SETAC NORTH AMERICA 45TH ANNUAL MEETING

20–24 OCTOBER 2024 ★ FORT WORTH, TEXAS

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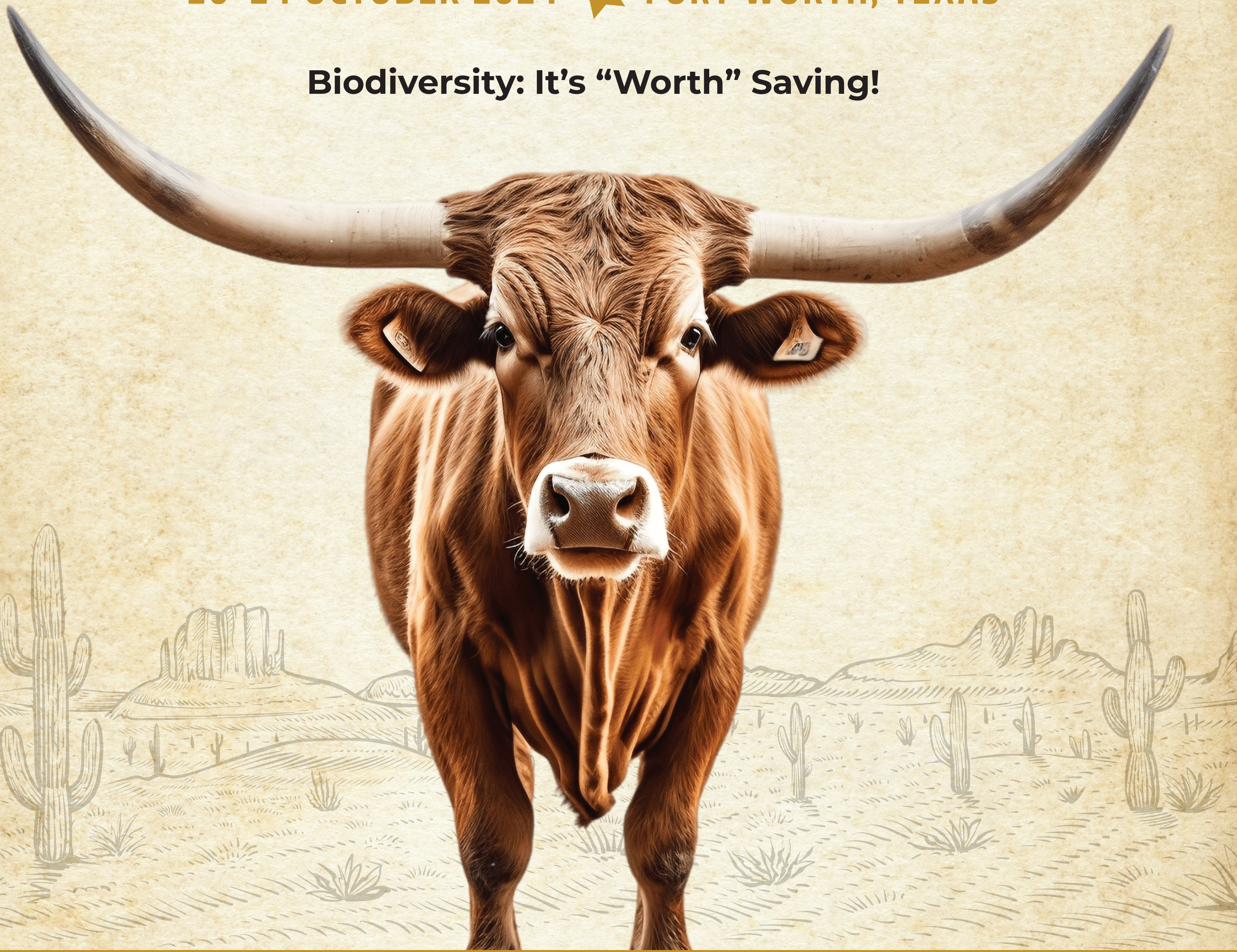

Abstract Book

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SETAC North America 45th Annual Meeting

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This book comprises the abstracts from the 45th annual meeting of the Society of Environmental Toxicology and Chemistry – North America (SETAC North America), conducted from 20–24 October 2024 in Fort Worth, TX, USA.

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About SETAC

The Society of Environmental Toxicology and Chemistry (SETAC), with offices in North America and Europe, is a nonprofit, professional society established to provide a forum for individuals and institutions engaged in the study, analysis and solution of environmental problems, the management and regulation of natural resources, environmental education, and research and development.

Specific goals of the society are:

- Promote research, education and training in the environmental sciences
- Promote the systematic application of all relevant scientific disciplines to the evaluation of chemical hazards
- Participate in the scientific interpretation of issues concerned with hazard assessment and risk analysis
- Support the development of ecologically acceptable practices and principles
- Provide a forum (meetings and publications) for communication among professionals in government, business, academia and other segments of society involved in the use, protection and management of our environment

These goals are pursued through the conduct of numerous activities, which include:

- Conduct meetings with study and workshop sessions, platform and poster presentations, and achievement and merit awards
- Publish scientific journals, a newsletter and special technical publications
- Provide funds for education and training through the SETAC Scholarship and Fellowship Program
- Organize and sponsor chapters and branches to provide a forum for the presentation of scientific data and for the interchange and study of information about local and regional concerns
- Provide advice and counsel to technical and nontechnical persons through a number of standing and ad hoc committees

SETAC membership currently comprises about 4,500 individuals from government, academia, business and nongovernmental organizations with backgrounds in chemistry, toxicology, biology, ecology, atmospheric sciences, health sciences, earth sciences, environmental engineering, hazard and risk assessment, and life cycle assessment.

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Environmental Quality Through Science®

Track 1: Environmental Toxicology and Stress Response

1.01.P-Tu Acquired Pollution Resistance: A Range of Mechanisms from Acclimation to Adaption, and Potential Fitness Costs

1.01.P-Tu-001 Differential DNA Methylation and Metabolomic Profiling of Killifish Populations Adapted to Contaminated Superfund Sites

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Populations of killifish species, such as *Fundulus heteroclitus* and *Fundulus grandis*, have rapidly evolved adaptations to local contamination with complex mixtures of toxicants. Several killifish populations inhabiting Superfund sites exhibit heritable tolerance to substances that induce the aryl hydrocarbon receptor (AHR) pathway, such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). This phenotype is linked to deletions in the AHR. However, there has been limited investigation into epigenetic and metabolic alterations in pollution-adapted killifish populations. To identify the involvement of epigenetic and metabolic regulation in the adaptive response of killifish (*F. heteroclitus*), we examined tissue- and sex-specific differences in global DNA methylation and metabolomic profiles between populations from New Bedford Harbor (NBH), a polluted site, and those from a nearby reference site, Scorton Creek (SC). Our results revealed evident liver-specific global DNA hypomethylation and different metabolic profiles in the NBH population. The sex-specific differences were not greater than the tissue-specific differences. Furthermore, we identified liver-specific enrichment of metabolic pathways, notably those associated with amino acid metabolic pathways converging into the urea cycle and glutathione metabolism, in the NBH killifish. Overall, our findings suggest potential metabolic mechanisms implicated in the adaptation of killifish surviving in NBH sites, underscoring the possible involvement of epigenetic regulation in these mechanisms. Follow-up work on epigenetic and metabolomic alterations in *F. grandis* (the sister taxon of *F. heteroclitus*) is underway to elucidate the functional significance of DNA hypomethylation in the transcriptional regulation and metabolism of adapted killifish. *This work was supported by the National Research Foundation of Korea grant funded by the Korea government (Ministry of Science and ICT) (NRF-2020R1A2C3006838).*

1.01.P-Tu-002 A Statistical Model to Identify Potentially Adapted Populations

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Upregulation of the Aryl Hydrocarbon Receptor (AHR) pathway and Cytochrome P450 (CYP450) enzymes for detoxification is a typical organismal response to xenobiotic exposure. Persistent organic pollutants (POPs) promote long-term xenobiotic exposure of species and thus persistent upregulation of the AHR. Induction of CYP450 may simultaneously exacerbate xenobiotic toxicity and produce reactive byproducts causing harm and disturbing organismal homeostasis -- intensified under persistent upregulation. However, organisms may evolve under changing environmental conditions, with wild populations' adaptive responses dependent on unique contaminant histories. For example, Gulf Killifish (*Fundulus grandis*) have been observed to adapt via a recalcitrant AHR pathway, resulting in reduced sensitivity to POPs.

