in the Emission Scenario Document (ESD) for wood preservatives. The exposure models used in this context have been developed mainly from the experiences gained on individual organic substances. As inorganics and metals behave differently this implies that the methodology/assumptions used cannot always be applied directly to metals without any kind of modification. The ESD currently ignores the adsorption of

chemicals onto bottom sediment, sedimentation processes and sediment burial processes which are important in slow flowing water body scenarios. In addition, although the ESD considers degradation for organic substances it ignores insolubilization processes relevant for metals such as binding to Acid Volatile Sulphides (AVS) and other relevant fate binding processes.Recently, advanced sediment models, such as the TICKET Unit world model, have been developed which are able to predict metal behaviour in sediments, by incorporating important sediment processes such as sedimentation, burial, binding to sulphides and remobilization. The current study aimed to evaluate the importance of these processes for the sheet piling scenario (ESD PT8) by environmental fate modelling in TICKET-UWM. Our study showed that incorporating more relevant metal environmental fate processes in the sheet piling exposure scenario for Cu via TICKET-UWM modelling resulted in a clear decrease in predicted environmental concentrations (PEC) compared to those predicted in the ESD-PT8 scenario. Sediment burial becomes an important process at long timescales, with approximately 60% of the Cu-input buried in the deeper sediment layers. Accounting for Cu-AVS binding lowered the bioavailable Cu concentration. The extent of the decrease in bioavailable Cu concentration depends on the AVS-concentration and the assessment period as a result of differences in the Cu-load. Overall, our study indicated that sedimentation, resuspension, sediment burial and AVS-binding processes are important in static or low flowing water bodies and should be included in the ESD PT8 scenario.

6.09PC.4

Depth-related analysis of sediment and pore water in microcosms using natural sediments

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Sediment toxicity testing of plant protection products (PPP) is gaining an increasing awareness within the scientific and regulatory community. In regulatory risk assessment of PPP, standardized composited (artificial) sediment is used in Chironomid toxicity studies acc. to OECD test guideline (TG) 218/219. Since, the artificial sediment provides standardized properties; it does not mirror certain variations of natural sediments. For example, in natural sediments providing a high organic matter, the spatial distribution and partitioning of active substances between sediment and water phase are expected to be different when comparing to the artificial sediment. Experiments acc. to OECD TG 218/219 were conducted with the artificial sediment to reveal the spatial distribution of three model compounds A ($K_{oc} \approx 0$ L/kg), B ($K_{oc} \approx 300$ L/kg) and C ($K_{oc} \approx 3900$ L/kg). To capture the stratification within the sediment, a sampling methodology enabling depth-related analysis of sediment and pore water was used multiple times within the test duration of 28 days. In the spiked sediment studies (OECD TG 218), the model compounds were homogenously distributed within the sediment layer at the start. While the polar compound A showed no adsorption on the sediment and migrated unhindered into the overlying water, the diffusion of compound B was retarded by sorption leading to decreasing amounts only in the top 5 mm of the sediment. Compound C was predominantly detected in the sediment extracts showing a homogenous distribution within the sediment layer at the last sampling interval. In the spiked water studies (OECD TG 219), compound A penetrated almost unhindered the sediment layer resulting in a homogenous sediment distribution. Model compound A was primarily found in the overlying water, revealing its low adsorption affinity. Due to the moderate sorption affinity – of model compound B, it was primarily found in the top 5 mm of the sediment. In course of the experiment, B migrated into the deeper layers and a depth-related concentration gradient was established. Compound C was primarily found in the top layer of the sediment due to its high adsorption affinity. During the meeting, we want to present experiments acc. to OECD TG 218/219 with two contrasting natural sediments. The model compounds are expected to show a lower mobility in the silt loam sediment (Wiehtalsperre), while the mobility in the sandy sediment (Anglersee) might be comparable with the artificial sediment.

6.09PC.5

Linking chemical concentrations in sediments to population and community effects based on monitoring and modelling

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One of the most densely populated areas worldwide is the North Sea region, encompassing catchments of several large rivers, including the Rhine, Elbe, Scheldt and Humber. Over the years, various assessment tools have been developed. Although detection techniques are continuously improved, chemical protocols are well-standardised allowing comparison across systems. By contrast, toxicological assays are not routinely applied, involve region-specific testing procedures and include a few species only. Ecological surveys differ even more across regions and over time. Similar differences apply to models. Decades of development have yielded chemical fate models that are well-established in science and well-embedded in management. So far, no ecotoxicological model with a similar scope has been embedded in international frameworks. As an alternative, the OMEGA model was developed. Yet, major knowledge gaps exist. Chemical, toxicological and ecological data from the same location and period often contradict each other due to the limited number of locations, substances, physical-chemical conditions, species and endpoints covered. Consequently, our aims were to compare and interpret chemical, toxicological and ecological monitoring across countries, authorities and systems using modelling tools linking chemical concentrations to biological diversity and ecosystems services. Along this chain, our focus was on bottlenecks for application in sediments, in particular, on equations for chemical speciation, databases for toxicological response and relationships to diversity, productivity and ecosystem services. Monitoring data were obtained from programs carried out in Flanders and the Netherlands. To link concentrations to effects, we used a recently compiled database containing median concentrations on 12836 chemicals. Total concentrations in sediments were converted to dissolved concentrations C in pore water. Based on the LC50 values from the toxicity database, we calculated the sum of the toxic units TU = $\Sigma C/LC50$ of all substances for a given species and compared these to actually measured survival in lab assays. Likewise, based on the dissolved concentrations obtained and HC50 values from the toxicity database, we calculated the potentially affected fraction PAF and compared these to actually measured macrofauna abundance in field inventories. Finally, as a first attempt, we translated potentially affected fractions to expected decreases in ecosystem services.

