The Belgian Society for Toxicology and Ecotoxicology

Annual Meeting 2023

Advances in the Risk Assessment of Chemical Mixtures

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Abstract Book

The Belgian Society for Toxicology and Ecotoxicology

Annual Meeting 2023

Abstracts Invited Speakers

Regulatory Implementation of a Scientific Approach for Mixture Risk Assessment

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There is growing societal and political concern about the risk of combined exposure to multiple chemicals, also referred to also referred to as mixture risk assessment. The European Chemical Strategy for Sustainability has highlighted the importance of mixture risk assessment and the European Food Safety Authority (EFSA) is working on a Roadmap on the Assessment of the Risk of Combined Exposure to Multiple Chemicals (RACEMiC).
The European Commission and the 27 Member States are funding the Partnership for the Assessment of the Risk of Chemical (PARC) to address future regulatory needs as described in the Chemical Strategy for Sustainability such as the need to perform mixture risk assessment for all chemicals. PARC will collect new human biomonitoring data, will improve hazard data collection of untested chemicals and will deliver new methods for regulatory risk assessment.
This Project Real-life mixtures aims to support regulatory risk assessment by providing methods, tools and concepts for human risk assessment of mixtures using data from HBM studies. Thirty-five institutes from 18 Member States joined forces to improve mixture risk assessment using HBM data.
The PARC project consolidate on international guidance from EFSA and the Organisation for Economic Co-operation and Development (OECD) describing the relevance of scientific grouping of chemicals affecting a target organ or a specific function of a target organ for conducting human risk assessment of mixtures . Furthermore, the PARC project consolidates on the recommendation and lessons learned from the EU funded HBM4EU project . Complementary to and in synergy with current activities at the European Agencies, the project developed practical tools, data, methods, and a general strategy to enable an effective mixture risk assessment based on HBM data.
HBM data from European studies have been listed in a data inventory. A process of harmonising the data from these partner studies has been initiated. Hazard data (including grouping into effect and toxicokinetic information) has started to be collected and organised. Statistical analysis (such as mixture identification) has been implemented in the Monte Carlo Risk Assessment (MCRA) toolbox and will be performed in a harmonised way in 2023-2024. Analysis is first carried out on prioritised chemical families (e.g. pesticides, heavy metals and PFAS) which are associated with specific health effects of concern (e.g. neurotoxicity, nephrotoxicity and immunotoxicity).
The PARC project Real-life Mixtures project will propose a coherent regulatory implementation of the data and models for evaluating the risk of mixtures by DG SANTE and DG ENVIRONMENT. This will go hand in hand with discussions with end-users being EFSA panels/ ECHA working groups, national experts and other relevant stakeholders such as the chemical industry.

The use of Mixture Assessment Factors (MAF) in Mixture Risk Assessment

Andreas Kortenkamp, Brunel University, London, UK

MAFs have been suggested as a pragmatic way of dealing with the issue of “cocktail effects” in chemical regulation. The idea is to include an additional factor for downward correction of “acceptable levels” for single chemicals to address the possibility of combined effects from large numbers of chemicals. Under discussion is the adoption of an a priori set factor of e.g., 5, but recently, calls for data-driven approaches for the sizing of MAFs have been growing. In this presentation, I will survey some mixture risk scenarios relevant to ecotoxicology but will then focus on data-driven approaches for human-relevant mixture scenarios, by way of specific case studies. These efforts show that MAFs larger than the currently proposed value of 5 are needed to afford sufficient protection. Data-driven approaches also open the way for estimating the residual risks associated with a priori set MAFs.

OECD Activities Using Relevant Effect Biomarkers and AOPs For Assessing Known and Unknown Mixture Risks.

Robert Pasanen-Kase (SECO, CH), Nancy B. Hopf (Unisanté, CH), Susana Viegas (ENSP/UNL, PT), Dan Villeneuve (US-EPA, US), Maryam Zare-Jeddi (Shell, NL), Rex FitzGerald (University of Basel, CH), Martin Wilks (SCAHT, CH), Radu Corneliu Duca (LNS, LU), Naoko Moritani (OECD, FR), Patience Browne (OECD, FR)

The OECD Occupational Biomonitoring Guidance Document (www.oecd.org/chemicalsafety/risk-assessment/occupational-biomonitoring-guidance-document.pdf) was published in 2022 as a joint activity under the Working Parties on Exposure and Hazard Assessment involving more than 40 institutes/organisations. The Guidance Document presents current regulatory and scientific approaches to derive occupational biomonitoring values and provides practical guidance on how to use them for risk assessment. In the OECD Guidance Document, the derived health-based human exposure biomarker assessment values are referred to as Occupational Biomonitoring Levels (OBLs) which are suitable for use in risk assessment and risk management. The methods described in the Guidance Document pave the way for high quality and globally harmonised occupational risk assessment, but are also limited to the availability of data sets, analytical capacities and speed of regulatory processes.
To be able to cope with these challenges, a new OECD effect-biomonitoring project using Adverse Outcome Pathways (AOPs) for mixture assessment was initiated in October 2022 for the following reasons:

(i) Effect-biomarkers are the only option to assess known & unknown exposures and mixtures in an integrative way.
(ii) Validated effect biomarkers can be used to address relevant health effect endpoints and Mode of Actions (MoAs) in humans and other organisms and can be linked to adverse effects using the developing Adverse Outcome Pathway (AOP) concept.
(iii) A systematic understanding of the relevance and interpretation of effect- biomarker data will lead to increased protection options for workers and environmental organisms.

The project includes around 80 experts from 20 countries and the work is divided among five drafting groups providing examples related to Endocrine Disruption, (Developmental)-Neurotoxicity, Genotoxicity & Oxidative Stress, Reproduction Toxicity and Exposure Assessment Quality Principles for effect-biomonitoring. The drafting group work is currently applied to already characterised effect-biomarkers and related case studies. The final project outcome will be practice-oriented guiding principles, including mixture threshold derivation concepts for relevant effect-biomarkers. With these practice-oriented mixture assessments, occupational and environmental exposures to chemical mixtures health effects can be addressed. These concepts are intended to be used for existing and future effect-biomarkers which can provide meaningful and robust links to adverse outcomes for an integrative risk assessment.

Relevance of Animal Studies in the Toxicological Assessment of Oil and UVCB Wax Hydrocarbons

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The human relevance of Animal Studies in the toxicological Assessment of food grade Oil and Wax UVCB Hydrocarbons will be presented by assessing the retention of "mineral oil saturated hydrocarbons" (MOSH) in human and animal tissues, with a particular focus on the liver as a target organ. Is the mere presence of MOSH in tissues considered adverse and appropriate for risk assessment and the derivation of health-based guidance values?
The assessment of UVCB hydrocarbons starts by understanding the compositional differences between oils and waxes, which are defined during manufacturing processes and have a direct impact in the final product specifications e.g. viscosity and associated regulatory requirements. Compositional differences determine what type of sub-fractions are important for hazard assessment in regards to assessing UVCB with variable compositions. These sub-class differentiation was a blind spot for decades in particular in the interpretation of repeated dose studies using the F344 or SD rat strains and their relevance to assess human health in the context of hepatic MOSH accumulation. While F344 rats exposed to mineral oil substances develop hepatic epithelioid granulomas, it is concluded that this effect is not relevant to humans. By using an AOP the mechanism of granuloma formation in rats is attributed to the retention of n-alkanes, which is not observed in the human liver. Furthermore, the retention of MOSH represented as a "gray cloud," in human and animal tissues' chromatograms, including the liver, does not lead to any adverse effects. It is concluded that the mere presence of MOSH, does not signify a hazard in and of itself. This is critical for risk assessment, as it underscores the distinction between hazard and exposure. An outlook on the latest EFSA assessment of MOSH is also presented. "

Cumulative Risk Assessment for Pesticide Residues. Status of EFSA-SANTE Action Plan

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EU Regulation 1107/2009 (placing of plant protection products in the market) and EU Regulation 396/2005 (on maximum residue levels in food and feed) require cumulative and synergistic effects of residues of plant protection products to be taken into consideration.
The European Food Safety Authority (EFSA) and the European Commission developed an action plan for gradual implementation of cumulative risk assessments for pesticides by EFSA and Member States from 2022 onwards . This plan provides that all toxicological effects of pesticides of relevance for CRA will have been identified by 2030 and cumulative assessment groups (CAG) in the respective organs and systems will have been established. This action plan on the implementation of CRA of pesticides and their residues will be part of the RACEMiC (Risk Assessment of Combined Exposure to Multiple Chemicals) project and will be integrated in the associated roadmap . Within this context, EFSA will further explore collaborations and synergies within the Partnership for the Assessment of Risks from Chemicals (PARC).
To ensure an optimal use of resources, EFSA is developing a prioritisation method which will allow the identification of pesticides and organ systems of relevance for dietary CRA. On the longer term, the implementation of the prioritisation method is intended to be repeated every three years.
It is estimated that between 8 and 15 organ/systems will require a comprehensive retrospective CRA. These assessments will provide a a baseline for the subsequent elaboration of prospective risk assessment (i.e. in view of regulatory decisions, e.g. on the approval of substances, authorisation of products or setting of MRLs). So far retrospective CRA have been performed for three organs: CRA for acute effects on the nervous system, CRA for chronic effects on the thyroid system and CRA for acute effects on craniofacial alterations . CRA for liver and kidney are ongoing. Examples of other CRA to be performed in the future are related to the reproductive (male and female), developmental toxicity, or developmental neurotoxicity.
EFSA will also focus on the integration of non-dietary exposure into retrospective CRAs, which will provide a clearer view on the risks associated to pesticides in use.