Statistical models that use existing data sets to explore mechanisms of population contaminant responses without "controlled experimentation", are increasingly important to ecotoxicology for rapid-response and appropriate allocation of resources. This is particularly true when investigating uncommon mechanisms like adaptation in population genetics, which may support comprehensive ecological risk assessment but be obscured in traditional toxicity approaches. A statistical methodology for exploratory data analysis (EDA) was developed to identify potentially adapted Great Lakes Tree Swallow (*Tachycineta bicolor*) populations, a common ecotoxicology model species. Individuals from these populations have unique and relatively consistent contaminant-histories, as Tree Swallows return to previous nest boxes post-migration, making them ideal for developing the model. The model identified potentially adapted populations via anomalies in Tree Swallow relative ethoxyresorufin-O-deethylase (EROD) response (as a biomarker of CYP450-1A induction) to polychlorinated biphenyl (PCB) exposures, via linear regression on approximately 800 individual nestling samples from populations with complex exposure histories. An idiosyncratically low EROD response slope with increasing PCB exposure – particularly at sites of substantial historical PCB contamination -- suggested potentially adapted Tree Swallow populations for targeted field and/or laboratory experiments. Innovative EDAs on existing ecotoxicity and environmental chemistry data can supplement research into specialized responses like adaptation where experimental toxicity testing may be ineffective or resource prohibitive.

1.01.P-Tu-003 Acute Exposure to the Polychlorinated Biphenyl Mixture Aroclor 1254 Causes Mortality, Growth, but Limited Behavioral Effects in Early Life Stage Zebrafish

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Polychlorinated biphenyls (PCBs) are a class of persistent organic pollutants known for their toxicity to humans and wildlife. NDL PCBs constitute the greatest proportion of PCB congeners in the environment and have been shown to negatively impact cognitive and physical behaviors in fish when assessed shortly after exposure. The longer-term effects of exposure to the PCB mixture Aroclor 1254 on mortality, growth, and the complex behaviors of feeding and shoaling in zebrafish were investigated. Embryos were exposed at 6 hours post fertilization (hpf) to aqueous Aroclor 1254 for 96 hrs without renewal at nominal concentrations of 100 and 500 µg/L. Larval zebrafish were monitored daily for mortality and growth measurement taken at 10, 12, 14, 18, 25, 34, and 45 days post fertilization (dpf). Larval zebrafish were assessed for feeding behavior at 14 dpf and shoaling behavior at 26 dpf. The 500 µg/L treatment group had a significant mortality event from 9-15 dpf. This mortality event acted as a filter, leaving surviving individuals with coping mechanisms and normal development. Growth within the 500 µg/L treatment was significantly lower at 10, 12, and 14 dpf but was not significantly different for the remainder of the study. Total number of interaction events was significantly lower in Aroclor 1254 treatments in feeding trials. Time spent outside of thigmotaxis zone was significantly higher in Aroclor 1254 treatments in shoaling trials. Data from this study demonstrate acute effects on mortality and growth but show limited effects on longer-term complex behaviors after exposure to a PCB mixture in zebrafish.

1.01.P-Tu-004 Phenotypic and Molecular Characterization of Newly Discovered Pollution-Adapted Populations of Gulf Killifish (*Fundulus grandis*) from the Corpus Christi Inner Harbor, Corpus Christi, Texas, USA

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The Corpus Christi Inner Harbor (CCIH), a heavily industrialized region on Corpus Christi Bay on the southern Texas Gulf Coast, has historically been shown to contain significant levels of persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). Gulf killifish (*Fundulus grandis*), a model estuarine species previously shown to have the capacity to develop resistance to the developmental and acute toxicity caused by PAHs and PCBs, inhabits the CCIH estuary. Previous research from our laboratory has demonstrated resistance to PAH and PCB-induced cardiac teratogenesis via a recalcitrant aryl hydrocarbon receptor (AHR) pathway in populations from the Houston Ship Channel (HSC), Galveston Bay, Texas, with a history of substantial POP exposures. Gulf killifsh were collected from two populations within the CCIH and from two populations that were previously characterized within the HSC. One HSC population, Smith Point, is a reference population, and the other, Vince Bayou, is a positive control representing a fully adapted resistant population. F1 embryos from both CCIH populations exhibited an intermediate level of resistance to PCB-induced cardiovascular teratogenesis. Ethoxyresorufin-*O*-deethylase (EROD) assays confirmed a reduction in basal and induced cytochrome CYP1A activity in CCIH populations. The results are consistent with previously described resistant populations of *F. grandis* and of the sister species, *F. heteroclitus*. Decreased levels of POP-induced cardiovascular teratogenesis and a decrease in CYP1A inducibility suggest CCIH *F. grandis* populations have adapted to anthropogenic contamination via a mechanism similar to *F. heteroclitus* and HSC populations of *F. grandis*. Ongoing genomic analysis will evaluate mechanistic similarities or differences between CCIH and HSC adapted *F. grandis* populations, specifically focusing on the role of a deletion in the AHR.

1.01.P-Tu-005 Adaption to Long-term Pollution of Aryl Hydrocarbon Receptor (AhR) Agonists in Mosquitofish Inhabiting An Electronic Waste Recycling Area

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Anthropogenic impacts on global environmental changes may enable adaptive evolution to organisms inhabiting under polluted conditions. Electronic waste (e-waste) recycling activities have severely polluted local aquatic environment with various aryl hydrocarbon receptor (AhR) agonists such as dioxin-like compounds, but adaptive phenotypes to e-waste pollution are largely unknown. Here, we found that western mosquitofish (*Gambusia affinis*) population residing in a site with over 30 years of e-waste recycling history exhibited adaptation to an AhR agonist (phenanthrene), which showed 2-fold more resistant based on external and internal median lethal concentrations compared to mosquitofish raised in a reference site. Adapted population exhibited hyperactivity under stress, which may result from intrinsic shifts in energetic allocation. Increased cytochrome P450 1A (CYP1A) inducibility was observed in adapted population, indicating potential shifts in phenanthrene biokinetics. Toxicokinetic experiments further confirmed enhanced phenanthrene phase I metabolism of adapted population both *in vitro* and *in vivo*. Additionally, we also observed elevated phenanthrene phase II metabolism *in vivo*, which may be connected to several AhR and cardiac signaling alterations, demonstrating stimulated metabolism and detoxification as another alternative for pollution adaptation. Our results

emphasized the significance on the role of different levels of biological organization in predicting the impact of environmental change due to long-term industrial activities.

1.01.P-Tu-006 Combined Use of Traditional TIE Methods and Semi-Quantitative Chemical Scans to Resolve *C. dubia* Toxicity

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(1)Sanitation Districts of Los Angeles County, United States, (2)Los Angeles County Sanitation Districts

Acute toxicity was observed to *Ceriodaphnia dubia* when exposed to the Los Angeles County Sanitation Districts' tertiary treated wastewater effluent. Sanitation Districts' staff used both traditional Toxicity Identification Evaluation (TIE) methods and a novel process using a suite of semi-quantitative chemical scans to rapidly identify the causative agent. The multiple lines of evidence included chemical-based work including treatment plant associated dissimilarity evaluations, seasonal trends analysis, and a comparison between effluent concentrations and water quality criteria information. The results were later confirmed and potential source was removed, returning the wastewater facility to compliance far sooner than would be expected using the traditional TIE method alone. This poster summarizes the investigation and the Sanitation Districts' findings, along with recommendations on how this novel process might be used to facilitate future toxicity identification efforts.

1.02.A.T Advances in Bioaccumulation Science and Assessment

1.02.A.T-01 A Comparison of In Vitro Metabolic Clearance of Various Regulatory Fish Species Using Hepatic S9 Fractions

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Bioaccumulation predictions can be substantially improved by combining *in vitro* metabolic rate measurements derived from rainbow trout hepatocytes and/or hepatic S9 fractions with quantitative structure activity relationship (QSAR) modeling approaches. Compared to *in vivo* testing guidelines OECD 305 and OCSPP 850.1730, the OECD Test Guideline (TG) 319A and 319B are *in vitro* approaches that have the potential to provide a time and cost efficient, humane solution, that reduces animal use while addressing uncertainties in bioaccumulation across species. This study compares the hepatic clearance of the liver S9 subcellular fraction of the rainbow trout, bluegill, common carp, fathead minnow and largemouth bass, discerning potential differences in metabolism between different warm- and cold-water species. With refinements to the *in vitro* metabolic S9 assay for high-throughput analysis, we measured *in vitro* clearance rates of seven chemicals crossing multiple classes of chemistry and modes of action. We confirmed data from rainbow trout liver S9 fraction metabolic rates can be utilized to predict rainbow trout BCF using an IVIVE model, as intended in the OECD 319B applicability domain per the bioaccumulation prediction. Secondly, we determined that OECD 319B can be applied to other species, modified accordingly to their environmental conditions (e.g. temperature). Once toxicokinetics for each species is better understood and appropriate models are developed, this method can be an excellent tool to determine hepatic clearance and potential bioaccumulation across species. This research could be leveraged prior to/in place of initiating *in vivo* bioconcentration studies, thus optimizing selection of appropriate fish species.