Refinement of the Risk to Birds and Mammals from Plant Protection Products: Higher Tier Studies and Approaches (PC)

6.10PC.1

Contributions of ECPA to the revision of the EFSA Guidance Document on birds and mammals

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The EFSA Guidance Document (GD) on Birds and Mammals is the key regulatory document on how to conduct risk assessments for the evaluation of active substances and registration of Plant Protection Products (PPPs) in the European Union (EU). This GD is now under revision by a Working Group of scientists. The reach and timelines are not yet clear but the regulators are evaluating a wide range of published and confidential information. Many data directly relevant for such revision have been generated by industry (i.e., several companies that are part of ECPA, the European Crop Protection Association) as part of the regulatory packages required by the relevant legislation and guidance. Beyond the required studies, industry has conducted additional studies and is working together to communicate the results of its work. A wide range of technical topics have been addressed by industry in the last few years, both relevant to toxicity and exposure, the two drivers of any risk assessment. In the toxicity area this includes work on relevant mammalian and avian toxicity endpoints, extrapolation factors for the acute LD50s for mammals and effects on bodyweight in laboratory mammalian studies. On exposure, residue decline in foliage and initial residues on fruits; focal species of birds in different crops and regions; the relevance of the vole as representative of small herbivorous mammals; the use of radio-tracking PT (proportion of time) data generated in field studies for the refinement of the long-term risk to birds and mammals. Bats is a novel area where ECPA plans to initiate some activities. Industry has contributed with data following different procedures: public communication of data and results in scientific conferences and by publications in peer-reviewed journals; industry has also provided studies and data directly to regulators, at the requests of the latter or proactively to EFSA. ECPA is developing and planning new activities, content and reach of which will be presented in the conference.

Keywords: risk assessments, Plant Protections Products (PPPs), guidance document, birds & mammals

6.10PC.2

Focal species for pesticide risk assessments - how to determine the correct wild mammals

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To determine the risk from pesticide uses for the registration process in the European Union the current Commission Regulation (EC) No 11/2007 referrers to

EFSA Guidance Documents (GD). The actual document for the Risk Assessment for Birds & Mammals is proposing a tiered approach for the determination of focal species (FS) for the exposure estimation. For bird focal species suitable methods are well described and established in regulatory documents for risk evaluations regarding pesticide registration in the EU. Not so for wild mammals. Wild mammals in Europe are often active only at night and hidden in warrens/burrows or dense vegetation during the day to avoid predation. Many species occurring in agricultural landscapes are also small (< 25 g bw) and live on/in the ground. Therefore, qualitative and quantitative assessments of FS with mammals is more challenging compared to birds and involves a higher level of expertise and skills from the researcher. For some mammal species, similar methodological approaches as for bird FS-determination in field studies ca be used. For smaller species, active at night and/or in dense ground vegetation, (live-)trapping is a suitable method to obtain information about their occurrence and abundance in specific crops. Also, the use of radio telemetry, GPS-logger-techniques or even motion-triggered cameras might be suitable for the data collection regarding mammal FS. Therefore, methods are available and with modern observation/field techniques even the specific difficulties in observing wild mammals can be solved. A different way of data analysis is necessary for mammals as for bird FS, because different field survey methods and the lower number of potential species have to be considered.

6.10PC.4
Residues decline in food items for birds and mammals
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The EFSA Guidance on the Risk Assessment of Birds and Mammals (ref) is the adopted guidance used for assessing the potential risks of specific crop protection products for birds and mammals. The tiered approach includes several possible “refinement” options at different tiers in order to make the exposure assumptions more realistic. One of the ways this is possible is by refining the dissipation time (i.e. half-life or DT50) of the residues present on the food items for the birds and mammals in question.. According to the guidance, specific studies are required in order to override the default value (i.e. DT50=10 days), which was based upon a large number of residues trials in various plant types and substances. How these targeted specific trials should be performed, evaluated, and used in the risk assessment, however, remains an issue where different member states and EFSA may not come to the same conclusions for the same trial or set of trials. Many of the issues surrounding residues decline studies were discussed at a recent meeting of the EU member state ecotoxicology experts intended to resolve some “recurring issues” in the assessment of active substances. The Central Zone has also discussed the use of residues data at Central Zone harmonization meetings. The bones of these discussions will be presented, together with some remaining issues and areas of uncertainty in the assessment of residues decline studies. The authors will provide further opinions and considerations when possible.