Chemicals Mixture Risk Assessment in the Environment: Regulatory Challenges and Scientific Opportunities for Industry

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As part of the Green Deal transition and in particular the Zero pollution strategy, the European Commission calls in the Chemicals Strategy for Sustainability for a systematic investigation of the impact of combined exposure into chemical risk assessments under REACH. To cover for such effects a default Mixture Allocation Factor (MAF) will be introduced to the risk characterisation calculated for each exposure scenario under REACH, hence reducing the safety limit of a single substance. The implementation of a MAF of 5 will have a large impact on the environmental risk assessment of inorganics (including metals and metalloids). Demonstrated by an extensive review of REACH exposure scenarios for metals, with a MAF of 5 2/3 or more of existing exposure scenarios would need revisions. However, exposure scenarios do not always reflect real life conditions as they describe generic conditions of use to cover for a broad set of manufacturing and use conditions, occurring in Europe. Additionally, given that inorganics are naturally occurring substances, the implementation of a default MAF results in some cases even in regulatory acceptance limits below natural background concentrations.

To anticipate the mixture toxicity correction under REACH, the sector of the non-ferrous metal industry, represented by Eurometaux, in collaboration with scientists from ARCHE Consulting and Ghent University, set up a dedicated research program on metal mixture toxicity in the aquatic environment as part of the Metals Environment Exposure Data (MEED)-program. The research program aims to deliver robust scientific evidence on the mixture assessment of naturally occurring substances, with the focus on those metals and inorganics that contribute most to the predicted overall risks of unintentional mixtures in Europe, i.e., the so-called Inorganic-Priority Contributing Substances (I-PCS). In a previous exercise using European monitoring data, the following inorganics have been identified as I-PCS for the aquatic ecosystem: Ag, As, Ba, Cd, Ce, Co, Cr, Cu, Dy, Er, Gd, Hg, La, Mn, Ni, Pb, Se, V, Y, and Zn. Key questions with respect to combined risk assessment are: I) are mixture effects of I-PCS relevant at regulatory and environmentally relevant exposure levels, II) can these effects be predicted based on standard mixture models, III) how do inorganics and organic substances interact when applied at environmentally and regulatory relevant concentrations, and IV) how eco-relevant are these assessments at local scale when measuring impact on biodiversity? The 3 first questions are addressed by reviewing the existing knowledge and by setting up of a dedicated research program investigating combined mixture toxicity of I-PCS at environmentally and regulatory relevant exposure levels. A review of existing studies has collected chronic metal mixture toxicity data for 24 different aquatic species and 14 metals in 33 combinations. Results show that chronic metal mixture effects can be predicted with the standard mixture reference models, concentration addition (CA) and independent action (IA), whereby CA results in more conservative predictions compared to IA. In addition, the best performing model depends on the trophic group considered. The literature review showed more non-interactive effects or antagonistic effects, while significant synergisms were less frequently observed, certainly when evaluated relative to the regulatory most preferred model CA. A Mixture Interaction Factor (MIF) has been proposed. The MIF quantifies the prediction performance of CA at low effect levels (i.e., 10% mixture effect), and is indicative of additive (MIF≈1), antagonistic (MIF>1) or synergistic (MIF<1) interactions relative to CA. For the extensive dataset, a median MIF of 1.3 has been derived, indicating that on average CA overestimates mixture toxicity at these regulatory and environmental relevant low effect levels. From a scientific perspective, the MIF may serve to refine for some of the conservatisms that are build-in in the regulatory MAF setting. The review demonstrated further that the absence of high-quality data on mixtures with anionic metals,

on more complex mixtures combining 4 or more metals, and on aquatic vertebrates, are main data gaps. In addition, only few studies investigated chronic mixture toxicity of combinations of inorganic and organic substances at environmentally and regulatory relevant combinations. Based on these data-gaps, a dedicated testing program has been developed aiming for filling gaps related to I-PCS metals to further evaluate the robustness and relevancy of the MIF correction factor for regulatory use under REACH. In parallel, MEED aims to develop a toolbox that allows validating the impact of mixture toxicity at local scale using a combination of mixture modeling, bio-monitoring and DNA/RNA monitoring techniques. All this should be ready before the mixture toxicity correction of REACH exposure scenarios should be introduced under the ongoing REACH update.

Towards a Harmonized Strategy for the Genotoxicity Assessment of Mixtures

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Humans are exposed to a wide variety of chemicals via food and environment, many of which are known or suspected to be genotoxic. Until today, the possible (geno)toxic risks of these chemicals are evaluated for each chemical individually. However, there is a growing societal concern regarding the combined effects following exposure to multiple chemicals. The need to extend risk assessment beyond the evaluation of individual chemicals and to include both intentional and unintentional mixtures has also been emphasized in the Chemical Strategy for Sustainability. As a result, several efforts have been undertaken to obtain more insights into the combined effects of chemicals. For non-genotoxic endpoints, the available evidence suggests that most chemicals affecting the same endpoint act without diminishing or enhancing each other's toxicity, also referred to as additivity. Synergistic and antagonistic effects, respectively defined as upward or downward deviations compared to this additivity, have also been reported. However, the methodologies applied in the studies reporting these types of combined effects often do not take into account the uncertainty of the individual data. Consequently, it is difficult to assess whether these effects are truly synergistic or antagonists or are instead still in line with the additivity principle when uncertainty is adequately considered. Despite all the past and ongoing efforts, tools and methods for mixture assessment thus need to be further improved to allow direct implementation in routine risk assessment.

Mixture genotoxicity is an example of a field where more work is required. One of the hurdles in assessing the combined effects of genotoxicants is that genotoxicity assessment so far mainly focuses on hazard identification, i.e. evaluating whether or not a chemical is genotoxic. A quantitative analysis of the data is needed to better understand mixture genotoxicity. Within this context, the benchmark dose (BMD) approach is of particular interest as it has been shown to allow the quantitative analysis of genotoxicity data and the evaluation of the combined effects in a mixture. In this presentation, a recently developed BMD-based approach to evaluate the combined impact of genotoxicants will be presented, and its strengths and limitations will be compared to other methodologies. Moreover, the application of the methodology in the field of mycotoxin mixtures will be discussed. Mycotoxin mixtures are particularly relevant as, on one hand, most fungi can produce a diverse cocktail of mycotoxins whereas, on the other hand, several fungal strains can contaminate the same food and/or feed matrix. Until now, data on the genotoxic effects of co-occurring mycotoxins are scarce, and therefore, more research is urgently needed.