1.02.A.T-02 An Evaluation of the Potential Impact of Per- and Polyfluoroalkyl Substances (PFAS) to Mink and Otters: Approaches and Considerations

***John L. Newsted**, Ramboll*

Perfluoroalkyl substances (PFAS) are synthetic substances that have been released into the environment during manufacturing processes, from commercial products, and applications. This has resulted in their presence being detected in avian and mammalian wildlife on a global scale. However, investigations into the toxicological significance of these exposures to wildlife are ongoing. Several approaches can be used to assess potential risks that PFAS in aquatic mammalian wildlife. First approach considers ingestion via drinking water and diet as well as inhalation of PFAS from their environment to estimate a total exposure, or the second approach is to use tissue-based concentration data that can be related to adverse effects, these are known as Toxicity Reference Values (TRVs). In the TRV approach, toxicity and exposure data from surrogate species are extrapolated to wildlife species, such as mustelids, but these extrapolations can be uncertain. This is especially relevant for PFAS that consists of a large, diverse group of chemicals that have varying capacities to bioaccumulate in wildlife and may have different tissue distributions profiles that can be species specific. To address this issue, a critical examination of toxicity data as well as bioaccumulation data from mammalian laboratory and field studies was conducted. The extent of this effort was limited to PFAS that have been identified and measured in mustelid species (mink, and otters), and that also have toxicity data with ecologically relevant endpoints that can be used to establish TRVs. While the focus of this effort has been on perfluorooctane sulfonate (PFOS) due to the availability of toxicity and bioaccumulation data in mink, insights gained from these efforts have been used to examine the potential effects of other PFAS including PFOA and PFNA. Normalization approaches that take into about the capacity of tissues to accumulate PFAS were also investigated to better evaluate underlying factors that can contribute to species related differences in toxicity and tissue accumulation. Finally, data gaps and uncertainties related to addressing potential risks of PFAS to mustelids will be discussed.

1.02.A.T-03 Bioconcentration of PFAS and Precursors in Fathead Minnow Tissues Environmentally Exposed to AFFF-Contaminated Waters

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Per- and polyfluoroalkyl substances (PFAS) exhibit ecological risks to wildlife and negative health effects in humans. For decades, the global scientific community has relied on empirical data on bioaccumulation and bioconcentration factors to evaluate PFAS biological partitioning propensity. Utilizing contaminated environmental compartments as sources for PFAS in dosing studies is underrepresented but may offer valuable insight to biological interactions of compounds in complex mixtures. For example, fire training activity at Joint Base Cape Cod, Massachusetts, incorporated the use of aqueous film-forming foam (AFFF) which resulted in long-term PFAS contamination of groundwater and hydrologically connected surface waters. It was here that a mobile laboratory was established to evaluate bioconcentration of PFAS from AFFF-impacted groundwater by flow-through design. Sexually recrudescent fathead minnows (n = 24) were exposed to PFAS in groundwater over a 21-day period and tissue specific PFAS burdens in liver, kidney, and gonad were derived at d1, d7, and d21. High resolution mass spectrometry was used for PFAS quantitation and suspect screening of additional fluorinated structures. Total PFAS (∑PFAS) concentrations in groundwater increased from approximately 10,000 ng/L at d1 to 36,000 ng/L at d21. Relative abundance of PFAS in liver, kidney, and gonad shifted temporally from majority perfluoroalkyl sulfonamides (FASA) to perfluoroalkyl sulfonates (PFSA). By d21, mean ∑PFAS concentrations in tissues displayed predominance in order of: liver > kidney > gonad. Generally, bioconcentration factors (BCFs) for FASA, perfluoroalkyl carboxylates (PFCA), and fluorotelomer sulfonates (FTS) increased with degree of fluorinated carbon chain length, but this was not evident for PFSA. Perfluorooctane sulfonamide (FOSA) displayed the highest mean BCF (8,700 L/kg) in d21 kidney. Suspect screening results revealed the presence of several perfluoroalkyl sulfinate and perfluoroalkyl sulfonamide compounds present in groundwater and in liver for which pseudo-bioconcentration factors are also reported. Pseudo-bioconcentration factors showed strong linear relationships with degree of perfluorinated chain length for compounds identified within the FASA subgroup. The bioconcentration observed for precursor compounds and PFSA derivatives detected suggests alternative pathways for terminal PFAS exposure in aquatic wildlife and humans.

1.02.A.T-04 Competitive Uptake of Three Rare Earth Elements (La, Ce, and Y) by *Chlamydomonas reinhardtii* in the Context of the Biotic Ligand Model

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Rare earth elements (REE) are increasingly used because of their unique optical, magnetic, and catalytic properties. With increasing mining and production, it is essential to determine the potential environmental impacts of the REE, which are nearly always found as mixtures. The biotic ligand model (BLM) can be used to predict metal bioaccumulation and toxicity. In theory, it can take metal competition into account, which is an important point when studying environmental mixtures. The objective of this study was to link the bioaccumulation measured under carefully controlled laboratory conditions to those measured in a potentially contaminated natural environment (REE mine effluent). Experiments were first performed in well-controlled synthetic waters in the laboratory. The bioaccumulation of three cations REE was measured to determine their biouptake constants for potential use in a BLM (La^{3+}: logK = 7.5 M^{-1}; Ce^{3+}: logK = 7.4 M^{-1}, Y^{3+}: logK = 7.5 M^{-1}). Subsequently, competitive interactions of REE metal pairs for uptake sites on the cell membrane were studied for *Chlamydomonas reinhardtii* (*C. reinhardtii)*. As the competition was well predicted using constants determined from the single metal experiments, it is likely that REE shared common transport sites. Given the similarity of the constants in the presence of competitors, the total REE bioaccumulation remained approximately constant for the different mixtures. Theoretical and experimental internalization fluxes for different mixtures of the three metals were also tested. Since similar constants were observed and given the similar constants, a simplified model based upon total metal concentrations could be prepared and a single Michaelis-Menten curve obtained under the assumption of a single uptake site. Finally, freshwaters were sampled and tested from the first REE mine in Canada (Nechalacho, NWT; Cheetah Resources). Internalization in these natural waters was verified against this simplified model, however, uptake was below detection limits, mainly due to the extremely low metal concentrations presently in the waters. This work will help predict the impacts of these high-technology metals on aquatic biota.

1.02.A.T-05 Higher Throughput Screening of Bioaccumulation Potential Using In Vitro and Isolated Perfused Liver Methods

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Bioaccumulation assessment in aquatic ecosystems typically relies upon *in silico* predictive modeling approaches based on *log* K_{OW}, with *in vivo* testing required to adequately predict bioaccumulation of chemicals which undergo biotransformation within an organism. In line with principles of reduction, refinement, and replacement (3R), approaches based on *in vitro* substrate depletion assays combined with *in vitro-in vivo* extrapolation (IVIVE) modeling have been proposed to meet regulatory bioaccumulation assessment needs. Such approaches have been met with concerns regarding chemical applicability domain, physiological relevance, and a lack of harmonised protocols between species. To address these concerns, an isolated perfused liver model representing an intermediate level of biological organisation was utilized to compare *in vitro* intrinsic clearance of benchmark chemicals with that of standardized *in vitro* substrate depletion assays. This approach was then applied to a diverse chemical mixture from the US EPA's Non-Targeted Analysis Collaborative Trial (ENTACT), with comparison data generated from rainbow trout isolated hepatocytes. This study has demonstrated that such *in vitro* approaches have both a broad chemical applicability domain, as well as the potential of higher throughput by utilizing mixture experiments. When combined with predictive IVIVE modeling tools, this *in vitro* intrinsic clearance data can be included in integrated assessment frameworks as part of a weight-of-evidence approach to assess bioaccumulation in aquatic organisms with both increased predictivity of *in vivo* outcomes, as well as dramatically reduced animal use.

1.02.A.T-06 Insensitive Environmental and Ecotoxicological Models

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Environmental and ecotoxicological models are often marked by insensitive predictions against the more variable observations. These predictions manifest as dense, horizontal striations of values in prediction-versus-observation plots. The presence of insensitive predictions means that the model is less effective in predicting the actual variability exhibited in reality. The issue of insensitive predictions is not addressed by standard regression theories as regression is justified primarily by the minimization or prediction error (or maximization of likelihood in projecting observations). This work sets out to: (i) demonstrate the widespread presence of insensitive models in environmental and ecotoxicological phenomena, (ii) uncover the general origin of insensitive predictions, and (iii) develop metrics or indicators for characterizing the extent of insensitivity for a given model. First, data and models on sorption, biodegradation kinetics, oral acute toxicity, and bioaccumulation of organic chemicals (n = 395, 969, 633, and 2513, respectively) are examined and used to illustrate the presence and different degree of model insensitivity. Next, theoretical evaluation demonstrates that insensitive predictions arise from the omission of influential predictors or attributes in the model, and thus model insensitivity is, in principle, extendable to all regression models or statistical machines. Lastly, a number of metrics are proposed to characterize model insensitivity, and they include range sensitivity (SenR), uniqueness sensitivity (SenU), overall sensitivity (Sen), and uncertainty-based sensitivity (U). The developed metrics are applied to the aforementioned data/models for illustration. This work concludes by reconciling model sensitivity with standard statistical theories and practices in regression or machine construction with a general workflow where model sensitivity and error

minimization (likelihood maximization) are used to advance both models and theories of the phenomena under investigation. This work calls for the general adoption and quantification of model insensitivity as a measure of model or theory inadequacy for environmental / ecotoxicological phenomena, which are typically situated in high-dimensionality data spaces.