6.10PC.5
Bats in pesticide risk assessment in the EU: Conservative assumptions, potential refinements and industry activities
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Bats have only recently come into the focus of authorities responsible for pesticide regulation in Europe. At the mandate of European Commission, an EFSA Working Group is currently conducting the revision of the EFSA Guidance Document (GD) on Risk Assessment for Birds and Mammals (EFSA 2009). Recent scientific work indicated that some bat species may warrant further investigation and /or a dedicated risk assessment. The EFSA PPR Panel (EFSA 2019) published a scientific statement (Statement) on bat risk assessment and concluded that “bats would not be adequately covered by the current risk assessment scheme”. In this paper, the Statement has been reviewed and further literature identified on the elements required for a preliminary RA and potential refinements. Published EFSA conclusions for 36 compounds (insecticides, fungicides and herbicides) were screened and are used in a preliminary risk assessment. Exposure estimates following the assumptions in the Statement for oral and dermal uptake scenarios were used. Toxicity exposure ratios were based on the available mammalian toxicity data. Some of the preliminary assumptions in the Statement are worst-case estimates based on field work that did not have pesticide risk assessment as a primary focus. They should be corroborated and/or refined by further published studies and by field data that are specifically designed to inform a potential bat risk assessment and to select appropriate focal species. The preliminary impact assessment for the 36 approved substances showed that the acute dietary risk to bats is low, even with conservative assumptions. The chronic dietary and especially the dermal exposure scenario in the EFSA Statement would lead to a failure rate of >50%, even with the best-case assumptions in the Statement. Further data should be generated before designing a risk assessment scheme for bats that will inevitably lead to a high failure rate even for compounds that are non-toxic to birds and mammals. Since bat-specific data are lacking, there would currently be no possibilities for a higher-tier risk assessment in cases where a lower tier assessment with conservative assumptions indicated potential concerns. We will present a brief summary of the activities that industry (the European Crop Protection Association) is developing in the area of bats.

Future Risks of Chemicals (PC)

7.03PC.1
ECORISK2050: Emission, fate, effects and risks of chemicals in aquatic ecosystems under global change
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The Innovative Training Network ECORISK 2050 is a EU Horizon 2020-funded training platform for the assessment of urban and agricultural chemical risks over future decades, working from the prediction of chemical emission scenarios and global change to measuring and modelling exposure, effects, and risk, and developing mitigation strategies to these risks. 13 PhD students are working across eight universities and research institutes, supported by fourteen industry and regulatory governmental partners. Over the course of the programme, these researchers will (1) assess the evolution of emissions, fate and transport and risk of urban and agricultural chemicals over North, Central and South Europe, and how changes will vary under different global changes scenarios, (2) identify potential adaptive and mitigation strategies and propose implementation based on global change scenarios, and (3) develop tools for industry and policymakers to assess and manage global change-mediated chemical risks.

7.03PC.2
Long-term sub-surface irrigation with sewage effluent: Trends in time, depth and space of micro-pollutants disclosed by non-target screening
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Alternative water resources such as the reuse of sewage treatment plant (STP) effluent through sub-surface irrigation (SSI) offer the possibility of making optimal use of soil processes while irrigation water is being provided to crops. Indeed, studies done in river bank filtration, managed aquifer recharge and constructed wetlands have proven that the soil may have the ability to act as a filter and buffer zone. However, these systems were constructed for purification functions, while SSI also serves as an irrigation system which may have shorter residence times with regards to crop uptake and longer residence times for groundwater flow. In this study we investigate the fate of contaminants of emerging concern (CoECs) for three consecutive years in a cropland currently sub-surfacely irrigated with STP effluent from the STP Haaksbergen, The Netherlands. STP effluent, surface water, soil moisture and groundwater samples are collected at different monitoring points in depth and time. The aim of this study is to detect major distribution trends across the sub-surface irrigation system transect and characterize the identities and fate of organic micro-pollutants by applying state-of-the art nontarget screening. This presentation will discuss preliminary data.

7.03PC.3
Probabilistic risk assessment of pesticides under future scenarios: A Bayesian Network approach
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By 2050, land-use and weather patterns will shift as well as the way water and food resources are obtained and managed all over the world. In turn, this will alter the use and distribution of pesticides in agricultural areas. Probabilistic risk assessment typically takes into account the variability of species sensitivities, but not the variability in chemical exposure. In this study, an alternative approach to

probabilistic risk assessment is explored with Bayesian Network (BN) modelling, which integrates the variability in both exposure and effects in the calculation of a probabilistic risk quotient. We have developed a BN model that focuses on environmental risk of pesticides in a case study region representative of northern Europe. Exposure and environmental data for the scenario modelled was provided by the Norwegian Agricultural Environmental Monitoring Program (JOVA, https://www.nibio.no/tema/miljo/jord-og-vannovervaking-i-landbruket). Moreover, BNs can serve as meta-models that link selected input and output variables from other models and information sources. A conceptual model of the BN is presented, which can link the risk quotient to outcomes from future climate and land-use scenarios, manifested as changes in chemical exposure and biological effects. In the long term, the use of tools based on BN models by industry and policy makers will allow for a more refined assessment and targeted management of ecological risks.

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