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Abstracts Young Scientist Competition
Oral Presentations

Differential Pulmonary Toxicity after Combined Exposure to Silica and DEP in two Mouse Strains

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Background: Inhalation of airborne particulate matter, such as silica and diesel exhaust particles (DEP), poses serious long-term respiratory health risks. Silica exposure can lead to silicosis and systemic autoimmune diseases, while DEP exposure is linked to asthma and cancer. Combined exposure to silica and DEP, common in mining, may have (supra)additive effects. This study investigates the separate and combined effects of silica and DEP on lung injury, inflammation, and autoantibody formation in two genetically distinct mouse strains.
Objective: The study objectives are to understand the interplay between genetic susceptibility, combined particulate exposure, and disease outcomes.
Methods: Silica and diesel exhaust particles were administered to C57BL/6J and NOD/ShilLtJ mice via oropharyngeal aspiration. Assessments included in vivo lung micro-computed tomography, lung function tests, bronchoalveolar lavage fluid analysis including inflammatory cytokines and antinuclear antibodies, and histopathology with particle deposition and colocalization using Raman spectroscopy.
Results: Silica exposure elicited a well-established inflammatory response marked by inflammatory infiltrates, release of cytokines, and chemokines, alongside limited fibrosis, indicated by collagen deposition in the lungs of both C57BL/6J and NOD/ShilLtJ mice. Notably, these strains exhibited divergent responses in terms of respiratory function and lung volumes, as assessed through micro-CT imaging. Additionally, silica exposure induced airway hyperreactivity and elevated anti-nuclear antibody levels in bronchoalveolar lavage fluid, particularly in NOD/ShiLtJ mice. Lung tissue analysis revealed DEP-loaded macrophages and co-localization of silica and DEP particles.
Conclusion: Mouse strain variations exerted a substantial influence on the development of silica-induced lung alterations. In contrast, the additional impact of diesel exhaust particles on these silica-induced effects was minimal.

Do Human and Rat Alveolar Macrophages share Common Responses to PSLT Overload?

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Background: Inhalation of poorly soluble low toxicity (PSLT) particles, e.g. titanium dioxide and carbon black, can induce chronic inflammation and lung cancer in rats at inhalation exposure levels leading to lung particle overload. Overload is associated with impaired function and mobility of alveolar macrophages, and although PSLT overload has been observed in several experimental species such as rats and mice, the rat appears to be the only one to develop chronic inflammatory and carcinogenic lung responses. Whether the responses of rats or mice can be expected in humans exposed to PSLT particles remains a source of debate, and has led to the classification of PSLT as a possible human carcinogen. To gain a better insight of the possible human carcinogenicity of PSLT particles, we compared the responses of human alveolar macrophages to those of rat and mouse alveolar macrophages, under PSLT overload.
Method: Primary alveolar macrophages from rats, mice and humans were exposed in vitro to titanium dioxide or carbon black particles at doses leading to control, non-overload or overload conditions. Four days after exposure, untargeted transcriptomic analyses were performed and the genome expression profile of the three species were compared.
Results and discussion: Following titanium dioxide or carbon black particle overload, rat alveolar macrophages were the most sensitive as, compared to mouse or human macrophages, hundreds of genes were significantly differentially expressed. Among these, we identified 18 genes that were observed and confirmed to be significantly modulated in the same way by titanium dioxide and carbon black particles, specifically under overload conditions (compared with control and non-overload conditions). The relative function and direction of expression modulation of these 18 genes were mostly relevant to overload, inflammation and cancer development, indicating that these genes may act as key mediators in the inflammatory and carcinogenic lung responses of rats exposed to PSLT overload. Modulation of these genes in rat macrophages did not correlate with modulation of orthologous genes in mouse macrophages, confirming the in vivo studies observing that rats and mice respond differently to PSLT overload. The modulation of these genes was, however, positively correlated when comparing rat and human alveolar macrophages, indicating that human and rat alveolar macrophages share common responses to PSLT overload.
Conclusion: To conclude, rat and human alveolar macrophages share common responses to PSLT overload indicating that inhalation of high doses of PSLT particles leading to overload may constitute a hazard for humans.

A Benchmark Dose-based Strategy for Evaluating the Combined Effects of Genotoxic Compounds

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Until today, chemical risk assessment strongly relies on exposure and toxicity data of single compounds. However, in reality, we are generally not exposed to one individual compound but instead to a variety of chemicals. In 2012, the European Commission already expressed its concerns about the risks related to the combined effects of chemicals. The three major types of these combined effects are additivity, synergism and antagonism. For mixtures of compounds affecting non-genotoxic endpoints, it is generally assumed that the principle of additivity applies. It is however not clear whether this principle also applies to compounds affecting genotoxic endpoints. Furthermore, risk assessment of mixtures is a complex challenge and no harmonized approach currently exist to evaluate the combined effects of chemicals.

This study aimed to develop an appropriate strategy for testing genotoxic mixtures in the in vitro micronucleus (MN) assay. To this extent, two types of binary mixtures were evaluated for their potential to induce chromosome damage in vitro. The first consisted of two genotoxicants with a similar mode of action (MoA), i.e. ethyl methanesulfonate (EMS) and methyl methanesulfonate (MMS), two DNA-alkylating agents. The second mixture contained two genotoxicants with a different MoA, i.e. MMS and etoposide (ETP, a topoisomerase II inhibitor).

First, in vitro MN data in TK6 cells for the individual compounds were collected in the absence of S9 metabolic fraction. Next, benchmark concentrations (BMC) of the two compounds inducing the same response level were calculated. Afterwards, the BMC values were used to select a range of binary mixtures expected to induce responses covering different parts of the concentration-response curve (low, moderate and high responses). The binary mixtures were also analysed in the in vitro MN test and the experimental data were compared to the responses predicted based on the data of the individual compounds using the PROAST dose-addition model 15 in R.

Both for the binary mixtures of EMS-MMS and those of ETP-MMS, the experimental data were close to the fitted curve based on the data of the single compounds assuming additivity. Our results thus demonstrate that the principle of additivity is applicable to genotoxic compounds with both the same and a different MoA. Moreover, as it appropriately takes into account the variability and uncertainty of experimental data to evaluate combined effects, the developed strategy will be of high value for the future work in the field of genotoxicity assessment of mixtures.

Using an Adverse Outcome Pathway Based-approach to evaluate the Thyroid Hormone System Disrupting Potential of Resorcinol in Fish

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Thyroid hormones (THs) are crucial for normal vertebrate development, as they are involved in regulating processes such as growth, brain development, and energy metabolism. Compounds capable of interfering with the TH system therefore raise concern for both human and environmental health. One such compound is resorcinol. Resorcinol is used for a variety of different applications, going from use in the rubber industry to use in pharmaceuticals. Previous research has shown that resorcinol is capable of inhibiting thyroperoxidase (TPO), an enzyme crucial for TH synthesis, in vitro. Additionally, resorcinol has been linked to thyroid hormone system disruption (THSD) in humans. There is however only limited information regarding the effects of resorcinol exposure on ecotoxicologically relevant species.

In this study, we assessed the THSD potential of resorcinol in the zebrafish embryo model using an adverse outcome pathway (AOP)-based approach. AOPs linking THSD to both impaired swim bladder inflation and impaired eye development in fish were previously developed. In a first step, in vitro assays were performed to determine the different modes of action through which resorcinol might interfere with the TH system. In addition to confirmation of potent TPO inhibition, two new mechanisms were identified: TH receptor (TR) antagonism and transthyretin (TTR) binding. In the next step, zebrafish embryos were exposed to resorcinol. Exposure resulted in a significant decrease of thyroxine (T4), the main synthetized TH, indicating that resorcinol can inhibit TPO in vivo. Furthermore, gene transcript analysis revealed that the TH system was disrupted at the mRNA level. Resorcinol exposure also resulted in a significant increase of uninflated swim bladders. This effect could not be rescued by T4 supplementation, indicating that the impaired swim bladder inflation is not due to TPO inhibition. It is therefore likely that the effect on the swim bladder is caused by one of the other two identified mechanisms: TR antagonism or TTR binding. Swimming performance was significantly impaired in larvae with uninflated swim bladders and the effect was concentration-dependent, indicating that resorcinol exposure might result in neurotoxic effects.

This study shows that resorcinol is a TH system disrupting compound, both in vitro and in vivo. Furthermore, this research illustrates how an AOP-based approach can be used to consider the contributions of multiple mechanisms when evaluating the THSD potential of a chemical.

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Abstracts Young Scientist Competition Posters

Y1: Which Organic Substances occur most frequently in Environmentally Relevant Mixtures with Metals in European Freshwater?