1.02.B.T Advances in Bioaccumulation Science and Assessment

1.02.B.T-01 Organochlorine Pesticide and Polychlorinated Biphenyl Exposure in Greek Children (Rhea Birth Cohort): Indications for Past and Continuing Indirect Exposure to DDT

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The study extensively characterized blood levels of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in a population of Greek children participating in the Rhea birth cohort study. The aim was to assess exposure levels, explore potential correlations with age and gender, and identify sources of exposure. Through the use of bivariate and multivariate statistical analyses, patterns of exposure and common sources of PCBs and OCPs were identified. A total of 947 blood samples from study participants were analyzed for OCP and PCB exposure: 375 samples from 4-year-olds, 239 from 6.5-year-olds, and 333 from 11-year-olds. Elevated levels of DDE were found in 6.5-year-old children compared to those reported in other European countries. Additionally, higher concentrations of DDE were observed in 4-year-old children, with the lowest levels found in the 11-year-old group. The DDT/DDE ratio remained consistently below 1 across all age groups, indicating historical exposure to DDT, possibly through dietary sources, leading to the formation of DDE. PCB 28 showed the highest concentration across the entire cohort, followed by PCBs 138, 153, and 180. The combined levels of the six indicator PCBs suggested low exposure levels for most of the cohort population. Spearman's correlations revealed strong associations between PCBs and OCPs, while principal component analysis identified two distinct groupings of exposure. DDE showed a positive correlation with a subset of PCBs (153, 156, 163, 180), suggesting a shared OCP-PCB source, and an inverse correlation with other PCBs (52, 28, 101), implying a different, possibly competing, source.

1.02.B.T-02 Parabens, Their Metabolites, and Halogenated Byproducts in Migratory Birds of Prey: A Comparative Study in Texas and North Carolina, USA

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Parabens are a group of the alkyl esters of p-hydroxybenzoic acid commonly utilized as preservatives in personal care products such as cosmetics and pharmaceuticals. Recent studies have discovered the presence of parabens in surface water and tap water due to their use as disinfection products for wastewater and drinking water treatment, and little is known about their ability to bioaccumulate or their occurrence in biological samples. This study examines the occurrence, and tissue distribution of parent parabens, their metabolites, and halogenated byproducts in the liver, kidney, brain, and muscle, in birds of prey from Texas and North Carolina, USA. Samples were analyzed by a solid- liquid extraction with ACN:MeOH, followed by SPE step and isotope dilution, LC-MS/MS. Detection frequencies revealed that MeP, PrP, and BuP were detected in over 50% of all the tissues studied, with the kidney exhibiting the highest concentration for MeP (0.65 ng/g -6.84 ng/g). PHBA, a primary metabolite, also showed the highest frequency detection >50% and concentration range in the liver, 4.64 to 12.55 ng/g. Chlorinated compounds, ClMeP and ClEtP, were found in over half of the samples across all tissues. However, Cl2EtP 1.01-5.95 ng/g and Cl2MeP 2.20-3.99 ng/g in the kidney showed the highest concentrations. Dibrominated derivatives, Br2EtP, were detected in more than 50% of samples, particularly in muscle and brain tissues, but Br2EtP 0.14-17.38 ng/g in the kidney and Br2PrP 0.09-21.70 ng/g in the muscle. Inter-species comparisons revealed no significant accumulation patterns of ∑P, ∑M, and ∑H between tissues in GHOW and MIKI. No considerable accumulation patterns were observed between species, suggesting constant exposure across different bird species. Positive correlations were observed in the liver between MeP, PrP, BuP, and PHBA, suggesting similar origins and metabolic routes. This work provides valuable insights into the occurrence and distribution of parent parabens, metabolites, and halogenated byproducts in bird tissues, highlighting the need for research on monitoring these compounds in bird species.

1.02.B.T-03 Predicting Rare-Earth Element Bioaccumulation in Natural Waters; Impact of Natural Organic Matter

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Rare-earth elements (REE) are in high demand given their applications in the medical, green energy and high-tech industries. Therefore, concentrations of REE are likely to be increasing in our landfills, waste-wasters, and natural waters and it is important to ascertain their risk for the natural environment. Current bioavailability models, such as the Biotic Ligand Model (BLM), have been developed mainly for divalent cations and therefore there is a need to validate them for the REE, which mainly form trivalent cations. In this study, the bioavailability of lanthanum (La) was evaluated through the use of internalisation fluxes for *Chlamydomonas reinhardtii* in the presence of 11 different natural organic matters (NOM). Lanthanum bioaccumulation indeed decreased in the presence of NOM, however, the decrease was much less important than what was predicted using calculated La^{3+} concentrations. Furthermore, impacts of the NOM varied with their chemical nature and thus the concentration of dissolved organic carbon was not sufficient to predict the effect of NOM on La bioaccumulation. NOM samples were extensively characterised in order to adjust speciation calculations with respect to NOM quality. In addition, free ion concentrations were estimated using an ion exchange resin. Results show that a modified BLM can be used to predict La bioavailability in the presence of NOM under controlled laboratory conditions. A final experiment was performed using waters and NOM isolates taken from the first active Canadian REE mine. These waters show high concentrations of NOM (4 to 43 mg C/L) and competitors (0,2 to 3,28 mM) as well as high pH values (7,26 to 8,95), all parameters that will contribute to lower internalisation.

1.02.B.T-04 Prioritizing PFAS for Site-Specific Aquatic Life Ecological Risk Assessment in Marine Surface Water

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Per- and polyfluoroalkyl substances (PFAS) are routinely detected in environmental media at hundreds of US Department of Defense facilities due to historical uses of aqueous film forming foams, leading to concerns over potential ecological and human health risks. The primary objectives of our research project are to fill two important data gaps for conducting ecological risk assessments at marine PFAS impacted sites, specifically: 1) the lack of ecotoxicological data on PFAS for aquatic life (i.e., fish, invertebrates, algae); and 2) the lack of an approach to evaluating the risks of PFAS mixtures. Laboratory acute and chronic toxicity testing of seven marine aquatic life species to 10 priority PFAS compounds (PFBA, PFHxA, PFOA, PFBS, PFHxS, PFDS, 6:2 FTS, and 8:2 FTS) in seawater indicated effect benchmarks (e.g., NOECs, LOEC, EC50s) that are typically > 1 mg/L while PFOS and PFDA indicated benchmarks for some species ranging from 0.2 to 1 mg/L. Additionally, mysid shrimp and topsmelt were collected after 7 days exposure to generate tissue bioconcentration factors (BCFs) for the 10 PFAS, and a subset of topsmelt were exposed chronically (28 days) to 100 µg/L to generate 28 day BCFs. BCFs in both species tended to increase with PFAS chain length, for example: the BCFs in topsmelt for PFBS, PFHxS, and PFOS are 0.2, 1.0, and 140 L/kg wet weight, respectively. The 7- and 28-day BCFs in topsmelt tended to be similar. PFBA, PFHxA, PFBS, PFDS, 6:2 FTS, and 8:2 FTS results indicate NOECs are greater than 10 mg/L, while PFOA and PFDA's NOECs are 4 and 0.4 mg/L, respectively. The NOECs for these eight PFAS are generally 1 to 2 orders of magnitude higher than upper ranges of concentrations in surface water that have been measured at AFFF sites. In contrast, 2 of the 10 PFAS tested (PFHxS, PFOS) have higher potential for toxicity at marine AFFF sites given the overlap of aquatic life benchmarks and observed concentrations. Next steps are assessing mixture toxicity with a vertebrate species (topsmelt fish (*Atherinops affinis*) and the most sensitive invertebrate species mysid shrimp (*Americamysis bahia*) following a classic toxic unit (TU) experimental design.