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Ecological risks of chemical mixtures are predominantly assessed within single compound groups rather than simultaneously accounting for different groups of chemicals. Few studies are available describing the chronic mixture toxicity of metals and organic pollutants and most of these studies have been conducted at concentrations that have neither environmental nor regulatory relevance. This study aims to prioritize organic substances that potentially pose an aquatic mixture risk in binary pairs with metals. An in-depth assessment of the European Environment Agency's Waterbase monitoring data set allowed to identify 16 metals and several organics as substances that potentially contribute to aquatic mixture risk. All organic substances, in Waterbase, with an individual CAS number were included in the analysis (625). 609 PNECs were derived from various open-access databases. 16 compounds were excluded because no PNEC could be found in any of the consulted databases, leaving 609 organic compounds for the analysis. To priority-rank the organic substances, we calculated the percentage of mixtures at risk – based on the Risk quotient (RQ = PEC/PNEC) – for each metalorganic pair. This percentage was calculated by dividing the number of samples showing mixture risk (sum RQ > 1) where both the organic and metal contribute at least 10% to the risk by the number of samples where both compounds were measured together. The mixtures for which >10% were at risk were selected for prioritization and ranked in decreasing order. Further, we applied the same method on Daphnia magna and Raphidocelis subcapitata by using species-specific EC10 and NOEC values instead of PNECs. The PNEC based prioritization revealed 55 organic priority substances, of which 14 were as well of relevance in the D. magna targeted prioritization and 13 substances in the prioritization targeting R. subcapitata. It is possible, though, that this prioritisation may be somewhat biased to include more organic substances for which high assessment factors have been used in deriving their PNECs. The top priority organic compounds selected belonged to various substance groups with different modes of action such as pesticides (imidacloprid, metolachlor), pharmaceuticals (diclofenac, venlafaxine), and Industrial chemicals (bisphenol-A, phenol). This prioritization study identified realistic metal-organic mixtures driving the risk to aquatic communities in the field based on PNECs and species-specific endpoints. This work is part of the comprehensive Eurometaux “Metals Environmental Exposure Data” program (MEED) as project 4.

Y2: Advancing Skin Sensitization Testing: Exploring Mixture Assessment within the Adverse Outcome Pathway Framework

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Skin sensitization testing is a critical component of safety assessment for various consumer products, including cosmetics, medical devices, and household products. In the pursuit of safety testing without the use of animals, the OECD has adopted integrated approaches that rely on in chemico and in vitro assays that cover specific key events within the skin sensitization Adverse Outcome Pathway. However, these new approach methodologies have predominantly been validated to assess single chemical compounds, while the assessment of mixtures or complex formulations, a scenario often encountered in real-world exposure scenarios, has remained a substantial challenge.

To bridge this gap, we investigated the potential applicability of two test methods, focusing on key events 1 (protein haptenation) and 2 (keratinocyte activation), in the context of mixtures. To this end, we simplified the inherent complexity of unknown mixtures into three distinct groups: combinations of two known skin sensitizers, mixtures of a skin sensitizer with non-sensitizing agents, and mixtures comprising multiple non-sensitizers. Besides the complex composition of these chemical mixtures, it's crucial to note that the test concentrations for these unknown mixtures would inevitably deviate from the required test concentrations of the in chemico and in vitro tests. Consequently, we conducted experiments using the in vivo reference value (EC3-concentration) for these chemicals, deviating up to 100-fold from the mandatory testing concentrations.

When assessing skin sensitizers in mixtures through in chemico and in vitro tests, sensitivity appears to play a more critical role than complex mixture interactions. For the latter, no significant interactions have been observed, indicating a promising shift towards evaluating mixtures rather than single chemicals. Clearly, sensitivity of the investigated tests remains paramount, as positive results may suggest the presence of skin sensitizers in mixtures, while negative outcomes should be interpreted cautiously to avoid potential false negatives.

Y3: Particle-induced Autoimmune Features in C57BL/6J and NOD/ShiLtJ Mice

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Introduction: Occupational exposure to crystalline silica is associated with an elevated risk of autoimmune diseases in susceptible individuals, yet underlying mechanisms, likely influenced by gene-environment interactions, remain unclear. We aimed to investigate the immunotoxicological effects of exposure to crystalline silica, and a co-exposure with diesel exhaust particles (DEP), considering the effect of genetic background in C57BL/6J and NOD/ShiLtJ mice.
Methods. C57BL/6J and NOD/ShiLtJ mice were oropharyngeally exposed to a total dose of 4 mg quartz (median size about 2 µm) and/or 40 µg DEP (size ranging from 5.3 to 110 µm) through repeated instillations with animal ethics committee approval of KU Leuven (P111/2021). Ten weeks post the last exposure, bronchoalveolar lavage fluid (BALF), lungs and serum were collected to evaluate inflammatory cell count, lung histology, lung fibrosis and antinuclear antibody (ANA) presence.
Results: Silica exposed mice developed a local inflammatory response characterized by inflammatory infiltrates, recruitment of macrophages/neutrophils and mild lung fibrosis, which was more pronounced in the NOD/ShiLtJ mice. In BALF, the silica-exposed NOD/ShiLtJ mice showed significantly high ANA scores with about 40% of mice reaching a score of 3-4, while silica-exposed C57BL/6J mice had lower scores, only reaching up to 2. Silica-exposed NOD/ShiLtJ mice also developed systemic ANAs present in serum, with scores reaching up to 4, whereas silica-exposed C57BL/6J mice only reached scores up to 2-3. This inflammatory response and the development of ANAs was not seen in mice upon DEP only exposure and neither aggravated in silica + DEP exposed mice for either mouse strains.
Discussion: The autoimmune-prone background of NOD/ShiLtJ mice characterized by an H2(g7) MHC haplotype and genetic variants impacting tolerance and T cell functioning seems to render them more susceptible to developing an inflammatory phenotype possibly facilitating the development of autoantibodies. A co-exposure with a 10 times lower dose of DEP, not able to provoke an inflammatory or autoimmune-like effect by itself, also did not intensify these effects in silica-exposed mice. This could be due to the dose being too low and therefore not accurately representing a daily DEP background exposure attributable to environmental pollution.
Conclusion: The autoimmune-prone genetic background of the NOD/ShiLtJ mice appears to be a critical factor contributing to their susceptibility to develop silica-induced features of autoimmune disease and a co-exposure with a low background-like dose of 40 µg DEP did not aggravate the observed effects.

Y4: Does the Concentration Addition Model become a more Conservative Predictor of Aquatic Metal Toxicity with increasing Number of Metals in the Mixture?

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Risk assessment of chemical mixtures is conveniently regulated following the concentration addition (CA) model. A more accurate alternative for chronic metal mixture toxicity is provided by the independent action (IA) model. A quantifier to assess the deviations of observed toxicity from toxicity predicted with CA at low effect levels is called the MIF (Mixture Interaction Factor), which indicates a trend toward synergistic (MIF<1) or antagonistic (MIF>1) interactions, relative to CA, if present. This study aims to test the hypothesis that IA generally predicts better metal mixture toxicity than CA. In addition, it will, based on theoretical and mathematical considerations, test the hypothesis that the MIF increases with the number of metals in the mixture. This work is part of the comprehensive Eurometaux MEED (Metals Environmental Exposure Data) program, as project 5.
Ag, As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, and Zn were selected for testing as they were estimated to be the major contributors to mixture toxicity for Raphidocelis subcapitata in European freshwaters. For this species, it was estimated that in a mixture experiment, five metals are usually sufficient to explain 90% of the toxicity of the risk of the whole mixture. Therefore, 3 environmentally relevant groups of the above-mentioned 10 metals were selected for testing (i. As-Cu-Pb-Ni-Cd, ii. Mn-Zn-Ba-Cr-Cd, iii. As-Zn-Pb-Ag-Cu). Each experimental design consisted of testing simultaneously the 5 individual metals separately and in mixtures. The set of mixtures consisted of a binary (2 metals), a ternary (3), a quaternary (4) and 2 quinary (5) combinations. The experiments were conducted following an equitoxic ray design based on EC10 values, with one additional quinary mixture at environmentally relevant concentration ratios.
Overall, the two models showed rather similar results, with no clear trend toward synergistic or antagonistic interactions relative to the models. However, at environmental and regulatory more relevant concentrations (i.e., at 10% effect level), CA generally overestimated the mixture toxicity, as confirmed by the MIF values being all above 1, with an average MIF of 1.59 ± 0.59 (±: SD). Finally, the MIF did not increase with the number of metals in the mixture. Possibly, the toxicity of the metal mixture (and the magnitude of the deviation from CA) is more influenced by the relative concentration of the metals in the mixture.