1.02.B.T-05 The Toxicokinetics of 6PPD-Q in Salmonids

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The tire rubber associated contaminant 6PPD-quinone (6PPD-Q) is linked to pre-spawn mortality in salmon. The concentration of 6PPD-Q in Seattle WA urban creeks with a history of pre-spawn mortality are frequently > 20 ng/L and occasionally approach 80 ng/L. Initial monitoring of juvenile salmon and caged mussels in Puget Sound observed low detection rates of < 20% with detected levels typically near or below 2 ng/g. This study seeks to better understand the relationship of field measurements with the toxicokinetics of 6PPD-Q in salmon such as juvenile anadromous rainbow trout (steelhead; *Oncorhynchus mykiss*) after controlled laboratory

exposures. Static water exposures at environmentally relevant concentrations for up to 96 h were used with exposure water repetitively sampled at various times for measurement of 6PPD-Q. At termination, plasma, liver, bile and carcass levels of 6PPD-Q were measured. The volume of exposure water (50-75 L) and fish size (40-70 g) was carefully chosen to achieve a loading density that was near 1 g/L. This experimental design combined with the aqueous stability of 6PPD-Q permitted mass balance calculations to assess bioconcentration factor (BCF) and recovery of 6PPD-Q after exposures. During all exposures, we observed rapid loss of 6PPD-Q with water concentrations that were typically 40-60% of the starting levels by 24 h. This suggests uptake of 6PPD-Q is rapid in fish and likely controlled by water flow over the gills (ventilation volume). After 24 h, loss of 6PPD-Q from exposure tanks proceeded in a linear manner with only 28% of the starting concentration remaining at 96 h. Carcass levels of 6PPD-Q in steelhead exposed for 96 h were very low, averaging 2 ng/g, which amounted to less than 3 % of the total amount of 6PPD-Q added to the exposure tanks. The estimated BCF for steelhead was below 100. During the 96 h exposures, approximately 72% of the added 6PPD-Q disappeared from the exposure system (not present in water, carcass or tissues). These observations imply steelhead have a large capacity to metabolize 6PPD-Q, which is limiting bioaccumulation. Ongoing intravascular dosing studies (with fish fitted with dorsal aorta catheters) are quantitatively measuring total body clearance to help determine interspecies differences in elimination and the contribution of biotransformation. These studies support exposure assessments and the potential for 6PPD-Q to bioaccumulate in marine food webs.

1.02.B.T-06 Trends in Uptake, Bioconcentration, and Critical Body Burdens for a Diverse Suite of Per- and Polyfluoroalkyl Substances Across Three Taxa

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Per- and Polyfluoroalkyl Substances (PFASs) are a diverse chemical class encompassing thousands of structures, many of which are persistent, bioaccumulative, and toxic. Understanding toxicity as a function of chemical structure and taxa is critical to estimating risk for data-limited PFASs using new approach methods and modelling techniques. Our group has measured sublethal toxicity for a diverse set of PFASs in four species: *Ceriodaphnia dubia*, *Hyalella azteca*, *Chironomus dilutus*, and *Pimephales promelas* (fathead minnow). Of these, *C. dilutus*, *H. azteca*, and fathead minnow are amenable to measurement of whole-body tissue residues and derivation of bioconcentration factors (BCFs). In this presentation, we discuss patterns in bioconcentration within these three species as a function of chemical structure and combine this information with water-column effect concentrations to infer internal potency. Among chemicals tested within the perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs), perfluoroalkyl sulfonamides (FASAs), fluorotelomer sulfonic acids (FTSAs), and others, logBCF increases linearly with perfluorinated carbon chain length, although off-sets between chemical classes vary across species and groupings shift when logBCF is related to chemical properties other than chain length. Estimated internal potencies (critical body burdens, CBBs) calculated by multiplying BCF by median water-column effect concentration (EC50) were generally constant across perfluorinated carbon chain length, with species- and class-specific differences hypothesized to represent distinct mechanisms of toxic action. One such mechanism is particular to *C. dilutus*, which is highly sensitive to the seven-, eight-, and nine-carbon PFSAs (PFHpS, PFOS, PFNS), with toxicity characterized by substantially lower CBBs relative to PFSAs in *H. azteca* and *C. dubia*, and PFCAs and FTSAs in any of these three species. Preliminary results imply that, in contrast to *C. dilutus*, the greater sensitivity of fathead minnow to PFSAs may be due to preferential uptake of these compounds rather than the greater internal potency observed in *C. dilutus*. This abstract neither constitutes nor necessarily reflects U.S. Environmental Protection Agency policy.

1.02.P-Mo Advances in Bioaccumulation Science and Assessment

1.02.P-Mo-001 Application of Avian In Vitro Substrate Depletion Assays to Study Biotransformation of Organic Chemicals

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Avian species play a critical role in aquatic and terrestrial ecosystems by providing many ecosystem services, and historically have been significantly impacted by the bioaccumulation of organic pollutants. Bioaccumulation is typically assessed in fish using B-metrics such as bioconcentration/bioaccumulation factors (BCF/BAF) depending on exposure route, however such methods are challenging to apply to air breathing organisms. These metrics are often determined using costly *in vivo* testing in fish and rats, with little focus on birds outside of field studies which have inherent modeling limitations. Recently, it has been suggested that a kinetic approach that views these B-metrics as the product of competing rates of whole-body uptake and elimination may be refined by incorporating tissue-specific biotransformation rates derived from substrate depletion assays using isolated hepatocytes or liver S9 subcellular fractions. The present study seeks to optimize, evaluate, and apply these assays to multiple relevant bird species using a diverse suite of benchmark compounds. In initial studies using mallard duck (*Anas platyrhynchos*) metabolic material, *in vitro* intrinsic clearance rates of pyrene and 4-*n*-nonylphenol were consistently determined across multiple assay conditions, with various thawing and handling protocols. Further research will apply an optimized assay protocol to additional relevant chemicals including anticoagulant rodenticides, polychlorinated biphenyls (PCBs), organochlorines, and emerging contaminants of wildlife concern. The goal of this study is to generate consistent data for tiered bioaccumulation assessment strategies, as well as lay the foundation for future ring trial or similar applications to inform wildlife risk assessment.

1.02.P-Mo-002 Are Current Regulatory log Kow Cut-Off Values Fit-for-Purpose as a Screening Tool for Bioaccumulation Potential in Aquatic Organisms?

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Persistent, Bioaccumulative and Toxic (PBT) and very Persistent and very Bioaccumulative (vPvB) are regulatory hazard categories that have been set to manage the possible risks to humans and the environment from these chemicals. In industrial chemicals regulations, their aquatic Bioaccumulation potential is usually assessed first with a screening based on the octanol/water partition coefficient (Kow). However, current log Kow cut-off values triggering classification, categorisation and/or further fish bioconcentration testing are not harmonised worldwide, and they have never been assessed for their regulatory relevance. In this study, the experimentally determined log Kow and fish bioconcentration factors (BCF) of 532 chemicals were compared. While the analysis underlined the robustness of using log Kow as a screening tool (5/532 were false negatives; log Kow: non-bioaccumulative, but BCF: bioaccumulative), it also demonstrated the conservatism of the cut-offs used worldwide. Indeed, many chemicals were deemed potentially Bioaccumulative based on log Kow when a fish bioaccumulation test showed no concern (false positives), therefore, leading to unnecessary use of vertebrate animals. Our analysis shows that the log Kow cut-off could be increased to 4.5 in all regions for all purposes without leading to a reduced protection of humans and the environment.

1.02.P-Mo-003 Bioaccumulation of Microplastics in Predatory Marine Species: Ingestion is Not the Sole Pathway for Trophic Transfer of Pollution

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Bioaccumulation of microplastics (MPs) within higher trophic level organisms through food web transfer is poorly understood for many marine species. Jonah crab (*Cancer borealis*) is a rapidly expanding commercial fishery species in New England, filling market gaps left by declining landings of established species such as lobsters. *C. borealis* are generally understood to be carnivorous and feed on a wide variety of prey which have been observed to contain MPs in recent surveys of wild populations, including mollusks, arthropods, polycheates, and other crustaceans. This predatory dietary preference may place them at risk of increased exposure and possibly biomagnification of MPs through the ingestion of prey-bound pollution. Experimental exposure trials were conducted during which crabs consumed bivalves whose tissues were inoculated with environmentally relevant quantities of polyester microfibers using a novel dosing method. Crab tissues (stomach, hepatopancreas, gills, hemolymph) were subsequently examined for MP accumulation. Crabs were observed to be highly efficient at removing MPs from the stomach, likely bypassing parts of the digestive system completely. However, crab gills were observed to be vulnerable to MP accumulation despite experimental MPs being presented to crabs within prey tissue only. The outcomes of this study highlight that uptake through ingestion is not the only significant means of MP entry into predatory organisms. MPs released during prey handling can become trapped within the feeding organism's respiratory tissues and accumulate in the body at a higher rate than MPs taken in through ingestion.

1.02.P-Mo-004 Biotransformation Assay Using Precision-Cut Tissue Slice of Common Carp (*Cyprinus carpio*)

Daisuke Kawaguchi, *Chiyoko Miyata and Takuo Fujisawa, Sumitomo Chemical, Japan*

The bioaccumulation potential of chemicals was primarily evaluated using the aqueous exposure test of fish (OECD TG 305-I). However, as the importance of bioaccumulation through the food chain has grown, the dietary exposure test has been incorporated as OECD TG 305-III since 2012. Subsequently, the number of applications using this dietary exposure test under the Chemical Substances Control Law has increased since 2018. The biotransformation rate is a critical determinant of predicted bioaccumulation potential in fish. Thus, *in vitro* assays have been established to measure the intrinsic clearance rate using rainbow trout hepatocytes (OECD TG 319A) or liver S9 sub-cellular fraction (OECD TG 319B), because it is assumed that chemicals taken up from the water via gills are predominantly metabolized in the liver. Conversely, in the dietary exposure, the biotransformation in the intestine, which is the pathway of chemical uptake, may be equally significant. In fact, some studies have demonstrated that the intestine and liver exhibit comparable metabolic activities in rats. As few studies on fish intestinal biotransformation have been reported, our study aimed to establish a method for evaluating biotransformation rate using precision-cut tissue slices (PCTS) from common carp (*Cyprinus carpio*). The advantage of PCTS is that it enables more accurate determination of biotransformation rates than *in vitro* methods utilizing hepatocytes or S9 fractions, largely due to the sustained preservation of various enzyme activity within the slices. In this study, we prepared 300 µm thick slices of carp intestine and liver using a vibrating microtome. These slices were then incubated in L-15 medium supplemented with 7-ethoxycoumarin or testosterone to measure the biotransformation rates. The results confirmed that both chemicals were metabolized in the slices of both organs. These results are reported in this presentation.

1.02.P-Mo-005 Comparison Between Measured and Estimated Bioconcentration Factors for Polychlorinated Biphenyls at Two USEPA Superfund Sites in Florida and Virginia

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Understanding the bioconcentration potential of contaminants (particularly hydrophobic organic contaminants) in aquatic organisms is crucial for assessing environmental risks and designing effective remediation strategies in contaminated sites, especially those designated as United States Environmental Protection Agency (USEPA) Superfund sites. The main aim of the study was to establish a comparative analysis between measured and estimated bioconcentration factors (BCF) for polychlorinated biphenyls (PCBs) at two active USEPA Superfund sites: Penniman Lake at Naval Weapon Station, Williamsburg, Virginia, and Wetland 64 near Naval Air Station, Pensacola, Florida., highlighting the accuracy and reliability of prediction models in assessing bioaccumulation risks. Eight sampling locations were selected at both sites based on prior PCB findings. Sediment samples from these sites were collected and homogenized, and a portion was allocated for laboratory investigations for ex-situ exposure. Solid phase microextraction (SPME) samplers coated with polydimethyl siloxane (PDMS) were deployed alongside sediment exposures for test organisms both in and ex-situ conditions. Filter-feeding clams and deposit-feeding worms were selected for bioaccumulation assessment. In-situ chambers were constructed using plastic buckets to accommodate the samplers and organisms. Both the samplers and organisms remained in the field and laboratory settings for a duration of

28 days. After 28 days, PDMS fibers and the test organisms were collected and processed for subsequent analysis. BCF refers to the ratio of a contaminant's concentration in an organism to its concentration in the surrounding water at equilibrium. These values can be directly determined using primary data obtained from lipid-normalized contaminant concentrations in organisms and porewater concentrations extracted from PDMS fibers. Moreover, BCF can also be estimated based on organic carbon-normalized sediment concentrations and the octanol-water partitioning coefficient (K_{OW}). In this study, both measured and estimated BCF values were computed for clams and worms. The findings indicate that K_{OW}-based prediction yielded more accurate BCF results than sediment organic carbon partitioning coefficient, K_{OC}-based prediction for PCBs.

1.02.P-Mo-006 Demonstration of a Commercially Available Peeper Passive Sampler for PFAS in Sediment

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PFAS are a concern at many Department of Defense (DoD) installations due to the historical use of aqueous film-forming foam (AFFF) containing PFAS and release of PFAS into aquatic systems. Sediment can represent a significant source of PFAS to aquatic life, aquatic dependent wildlife, and human receptors, and there is a critical need to understand the fate and bioavailability of PFAS in sediment for site-specific risk assessments. Passive samplers have shown to be a powerful tool for providing measurements of the freely dissolved concentration (Cfree) that can be used to improve site-investigations; however, there are limited examples of applying commercially availability passive samplers at PFAS sites. The overall objective of this project is to demonstrate and validate the use of PFASsive™, a commercially available dialysis-based passive sampler ("peeper") for measuring PFAS in sediment porewater and surface water. Specific goals are: 1) Demonstrate an in situ field deployment of the peeper samplers in PFAS-impacted sediment and water at a freshwater DoD site and a marine DoD site; 2) Validate the ability of the in situ passive sampler results to measure availability of PFAS in sediment porewater via comparison of the Cfree of PFAS measured with the passive samplers to concentrations of PFAS in mechanically extracted co-located porewater samples and concentrations of PFAS in benthic organisms exposed in co-located sediment; and 3) Validate the ability of the in situ passive sampler results to measure availability of PFAS in surface water via comparison of the Cfree of PFAS measured with the passive samplers to concentrations of PFAS in co-located surface water grab samples and concentrations of PFAS in aquatic organisms exposed in the surface water column. The first demonstration is taking place at the freshwater site in May 2024 with data analysis being completed Summer 2024. This presentation will highlight benefits of passive sampling for PFAS, sample design, field work, and discuss the results of Cfree measured in the passive samplers compared to measured PFAS in bulk media and organism tissue.

1.02.P-Mo-007 Development of a Toxicokinetic Model for Maternal-Child Transfer of PFAS

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Past work has shown that maternal transfer of per- and polyfluoroalkyl substances (PFAS) to young children is their dominant exposure source, and that children who are breast-fed have higher PFAS exposures than formula-fed children. While several toxicokinetic models have been developed for relating external PFAS exposures to serum PFAS concentrations in adults, limited tools are available for attributing exposure sources for young children. To address this gap, we developed a generalizable toxicokinetic model for maternal-child PFAS transfer to better understand the impact of breastfeeding on the levels of PFAS in children, and how it changes with age after cessation of breastfeeding. We parameterize the model using data from past studies on dietary preferences, PFAS concentrations in environmental media, and measured internal PFAS exposure levels for multiple cohorts. We use the model to evaluate the contribution of multiple exposure sources on the internal levels of PFAS among both the general population and those highly exposed to PFAS. Preliminary results show time-dependent changes in perfluorooctane sulfonate (PFOS) concentrations in mothers during pregnancy and lactation, as well as in milk and children. Sensitivity analysis using time-varying area under the curve indicated that the duration of breastfeeding (1, 6, and 12 months) has the greatest effect on accumulation of PFOS in children at 10 months, 2 years, and 4 years of age, respectively. Among the exposure sources considered, preliminary results suggest dust ingestion is also contributes substantially to PFOS exposures during childhood and adolescence. This study highlights the extended influence of breastfeeding on PFAS accumulation in the human body. Future research will analyze the effects of different infant feeding scenarios (such as exclusive and partial breastfeeding) and develop maternal-child transfer models for novel PFAS.

1.02.P-Mo-009 Mechanistic Modeling to Assess the Relevance of Gill Membrane Permeability to Bioaccumulation of Perfluoroalkyl Acids (PFAA) in Fish

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Perfluoroalkyl acids (PFAA) are a well-studied subclass of per- and polyfluoroalkyl substances (PFAS). Some PFAA bioaccumulate in food webs, and fish are an important vector of human exposure for seafood consumers. However, mechanistic studies investigating the importance of tissue binding, kinetic transport mechanisms, and excretion of ionogenic chemicals are limited. Here we examine gill membrane permeability as a potential rate-limiting mechanism for PFAA bioaccumulation in fish using a physiologically based toxicokinetic (PBTK) model. The model is used to replicate laboratory and environmental conditions and accounts for co-transport of PFAA across the aqueous boundary layer and with dissolved organic carbon (DOC). Model simulations for PFAA containing four to ten perfluorinated carbon atoms (η_{pfc}=4–10) agree with previous experimental bioaccumulation factors within one standard deviation of the median. Model results suggest the gill accounts for 5–47% of the total tissue bound PFAA, predominantly due to binding with proteins and phospholipids in the gill (>99%). Sensitivity analyses of toxicokinetic processes show bioaccumulation is most affected by differences in tissue binding (normalized sensitivity coefficients [NSC]: 42–104%), followed by gill permeability (NSC: 6–11%) and elimination (NSC: 1–15%). Among gill permeability parameters, aqueous boundary layer thickness is the most sensitive factor (NSC: 2–7%). Aqueous boundary layer thickness and DOC concentrations have a greater influence on long-chain ($\eta_{pfc} \geq 6$) PFAA accumulation compared to short-chain PFAA ($\eta_{pfc} < 6$). The sensitivity of gill membrane thickness and gill membrane binding decreases as a function of increasing number of perfluorinated carbon atoms. Lab tests suggest high DOC concentrations may reduce bioconcentration factors by up to 21%. Model results show gill permeability is affected by water flow rates and DOC concentrations. Overall, this work improves understanding of major factors driving PFAA bioaccumulation in fish and could be expanded to novel PFAS in future work.