Y5: Occupational Exposure of Firefighter Instructors to Polycyclic Aromatic Hydrocarbons in a Hot Training Exercise

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Firefighters are exposed to many hazardous substances, such as polycyclic aromatic hydrocarbons (PAHs), which are produced during incomplete combustion of organic matter. The compound benzo(a)pyrene, as well as the occupation “firefighter”, is classified by IARC as carcinogenic. Metabolites of PAHs can serve as biomarkers of exposure in human biomonitoring. In this study, we investigated the exposure of PAHs of firefighter instructors during two hot training exercises of 20 minutes. The training exercises existed of burning wood pallets in a closed container and in a two-level house. The personal protective equipment of the firefighter instructors consisted of turnout gear, thermal clothing, boots, structural firefighting gloves, nitrile gloves, hood, helmet, and Dräger PSS100 breathing apparatus. Between exercises, the firefighter instructors were wearing FFP3 masks. The ambient air of the hot training site and the nearby office workplace (control group) was assessed. A high-flow pump was connected to a sampling train consisting of an IOM cassette (PTFE filter) to assess the inhalable particle fraction and an absorbent silica tube to measure the volatile gaseous fraction. In addition, the concentration of ten PAH metabolites was assessed on three urine samples (0h, 8h and 24h) of eight firefighter instructors (FF) and eight office workers (OW). The compliance of the biological exposure index (BEI) for 1-OH-pyrene was carried out using the Altrex Biomonitoring. Each study participant completed a questionnaire about demographic characteristics, fire interventions, smoking and diet behaviour, and medical history. For the ambient air measurements, benzo(a)pyrene, benzo(a)anthracene and indenol(1,2,3-cd) pyrene were mainly found for the particulate phase and naphthalene was the most prominent PAH that was found in the gaseous phase. For the urinary metabolites, a difference in concentration of 1-OH-naphthalene between FF and OW (8h) was found to be significant (46.25 µg/g versus 0.88 µg/g creatinine). For 2-OH-naphthalene, a significant difference was found between the FF and the OW after 8h (39.79 µg/g versus 0.87 µg/g creatinine) and after 24h (2.12 µg/g versus 1.47 µg/g creatinine). For 1-OH-pyrene, significant differences between FF and OW were found after 8h, and four FF even exceeded the BEI, also after adjustment of the pyrene/benzo(a)pyrene ratio (0.54) found on the ambient air measurements (FF = 3.68 µg/L, OW = 0.74 µg/L, and BEI = 1.35 µg/L). This study demonstrates that firefighter instructors and, in general, firefighters can have substantial PAH uptake during fire interventions, showing the need to improve exposure prevention further.

Y6: Bronchial Epithelial Spheroids for Respiratory Toxicology: Development and Validation

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Background and Aim: Driven by ethical, scientific, and economic concerns, several fields of biomedical research have aimed to shift from the frequently used laboratory animals to the use of validated alternative methods. This has led to a vast increase in the use of two-dimensional (2D) cell cultures. Nevertheless, it is well known that the expression of genes and the cell-cell interactions are altered in 2D-cultured cells, influencing their response to external stimuli. As a result, the translatability of the obtained results to the in vivo setting can be questionable. Alternatively, great strides have been taken in the development of more complex three-dimensional (3D) cell cultures, which can better recapitulate the tissue-like physiological and morphological features. The use of 3D cell cultures can result in a more accurate prediction of human outcomes, caused by exposure to environmental contaminants. Even though the prospect of a more accurate risk assessment is enticing, no guidelines or standard operating procedures for the use of 3D cell cultures in regulatory toxicity assessment currently exist. Our aim was to develop a high throughput and cost-effective 3D cell culture comprised of human bronchial epithelial cells, useable for assessing key endpoints commonly assessed in respiratory toxicology.
Methods: We generated stable spheroids by culturing 16HBE14o- cells as a hanging drop. Spheroids of hanging drops were collected and transferred to an agarose-coated well plate for further culturing and exposure. For characterization of the spheroids, the dissociation of the spheroids to single cells was optimized which allowed for the analysis of the cellular growth via flowcytometric cell-cycle analysis. The primary goal of this research was to be able to use the spheroids to study respiratory toxicological events. Therefore, spheroids and protocols were made compatible with existing assays to asses genotoxicity, cytotoxicity, oxidative stress, apoptosis, expression of genes, and more. For validation, spheroids were exposed to varying concentrations of DMSO or MMS, and a comparison with 2D cultured cells was made.
Results and Conclusion: We found that spheroids showed an increased expression of CLDN1 as well as signs of G0/G1 arrest. Moreover, spheroids were successfully used to assess the genotoxicity, cytotoxicity, oxidative stress, and apoptosis caused by DMSO and/or MMS. In addition, spheroids exposed to high concentrations of DMSO appeared to lose their tight conformation and appeared to disintegrate. Eventually, these bronchial epithelial spheroids can promote the use of 3D cell cultures for regulatory respiratory toxicity assessment.

Y7: Airborne Micro and Nano Plastics in Indoor Areas

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Background: Micro and nano plastics (M/NPs) are widespread, even in remote areas, raising significant health concerns. While the majority of research on M/NPs has predominantly focused on marine and soil environments, the underestimated threat of exposure to airborne M/NPs (aM/NPs) is concerning. These particles, characterized by their low density and small size, can pose potential health risks through inhalation and ingestion. One of the major challenging aspects in comprehending these risks pertains to the complexities of sampling, measurement, and analysis of these particles in the atmosphere. To address this issue we critically screened the advantages, limitations, and practical applicability of various techniques and methods employed so far for the assessment of aM/NPs. Subsequently, In line with our study's primary objective of assessing human risk, a trial experiment was conducted to investigate indoor exposure to aM/NPs, considering human activity intensity.
Materials and Methods: We systematically searched PubMed and EMBASE to extract data up to the year 2022 regarding sampling strategies, identification methods, and reporting data for aM/NPs quantification. MP deposition samples were collected from 3 types of rooms within a laboratory in Leuven, Belgium using glass funnels attached to Erlenmeyer flasks over a period of 10 working days. This experiment was executed during 4 different periods: Low activity/Cold season, High activity/Cold season, Low activity/Warm season, and High activity/Warm season. Concurrently, we monitored environmental parameters, including CO2 concentration, flow rate, relative humidity, and temperature. For quantification, macro imaging and ImageJ software were employed, while morphological attributes were examined using a stereomicroscope. To identify the chemical composition, reference materials were used to optimize qualitative analysis through µ-Raman, Pyro-GC/MS, and high spectral imaging techniques.
Results and Discussion: Our prior study highlighted the importance of careful consideration in five critical steps: pre-sampling, sampling, post-sampling, analysis, and contamination control, all of which are integral to yielding precise results regarding human exposure to aM/NPs. Our findings consistently revealed the highest M/NP concentrations in the kitchen and dining area across all seasons, notably in the cold season. During the cold season, human activity drove aM/NP levels in offices, kitchens, and dining areas, while in the warm season, external sources, combined with human activity, increased particle counts. Laboratories predominantly experienced exposure due to human activity.
Conclusions: A comprehensive assessment of aM/NPs necessitates diverse techniques and contamination control measures. Human exposure to particles is influenced by factors such as human activity, cleaning schedules, and air quality.

Y8: Boscalid, Pyraclostrobin and their Mixture induce a Mitochondrial Dysfunction in Human Hepatocytes

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Fungicides are extensively used in agriculture for crop protection. The most commonly used classes of fungicides are inhibitors of the electron transport chain, including succinate dehydrogenase inhibitors (SDHIs) and strobilurins which act by blocking complex II and complex III, respectively. In addition, to deal with the emergence of resistance to conventional pesticides, the agrochemical industry resorts to the use of cocktails of phytosanitary products.
In a first study, we analyzed the effect of boscalid and bixafen (two SDHIs) on the mitochondrial function of human hepatocytes. We observed that both SDHIs (1 µM concentration) induced a decrease in oxygen consumption rate (OCR) and an increase in mitochondrial superoxide. Flow cytometry revealed an increase in the number of early apoptotic cells in human hepatocytes exposed to both SDHIs [1].
In a second study, we analyzed then the impact of the exposure of human hepatocytes to pyraclostrobin, a fungicide belonging to the class of strobilurins. Using electron paramagnetic resonance (EPR), we observed a decrease in OCR and an increase in mitochondrial superoxide levels after 24 h exposure to 0.5 µM concentration. As a consequence, the content in ATP amount in the cells was reduced, the ratio reduced/oxidized glutathione was decreased, and a decrease in cell viability was observed using three different assays [2].
As SDHIs and strobilurins are commonly associated in commercial preparations, we evaluated a potential “cocktail” toxic effect. For this purpose, we selected low concentrations of boscalid (0.5 µM) and pyraclostrobin (0.25 µM) that did not induce a mitochondrial dysfunction in liver cells when used separately. In sharp contrast, when both compounds were used in combination at the same concentration, we observed a decrease in OCR, an increase in mitochondrial superoxide production, a decrease in the ratio reduced/oxidized glutathione, and a decrease in cell viability in three different assays [2].

D. d’Hose et al, Molecules 26, 5842, 2021
M. Carbone et al, Molecules 28, 7013, 2023

Y9: Short-term and Long-term Effects of Marine Algal Toxins on Copepod Nitokra spinipes under Fluctuating Thermal Conditions

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Harmful algal blooms are proliferated algae densities that are often able to produce different kinds of toxic metabolites. These blooms have been found in increasing quantities in northern ocean. Research has established that sudden increases in nutrients — often from agricultural fertilizers — contribute to HAB occurrence and it has also linked warming temperatures to individual events. But the broader influence of climate change on these outbreaks is less well quantified. Several studies have indicated that temperature variations can markedly influence harmful algal growth and toxin content. However, limited research has been conducted on the toxicity of metabolites from harmful algae under fluctuating temperatures. This is especially relevant for zooplankton, a crucial component of aquatic ecosystems. They do not only consume algae but also serve as prey for organisms at higher trophic levels, hence, playing a pivotal role in energy transfer and nutrient cycles in marine food webs. Therefore, we aimed to examine the impact of marine toxins on marine zooplankton in the context of climate change. This will furnish critical insights into the interaction between climate change and toxins in marine ecosystems. Hence, in this study, we designed a series of experiments to assess the toxicity of four commonly occurring algal toxins on the model organism, Nitokra spinipes, exposed to three different temperatures. We first evaluated acute toxicity of domoic acid and yessotoxin, respectively. Adult females were exposed to these toxins at 15, 20, and 25°C for 48 hours, while nauplii aged 48 to 72 hours were exposed at 18, 20, and 22°C for the same duration. The results revealed no significant difference in mortality across the three temperature levels. In the long-term toxicity test, the developmental and reproductive toxicity of domoic acid, yessotoxin, saxitoxin, and microcystin-LR were examined at 18, 20, and 22°C. Laval development ratio, brood size and inter-brood time were recorded.

Y10: Nanoplastics stimulate Extracellular Polymeric Substance (EPS) Production in Marine Algae and Adversely affect Population Density

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In context of the ever-increasing plastic waste accumulation in the marine environment, it is important to understand the interaction between microalgae and nanoplastics (NP), and the role of extracellular polymeric substances (EPS) within this. EPS production is a known algal stress response, and its adhesive properties may induce aggregation of both algae themselves and the hetero-aggregation with plastic particles, which in turn may affect ecological and hydrodynamic processes such as the trophic transfer of nanoplastics or their vertical transport. In this study, the impact of fragmented, polydisperse polyethylene terephthalate (PET) nanoplastics on algae growth and the production of EPS was analyzed, through the exposure of the marine algae Rhodomonas salina to low NP concentrations (10, 100 1000 and 10000 NPs ml-1) during their entire growth cycle. Both virgin and aged PET nanoplastics were used, to assess the effect of weathering on the toxicity of plastics. Kaoline particles were included in the experiment to control for particle effects. The data was analyzed by means of log-logistic growth-models fitted to the data. Both exposure to virgin PET and aged PET resulted in significant effects on algae growth rate, and decreases in algae population density. Interestingly, the effect size was significantly higher for the aged PET particles. Furthermore, the observed adverse effects on growth were accompanied by and proportionate to significant increases in the production of EPS. We hypothesize that the EPS-production as a stress response requires metabolic energy that can no longer be attributed to growth. This research raises interesting questions about the production of EPS as an algal protection mechanism and indicates a role for EPS in the algae-NP interaction and their respective fates. Furthermore, it highlights the importance of using environmentally realistic test-materials, as their effects significantly differ from virgin test-materials.

Y11: Analysis of Preservatives and Fragrances in Topical Medical Devices: The Need for More Stringent Regulation

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Introduction: Medical devices and cosmetics have a long history of use, and come with regulatory frameworks to ensure user safety. Although topical medical devices might be applied to damaged skin, their composition is often very similar to that of cosmetics applicable to intact skin, especially in terms of preservatives and fragrances. However, unlike cosmetics, these products are not subject to compound-specific restrictions when used in medical devices.
Objective: This study aimed to identify and quantify preservatives and fragrances in class I and IIa topical medical devices and subsequently assess their safety towards the Cosmetic Regulation (EC) 1223/2009.
Method: A total of 69 topical medical device products available on the EU market were subjected to previously established and validated LC-MS/MS and GC-MS methods to identify and quantify occurring preservatives and fragrances.
Results: Findings revealed that 22 of the examined medical devices did not provide comprehensive ingredient lists, leaving users uninformed about potential risks associated with product use. Furthermore, 20 of these medical devices would not meet safety standards for cosmetic products and, most significantly, 9 of the analyzed samples contained ingredients that are prohibited in leave-on cosmetics.
Conclusion: Our results highlight the pressing demand for more stringent requirements regarding the labeling and composition of medical devices to protect patient safety. Improved regulation and increased transparency can mitigate potential health risks associated with the use of topical medical devices, while also ensuring they adhere to the same safety standards as cosmetics.

Y12: Potential Public Health Risks of Trace Metals across the Drinking Water Supply Chain of Addis Ababa, Ethiopia

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These days, the global distribution of trace metals in the environment is increasing as a result of industrialization and other anthropogenic influences including urbanisation, agricultural activities and mining. The negligence in implementing, enforcing and monitoring environmental regulations, especially in low and middle income countries like Ethiopia, makes this problem even worse. Humans can be exposed to trace metals through different exposure routes like ingestion, dermal contact and inhalation. Chronic exposure has been associated with an increasing number of noncommunicable diseases including cardiovascular disease, neurological disorders, nerve damage, renal failure and even cancer. Furthermore, as reported by World Health Organisation (WHO), 80% of human diseases are associated with water pollution particularly in low and middle income countries. Thus, this research focuses on the exposure to dissolved trace metals via the drinking water.
Addis Ababa, the capital of Ethiopia, is the primary city with more than 5 million inhabitants. Despite this large number of people, very little attention is given for drinking water supply infrastructures and quality control measures. Studies reported poor water quality in terms of biological parameters, but little is known about the concentration of dissolved trace metals across the water supply line to the public taps.
Herein, the concentration of trace metals both in municipally supplied drinking water and bottled water in Addis Ababa are determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Moreover, this study tries to appraise the patterns of variability in trace metal concentrations across the drinking water production lines in different seasons, linked to the amount of rainfall. Accordingly, water samples are collected from reservoirs that fed the drinking water treatment plant, from the inflowing raw water and treated water outlet of the treatment plant, from storage tank and from municipal drinking water taps both in standard and slum areas of the city both during dry and wet seasons.
Having a full image of understanding the source, pattern of variability in concentration of trace metals and contamination level of drinking water sources will be a key factor in identifying key sources of exposure risks and develop appropriate mitigating measures.

Keywords: Trace metal, drinking water, public health risks, Ethiopia

Y13: Combined Western Diet and Bisphenol A Exposure induces an Oxidative Stress-based Paraoxonase 1 Response in Larval Zebrafish

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Paraoxonase 1 (PON1) is an enzyme linked to metabolic disorders by genome-wide association studies in humans. It functions as a high density lipoprotein (HDL)-bound antioxidant enzyme with the additional ability to metabolize some metabolic-disrupting chemicals (MDCs). MDCs, such as bisphenol A (BPA), together with genetic and dietary factors, can increase the risk of metabolic disorders. Taken together, these factors show the potential for PON1 to play an important role in metabolic disruption and the resulting metabolic disorders. However, the interaction between metabolic disruption and PON1 remains poorly studied, especially in non-human vertebrates. The objective of this study was to investigate how PON1 responds to the metabolic changes and oxidative stress caused by a western diet in larval zebrafish, and whether exposure to BPA alters the metabolic and PON1 responses. Zebrafish larvae at 14 days post fertilization were fed a custom-made nutritionally balanced diet without exposure to BPA or a western diet with and without aquatic exposure to two analytically confirmed concentrations of BPA for 5 days. A combination of western diet and 150 µg/L BPA exposure resulted in a stepwise increase in weight, length and oxidative stress, suggesting that BPA amplifies the western diet-induced metabolic shift. The positive correlation observed between the transcription of acetyl-CoA carboxylase, an enzyme responsible for fatty acid biosynthesis, and internal BPA concentration provides additional evidence for this amplifying effect of BPA. PON1 arylesterase activity was increased in all western diet and BPA exposure groups and PON1 lactonase activity was increased when western diet was combined with exposure to 1800 µg/L BPA. Both PON1 activities were positively correlated to oxidative stress. Based on our observations we hypothesize that the western diet caused a shift towards fatty acid-based metabolism, which was amplified by BPA exposure. This shift resulted in increased oxidative stress, which in turn was associated with a PON1 activity increase as an antioxidant response. The metabolic shift may also have directly contributed to the PON1 response by increasing HDL levels or the activity of lipid metabolism regulators such as the peroxisome proliferator-activated receptor gamma, both of which are known to stimulate PON1 activity. However, these mechanisms were not directly observed in this study. This is the first exploration of PON1 responses to metabolic challenges in zebrafish, and the first study of PON1 in the context of MDC exposure in vertebrates.