1.02.P-Mo-010 Metabolic Activities in Rainbow Trout (*Oncorhynchus mykiss*) S9 Fractions from Liver and Extrahepatic Organs as an Alternative In Vitro Ecotoxicity Assessment Approach

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Whole body biotransformation rate constants can be calculated using an appropriate in vitro to in vivo extrapolation (IVIVE) model. These models use $CL_{\text{IN VITRO, INT}}$ rates derived with OECD Test Guideline 319B or 319A to estimate liver clearance rates, which are then extrapolated to a whole-body (in vivo) biotransformation rate constant. However, beside the liver, extrahepatic organs may also display Phase I and Phase II biotransformation activities and thereby play a role in metabolic clearance and bioaccumulation of compounds. To address these questions, we have maintained rainbow trout (Oncorhynchus mykiss) under controlled housing conditions according to OECD 319A/B. Different organs from eight sexually immature animals were collected and pooled, including liver, gill, intestine, brain, heart and spleen. S9 fractions were prepared to determine the Phase I and Phase II enzyme activities by Liquid Chromatography-Mass Spectometry (LC/MS) analysis. Cytochrome P450 activities, glucuronidation and sulfation activities were analyzed. The liver displayed the highest Cytochrome P450 activities of all organs tested. Chorzoxazone Hydroxylase activity was only detectable in liver, 1-OH-Midazolam Hydroxylase activity was mainly restricted to liver, minor activities could be detected in intestine. However, Phenacetin-O-Deethylation was also detectable in other organs, with intestine, gill and spleen contributing 34, 18 and 11% of the total enzyme activity. Diclofenac-hydroxylase activity was present in all organs, as well as Bupropion-4-Hydroxylase activity, which was more or less evenly distributed among all organs. Phase II activities were detected in the liver, gill, intestine and heart, but not in spleen or brain. In summary, the liver is the major organ for detoxification of compounds. However, extrahepatic organs, mainly intestine and gill, but also the brain, heart and spleen exhibit certain cytochrome P450 activities. Phase II enzyme activities were also detected in the intestine and gill. Our results suggest that extrahepatic organs, mainly intestine and gill, should also be taken into account when bioaccumulation and in vitro clearance rates are determined for IVIVE modeling in rainbow trout.

1.02.P-Mo-011 Nano- and Microplastic Particles as Vectors of Exposure for Plastic Additive Chemicals: Modifications to the ACC-HUMAN Food Web Model and Implications for Evaluating Human Health Risk

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Nano- and microplastic particles (NMPs) may present a hazard in various ways, including a direct biophysical response to physical particle properties (e.g. size and shape), or as vectors for chemical exposure (e.g. plastic additive chemicals and/or those that might become sorbed to the particles from the surrounding environment). Evaluating risk requires reliable and relevant characterization of both exposure and hazard. The leaching of chemicals from NMPs has been identified as a potential exposure pathway in studies reporting effects associated with both *in vivo* and *in vitro* test systems. However, it remains unclear whether this exposure pathway is relevant to human health under environmentally relevant conditions. Previously, the bioaccumulation food web model (ACC-HUMAN), has been used to estimate human exposure to hydrophobic organic chemicals as a result of consuming contaminated food and beverages obtained from the terrestrial and marine environments. Here, we describe a modification of the steady-state version of the ACC-HUMAN model to include dietary exposure to NMP containing either accumulated chemicals from the surrounding environment or chemical additives embedded in the plastic. Chemical transfer to the organism is described using a two-film resistance equation assuming spheroidal particles of different sizes. Various scenarios are presented, including the ingestion and subsequent bioaccumulation of chemicals associated with NMPs in terrestrial and marine food webs, with tissue concentrations accumulated in humans compared against a toxicological threshold of concern (TTC). Results suggest that exposure to NMPs with an inclusion level of plastic additive chemicals of 5% wt/wt with varying physicochemical properties needed to exceed a TTC, would be significantly greater than current estimates of exposure to NMPs. The model can, thus, provide the basis for enabling a tiered evaluation of the relative human health risks posed by exposure to NMPs with varying levels of plastic additive chemicals.

1.02.P-Mo-012 Occurrence and Maternal Transfer of Per- and Polyfluoroalkyl Substances (PFAS) in Various Sharks

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Per- and polyfluoroalkyl substances (PFAS) represent a concerning class of environmental contaminants due to their persistence, bioaccumulation, and potential adverse effects. This study investigated PFAS levels in the muscle and liver of Great hammerhead (*Sphyrna mokarran*), Tiger (*Galeocerdo cuvier*), Bonnethead (*Sphyrna tiburo*), and Atlantic sharpnose (*Rhizoprionodon terraenovae*) sharks and their corresponding embryos. Preliminary results indicated detectable PFAS concentrations in both muscle and liver tissues, with the liver exhibiting higher levels compared to muscle across all species. The levels of PFAS detected in embryos, particularly the presence of long chain perfluoroalkyl carboxylic acids (PFCAs), provides compelling evidence supporting the maternal transmission hypothesis. These findings offer valuable insights into the dynamics of PFAS exposure in shark populations, highlighting the potential for maternal transfer and accumulation in developing embryos. Understanding these pathways of contamination is crucial for devising effective conservation strategies to mitigate the impacts of PFAS on vulnerable marine species and their ecosystems. Further research is warranted to elucidate the mechanisms and consequences of maternal transfer in shark populations and its implications for ecosystem health.

1.02.P-Mo-013 Over-Riding log Kow: Understanding Biotransformation Through In Vitro Depletion Assays as a Means of De-/Prioritising Bioaccumulation Testing of Chemicals

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The simple physico-chemical parameter n-octanol/water partition coefficient (Log K_{OW}) is the Tier I driver for the screening of bioaccumulation (B) potential for neutral organic chemicals on a global basis. The threshold value indicating potentially B varies regionally between a Log K_{OW} of 3.0 and 4.5. Although the Log K_{OW} informs on the lipophilicity of a chemical, it does not inform on the potential of the chemical to undergo biotransformation in an organism, which is considered to be the dominant elimination mechanism for hydrophobic substances, significantly reducing their bioaccumulation potential. Existing computational models are relatively poor in assessing the significance of biotransformation on the attenuation of bioconcentration. Thus, Log K_{OW} often results in follow-up testing for B where there are no regulatory accepted intermediate Tiers of evaluation to permit avoiding *In vivo* testing. This study will demonstrate the utility of *In vitro* assays using fish liver S9 fractions (OECD Test Guideline 319B) to quantify the rate of biotransformation of chemicals and how such studies are useful in de-/prioritising further *In vivo* testing requirements. Predicted BCFs based on *In vitro* intrinsic clearance (TG 319B) and two different extrapolation models are compared to BCFs from a substantial data-set of *In vivo* studies for different fragrance ingredients across diverse chemistries and broad physico-chemical characteristics. The presentation will further discuss adaptations of the assay which permit a reduction in the uncertainty of the determination. *In vitro* biotransformation assays may be integrated as a Tier II approach to B assessment as a means of identifying where additional higher-tier testing may be required, and, moreover where they may be waived, while respecting the 3Rs (replace, reduce, refine) principles.

1.02.P-Mo-014 PFAS Bioaccumulation in Stocked Brook Trout

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Per- and polyfluoroalkyl substances (PFAS) have been shown to bioaccumulate in fish. Bioconcentration factors have been computed for certain PFAS in laboratory studies with controlled doses of PFAS, however it is uncertain if wild fish experience similar bioaccumulation rates. Field studies including PFAS measurements in water and fish have also been used to compute bioaccumulation factors (BAFs), however many aspects of waterbodies and wild fish are inherently variable. The current study examined PFAS bioaccumulation of hatchery-raised brook trout that were stocked in the late fall in two small ponds with high concentrations of PFAS in the water. The age of the fish and duration of exposure were controlled but the fish were in a natural environment rather than a laboratory setting. Two age classes of trout were included in the study, including fall fingerlings (½ year old) and fall yearlings (1 ½ year old). Fish and water samples were collected before stocking and 1, 2, 4, and 10 weeks after stocking. In addition, passive PFAS samplers were deployed in one of the ponds. Twenty-eight kinds of PFAS were analyzed for the fish and water samples. The PFAS with the highest concentrations in the fish included PFOS, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, and PFOSA. PFOSA and PFUnDA had the highest BAFs. The yearling trout had lower concentrations of PFAS and lower BAFs compared to the younger, fingerling trout. Fish collected later in the study had higher PFAS concentrations and BAFs than fish collected earlier in the study. The BAFs observed in this study were generally less than BAFs computed in other studies, perhaps due to the cold water and short duration of the study. BAFs were also computed with the passive sampler data. Overall, this study demonstrated that brook trout that are stocked in waterbodies with high concentrations of PFAS can rapidly accumulate unsafe levels of PFAS. A "do not eat" fish consumption advisory was issued for the ponds.