Y14: Impact of Endocrine Disruption on the Number of Hair Cells in Neuromasts of the Lateral Line Organ of Zebrafish Larvae (Danio rerio)

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Chemical pollution is a major public health concern since it causes a wide range of disorders (e.g. neurological and endocrine disorders). Especially the developing neurosensory system is a vulnerable target due to critical developmental processes (e.g. differentiation and migration). Endocrine disrupting chemicals (EDCs) can adversely affect this system and can therefore contribute to impaired neurodevelopment. However, there is limited information available on the adverse effects these chemicals may have on neurodevelopment. Therefore, new endpoints and test methods need to be developed in order to characterize these chemicals. A promising endpoint is the lateral line (LL) organ in fish. This organ comprises of neuromasts that detect changes in water motions and pressure gradients in the surrounding medium. One of the cell types present in these neuromasts are hair cells (HCs), which are mechanosensory receptors that convert mechanical stimuli into neural signals. Since the LL system is located at the epidermal surface of the fish, it is a favorable endpoint to use in order to determine adverse neurosensory effects of chemicals. In the last decades, LL research has mainly focused on aminoglycoside antibiotics and heavy metals while EDCs have received less attention. The goal of this research is therefore to determine the potential adverse effects of EDCs on the number of HCs of the LL in order to potentially develop a new approach methodology (NAM). This NAM would help in assessing the risk chemicals pose to neurodevelopment, while complying with the 3R principle. In this study, zebrafish embryos were exposed to a positive control (copper sulfate pentahydrate), estrogen receptor agonist (17α-ethinylestradiol), estrogen receptor antagonist (fulvestrant), two different thyroid hormone system disruptors (iopanoic acid, a deiodinase inhibitor, and methimazole, a thyroid peroxidase inhibitor) and an aryl hydrocarbon receptor agonist (β-naphthoflavone). Exposure lasted from <1 hours post fertilization (hpf) to 120 hpf, after which a membrane dye (FM1-43) was used to visualize and count the HCs of four neuromasts (two of the anterior LL and two of the posterior LL). From the EDCs, only methimazole reduced the number of HCs in all neuromasts. This research shows that there is a potential adverse effect on HC development due to thyroid hormone system disruption. Additional studies, using various test methods to study neuromast development and function as well as chemicals with additional modes of action, may help better characterize the potential link between HC development and endocrine disruption.

Y15: Alterations of Global DNA methylation, 8-Hydroxy-2'-deoxyguanosine and Telomere Length in Workers Occupationally Exposed to Hexavalent Chromium

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The European human biomonitoring initiative (HBM4EU) aimed to harmonise human biomonitoring and to improve chemical risk assessment. The HBM4EU chromates study involved seven European countries to characterize occupational exposure to Cr(VI). We applied a cross-sectional study design and used chromium in urine as the primary biomonitoring method for Cr(VI) exposure. Furthermore, the effect of occupational Cr(VI) exposure on 8-Hydroxydeoxyguanosine (8-OHdG), global DNA methylation, global DNA hydroxymethylation and telomere length in blood was investigated. Workers with potential exposure to Cr(VI) were included (n=254). As controls (n=114), healthy adult (18–70 years) office workers from the same companies as the exposed workers (referred to "industrial controls") or from other companies with no activities that were associated with Cr(VI) exposure (referred to "other controls") were recruited. In general, each exposed subgroup displayed significantly higher mean urinary Cr levels than the total control group, the industrial control (within company) or the other control (outwith company) subgroups ($p = 0.007$, MW test). The industrial controls (within company) had significantly higher internal exposure levels than the other controls (outwith company). The other controls (outwith company) had significantly higher global DNA methylation levels, lower levels of 8-OHdG and longer telomere length than all other exposure subgroups. In workers exposed to Cr(VI), global DNA hypomethylation, increased levels of an oxidative stress biomarker 8-OHdG and shorter telomere lengths were observed. Overall, these findings reinforce the results of exposure biomarkers, highlighting that in Cr-related industries, (office) workers exposure to Cr is associated with detectable alterations in biological effect markers.

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F1: Utilising the THSD-AOP Cross-species Network to explore the Use of Zebrafish Embryos as NAMs for extrapolating to Mammalian THSD

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Thyroid hormone system disruption (THSD) caused by anthropogenic substances is considered a human and environmental health threat. Consequently, multiple efforts are underway to develop fast and reliable test methods to detect compounds causing THSD. These efforts aim to reduce the need for rodent-based testing strategies by introducing new approach methodologies (NAMs) like zebrafish embryos. Under the current EU legislation, zebrafish embryos are not protected up to five days post fertilization. Further, the hypothalamic-pituitary-thyroid axis of vertebrates is highly conserved and thus, zebrafish embryos could be employed as in vivo NAMs to support the extrapolation of THSD effects to mammals. The adverse outcome pathway (AOP) framework is especially suited to support these extrapolations as it provides information about the linkages between a molecular initiating event (MIE) and an adverse outcome (AO) via causally linked key events (KEs). Combining single THSD AOPs, developed in different species, results in an AOP network for which we previously evaluated the cross-species applicability.

This cross-species AOP network forms the basis for a case study in which we are exploring the use of zebrafish embryo tests as NAMs for extrapolating THSD effects to mammals, incl. humans. In a first step we investigated whether the thyroid hormone system of mammals and (zebra)fish responds similar to THSD model compounds (methimazole, 6-propyl-2-thiouracil, perchlorate, iopanoic acid). This was done by means of a literature review which focused on the identification of MIEs, KEs and AOs that responded similar in mammals and (zebra)fish. The results support the suitability of KEs and AOs linked to impaired neural development and eye development for cross-species extrapolations. Secondly, altered thyroid hormone (TH) levels were identified as hub KEs in the THSD AOP network and therefore data on triiodothyronine (T3) and thyroxine (T4) levels were extracted from the literature. Baseline T3 and T4 levels were established for mammals and (zebra)fish and we are investigating TH levels of exposed/unexposed rats and zebrafish to evaluate whether changes in both species are comparable in direction and magnitude.

With our study we provide support for the use of KEs and AOs with cross-species relevance in an Integrated Approach to Testing and Assessment (IATA) utilizing zebrafish embryos as in vivo NAMs to raise concern for both human and environmental health. In particular, our semi-quantitative approach to compare TH level alterations across species will facilitate the effort of bridging THSD effects between (zebra)fish and mammals. We also identified data gaps that currently impede cross-species extrapolations.

F2: Ecotoxicological Assessment of Banned and Novel PFAS as Individuals or in Mixture– Introduction to a MSCA Project

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Per- and polyfluoroalkyl substances (PFAS) are synthetic organofluorinated compounds with strong hydrophobic and lipophobic properties, making them highly suitable for many applications. However, they are part of the emerging chemicals identified to be persistent and bioaccumulative. They are very stable, non-degradable, and present all around the world. More than 9000 distinct individual compounds make up the PFAS group, with a carbon chain backbone between 4 and 14 C atoms in length, and a charged functional moiety: sulfonate or carboxylate. The two principal representatives and studied PFAS (perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS)) are banned in many countries, leading the major global manufacturers to find replacement chemistry with multiple ether-oxygens inserted between the perfluorinated carbon backbones such as perfluoroalkyl ether carboxylic acid (PFECA) [e.g. hexafluoropropylene oxide trimer acid (HFPO-TA) and Perfluoro-3,6,9-trioxadecanoic acid (PFO3-3-6-9-DoA)] and perfluoroalkyl ether sulfonic acid (PFESA) [e.g. Perfluoro-3,6-Dioxa-4-Methyl-7-Octene-1-Sulfonic Acid, PFESA1 (Nafion By-product 1) and 7H-Perfluoro-4-methyl-3,6- Dioxaoctane Sulfonic Acid, H-PMO2OSA (Nafion By-product 2)], allowing the formation of non-covalent hydrogen bonds with a water molecule, which makes them more labile to degradation. However, these new chemicals have been identified in various environmental matrices are now becoming global contaminants. But our understanding of the toxicity of emerging replacement chemistry is lacking in aquatic organisms and even not unexplored. Given the detection of these emerging PFAS as complex mixtures (terminal compounds, degradation products, precursors, and metabolites) in diverse environmental matrices and sampled human blood, this project aims at developing a molecular Adverse Outcome Pathways (AOP) for the replacement chemistry (PFO3-3-6-9-DoA, HFPO-TA, H-PMO2OSA and PFESA1) at individual and whole mixture levels (PFOA+PFOS+PFO3-3-6-9-DoA+PFESA1+HFPO-TA+H-PMO2OSA), and lean on an AOP approach to predict the possible risks associated with the current anthropogenically-driven increase in their concentrations in aquatic organisms. To that purpose, our project will investigate the toxicity (acute, developmental, cellular, system and genetic) pathways for the replacement chemistry in three freshwater species (Daphnia magna, Gammarus fossarum and Danio rerio) as well as investigate the possibility of the PFAS replacement chemistry acting simultaneously with legacy PFAS to induce additive or synergistic or antagonistic effect. This project will build on the expertise of the researchers involved together with the acquired data to provide a basis for a better understanding of the influence of the effects on individual and population fitness to support a broader integration of these data into risk assessment frameworks.

Keywords: Replacement Chemistry, AOP, Mixture toxicity, Freshwater Aquatic Organisms

F3: Relative Fate Factor: Regulatory Framework for Soil Contaminated with Mixtures of PFAS

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The past and current use of per- and polyfluoroalkyl substances (PFAS) have resulted in increasing levels of environmental contamination, posing a threat to both human health and ecosystems. At present, the PFAS-family consists of several thousand different congeners, yet not much is known about their exact toxicological properties. This has significant implications for chemical risk assessment given that toxicological data is classically used for deriving regulatory threshold values. In Flanders for instance, regulatory values (i.e., soil-remediation values, SRVs) could only be derived for two congeners, namely PFOA and PFOS. There is thus an urgent need for a framework through which regulatory thresholds can be derived and risks of PFAS-mixtures can be assessed. In this study, a pragmatic framework is proposed through which the risk of PFAS-mixtures can be assessed; this is done by allocating weighing factors (the relative risk factor, RFF) to each congener, which are further combined with the concentration of each congener and aggregated over all congeners in a mixture, the result of which can be compared to SRVs. This concept is illustrated below for the perfluorocarboxylates (Eq. 1) and the perfluorosulphonates (Eq. 2):

$$\sum_{i=1}^{n} RFF_i \times C_i \leq SRV_{PFOA} \quad \text{(Eq. 1)}$$

$$\sum_{J=1}^{m} RFF_j \times C_j \leq SRV_{PFOS} \quad \text{(Eq. 2)}$$

Conceptually, the RFF-model will be constructed using *in silico* data and validated using experimental data. This way, the fate factor of less studied congeners can be simulated. Of note is that the proposed RFF-model is a tentative model that can further be expanded by additional physicochemical/fate-based parameters.

In a first-generation RFF-model, two parameters are combined namely the binding affinity of PFAS for human serum albumin (measured by ΔG_{bind}) and the biotransfer factor to milk (BTF_{milk}, as a proxy for elimination half-life).

The RFF-model would conceptually be constructed as the product between ΔG_{bind} and BTF_{milk}, both of which are mathematically transformed in such a way that their product is of relevance regarding environmental and human fate. This led to the development of the following model for the perfluorocarboxylates, in which ΔG_{bind} and BTF_{milk} are normalized according to the indicator-congener, PFOA:

$$RFF = \left(\frac{\Delta G_i}{\Delta G_{ind}}\right)^2 \times \sqrt{\frac{\frac{BTF_i}{BTF_{ind}}}{\frac{BTF_i}{BTF_{ind}} + 1}}$$

This model serves as a proof of concept for the development of future pragmatic risk assessment frameworks. The first-generation RFF-model described above consists of two terms; naturally, this can be expanded by other relevant properties so that PFAS-behavior in soil and the transfer thereof into humans can better be approximated.

This project is co-financed by the Flemish Waste Management Authority (OVAM) and the European Partnership for the Assessment of Risks from Chemicals (PARC)

F4: Spatio-temporal Patterns in the Gene Expression of the Copepod Temora longicornis in Response to Chemical Pollution

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

F5: Accelerating the (regulatory) Uptake of New Approach Methodologies via the RE-Place Project

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The implementation of the 3Rs Principle (Replacement, Reduction and Refinement) in EU Dir 2010/63 resulted in a boost of the development of methods (partially) replacing animal testing. The field of toxicology played a pioneering role in this respect. For certain endpoints, including skin corrosion, irritation and sensitisation, a full replacement of animal testing is already possible for regulatory purposes. However, the situation is more complex for other (regulatory) endpoints, such as systemic and reproductive toxicity, which currently require a complementary approach combining in vitro and in vivo experiments.
Methods linked to animal replacement are also referred to as 'New Approach Methodologies (NAMs)'. These include, amongst others, sophisticated cell- and tissue cultures such as organoids and organ-on-chip, computational modelling techniques, high throughput testing strategies, and the use of -omics. Generally, these methods do not involve the direct use of animals, although the term NAMs is sometimes also used to refer to animal methods that use less animals. While the importance of NAMs is increasingly recognized worldwide, their adoption and integration into (inter)national regulations is often lagging behind. Encouraging the development and use of NAMs may benefit from a bottom-up approach such as the 'RE-Place' project, which collects all existing expertise on the use of non-animal NAMs contributing to the reduction and replacement of animal testing in Belgium in one central, open-access database. This database, available via www.RE-Place.be, provides an up-to-date overview of the existing non-animal NAMs and links this information with the names of experts and institutes where the methods were developed or are currently applied.
At present, the RE-Place database contains 263 methods covering a wide array of applications in biomedical research, regulatory testing and education. Among these, approximately 100 methods are linked with toxicology including viability assays, organ-on-chip, machine learning and structure-activity relationship models. However, this is only a fraction of the expertise available in Belgium. The RE-Place team encourages scientists to submit their knowledge to facilitate the exchange of information and practical know how in order to reach the full potential of these technologies.
The RE-Place team also supports scientists to increase the visibility of their work by promoting it on social media and giving them the opportunity to present on symposia, study days and webinars. RE-Place hence promotes the development and use of non-animal NAMs in order to accelerate their regulatory uptake and contribute to the reduction and ultimate replacement of animal testing in the long term.

F6: Silicone Wristbands as Passive Samplers for Personal Exposure Assessment of Legacy Persistent Organic Pollutants: A Pilot Study

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Persistent organic pollutants (POPs) are a diverse group of chemicals characterized by toxic properties, resistance to degradation, bioaccumulation, and widespread transport through air and water. Despite global regulatory efforts, these substances are persistent in the environment, resulting in widespread contamination long after restrictions are in place. The continuous, ubiquitous exposure of individuals to these environmental chemicals highlights the critical need to assess personal exposure to legacy POPs, making it an emergent exposome-related research topic.

This pilot study presents a novel approach using silicone wristbands (SWBs) as passive samplers for the measurement of time-weighted average exposure to legacy POPs. In particular, organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) will be investigated. Challenges arise due to the presence of polychlorinated alkanes (PCAs), the predominant component of chlorinated paraffins (CPs). These interfere with the signal of POPs in the applied GC-NCI-MS method. In future studies, this was resolved by applying a GC-EI-MS/MS method.

The results (Fig. 1) showed that hexachlorobenzene (HCB) was consistently detected in all samples. The median concentration reached up to 150 picograms per gram of wristband (pg/g wb). In addition, PCB 153 (median 54.0 pg/g wb) and BDE 99 (23.9 pg/g wb) were found to have the highest concentrations of PCBs and PBDEs, respectively, among all worn SWBs. These results demonstrate the existence of individual differences in exposure and highlight the variability in external exposure patterns among the study participants. Hierarchical clustering analysis shows that the SWBs worn by participants PS03, PS09 and PS12 stand out as outliers, while the rest of the worn SWBs show similar exposure patterns to field samplers (FS) used in indoor and outdoor microenvironments.

Results from this pilot study contributes to the overall goal of establishing a universal sampling protocol for SWB-based personal exposure monitoring in general population and occupational scenarios. These findings have the potential to improve exposure assessment methodologies and deepen our understanding of the impact of environmental contaminants on human health. In addition to advancing the field of environmental monitoring, the study highlights the importance of developing robust tools for assessing chemical exposures in an era characterized by the persistent presence of POPs in the environment.

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