1.02.P-Mo-015 Potential for Dietary Accumulations of Chemicals in Common Carp and Their Relationship with Octanol-Water Partition Coefficient

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Fish can absorb chemicals from the various tissues, with the primary routes are considered to be the uptake of waterborne chemicals through the respiration via the gill, and ingestion of ones contained in the diet during feeding. In the previous studies, there have been many information for accumulations of chemicals absorbed through the respiration and, as a result, bioconcentration factors (BCFs) estimated from the exposure examinations are frequently shown. Generally, hydrophobic substances adsorb to the suspended solids in aquatic environment, and, at last, settle on bottom sediments. They are absorbed by the various organisms through the food chain. Additionally, hydrophobic substances tend to accumulate in the adipose tissue, and cause often high bioaccumulation. Many research reported that bioconcentration factor (BCF) is often obtained by conducting exposure test which are performed with dissolved even hydrophobic substances into water using solvents. In fact, in order to accurately evaluate the uptake of hydrophobic substances into the organisms, it should be desirable to be evaluated them using dietary exposure tests. However, there are currently limited data on dietary exposure tests. Actually, it is considered that there is a certain correlations between the logKow of a chemical substance and its BCF or biomagnification factor (BMF), the empirical evidence supporting a consistent correlation between logKow and BMF is currently underrepresented. In this study, we explored the possibilities of biomagnification for some substances using common carp (*Cyprinus carpio L.*) as test fish, and simultaneously exposed to some substances with similar structure and logKow added into feed through feeding. The range of logKow were within 3.5 to 5, or 5 to values more than 5. The target substances for exploring in the study were such as PAHs, terphenyls, and pesticides. As a result, the substances with high logKow values could not be accumulated and were not almost shown high BMFs. Meanwhile, a few substances such as phenanthrene showed slightly higher BMF despite being below 1. These suggests that BMFs of chemicals should not be particularly determined only by lowKow. Perhaps, we should examine the relationship with the various factors such as their structures, pathway of metabolisms in the organisms, and others.

1.02.P-Mo-017 The Influence of Sediments on the Bioaccumulation of Per- and Polyfluoroalkyl Substances (PFAS) in Great Lakes Benthic Organisms

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The widespread presence of per- and polyfluoroalkyl substances (PFAS) in the Laurentian Great Lakes, which collectively form the largest freshwater system globally and provide crucial water resources for approximately 30 million people in surrounding communities across North America, is of significant concern. PFAS exhibit varied distribution in aquatic sediments where they serve as potential sources to benthic biota and can enter higher levels of the aquatic food web through benthic invertivores like sculpins (*Cottoidea*) and invasive round goby (*Neogobius melanostomus*). Understanding PFAS bioaccumulation patterns in sediment and benthic organisms is important for assessing ecosystem health because PFAS are persistent in freshwater environments and can potentially biomagnify along trophic pathways within the food chain. This research investigates the mechanisms driving PFAS accumulation in benthic organisms and contextualizes the importance of benthic-sourced PFAS in relation to the overall food web. We collected sediment, pore water, overlaying water, and benthic organisms from various locations across the Great Lakes and analyzed them for PFAS and light stable isotopes. We hypothesize that longer-chained PFAS (≥8 carbons for perfluoroalkyl carboxylates and ≥6 carbons for perfluoroalkyl sulfonates) will be most abundant in sediment and thus reflected in benthic organisms reliant on benthic-produced energy sources (e.g., *oligochaetes*). Conversely, PFAS with shorter chains are expected to prevail in water and bioaccumulate in species reliant upon pelagic primary production (e.g., *Dreissenid* and *Diporeia hoyi*). We further hypothesize that bioaccumulation factors will depend on sediment and porewater carbon content, invertebrate diets, and the relative abundances of PFAS within the sediments, which are spatially heterogeneous. This research contributes valuable insights into PFAS contamination dynamics in freshwater ecosystems, aiding in ecological risk assessment and the development of PFAS mitigation strategies in the Great Lakes. This abstract does not necessarily reflect the views or policies of the USEPA.

1.02.P-Mo-018 Toxicokinetics of Ionizable Organic Chemicals in the Early Life Stage Zebrafish (*Danio rerio*)

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Highly bioaccumulative chemicals can pose potential risks to public health and the environment. Previous investigations on chemical bioaccumulation have mainly focused on hydrophobic nonionizable organic chemicals, while ionizable organic compounds (IOCs), which constitute a substantial portion of commercial chemicals, have received insufficient attention. Moreover, it remains unclear how the bioaccumulation of IOCs in fish is affected by developmental stages. Herein, we selected eight pharmaceuticals with diverse physicochemical properties (molecular weight <500, log K_{ow}: 0.51−4.92) as representatives of IOCs and investigated their toxicokinetic processes and bioconcentration potentials across two early life stages of zebrafish (embryo and larva). First, the influence of individual or mixture exposure on toxicokinetic parameters and the bioconcentration factor (BCF) in zebrafish embryos was compared. The deviation of toxicokinetic parameters and BCFs of studied IOCs under individual and mixed exposures was within one order of magnitude, suggesting acceptable variance in experiments using the mixed exposure method, which is more environmentally relevant and includes animal welfare benefits. The selected IOCs showed varied toxicokinetic parameters and BCFs in the two early life stages of zebrafish, including influences of dissociation form and hydrophobicity. The uptake rate constant (k_u), elimination rate constant (k_e), along with BCF of IOCs were ranged from 0.30 to

3.8 mL/g/h, 0.085 to 29 1/h, and 0.0766 to 112 L/kg ww for embryos, and 0.0084 to 47 mL/g/h, 0.022 to 1.92 1/h, and 0.013 to 2132 L/kg ww for larvae, respectively. In terms of toxicokinetics across developmental stages, larvae exhibited at least 3.2 times faster uptake and 1.5 times slower elimination of cationic pharmaceuticals compared to embryos, while larvae showed at least 3.2 times slower uptake and 15 times slower elimination of anionic compounds, except for diclofenac, compared to embryos. Notably, the BCFs of highly hydrophobic IOCs, which ionize as cations in zebrafish larvae, were higher than zebrafish embryos, indicating greater bioaccumulation potential of cationic pharmaceuticals in zebrafish larvae compared to embryos.

1.02.P-Mo-019 Trophic Transfer and Dietary Kinetics of Per- and Polyfluoroalkyl Substance Mixtures in Amphibians and Mammalian Consumers

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Per- and polyfluoroalkyl substances (PFAS) are identified in environmental matrices globally. They are also observed in plants and animals that may be relevant for human and ecological risk assessments. As some PFAS show high persistence and observations of terrestrial trophic transfer in the field, it is plausible to expect human health and ecological risk assessments to require data supporting terrestrial exposure models focused on higher order consumers. Accordingly, to ensure control of confounding factors common to field studies, uniform concentration PFAS-spiked soil was generated and then (1) plants were grown in the soil; (2) worms were grown in the soil; (3) exposed plants were fed to rabbits; and (4) exposed worms were fed to American toads. The dietary exposure of toads and rabbits follows the design of Organization for Economic Cooperation and Development (OECD) Technical Guide #305 with a 28-day uptake period where animals were fed PFAS-exposed diets and a 28-day elimination period where animals were fed control diets. Tissues from rabbits and toads were sampled periodically to develop toxicokinetic models and inform trophic transfer coefficients (TTCs) for the diet to consumer trophic transition. TTCs are toxicokinetic model uptake rate divided by elimination rate, subsequently controlling for volume of distribution and representing an integrative measure of PFAS-taxa combination-specific affinity. Multiple model types were evaluated and leave one out cross validation was used to identify the model with the most predictive power. In rabbits, PFOS, PFNA, PFDA, PFHpS, and (unexpectedly) 8:2 FTS showed substantially slower elimination rates than uptake rates and would be considered bioaccumulative and potentially trophic magnifiers. In toads, PFOS, PFUdA, and PFDA show potential for bioaccumulation and trophic magnification. In toads, PFHpS and 8:2 FTS showed high elimination rates. Clearly, 8:2 FTS and PFHpS showed opposing interpretations across taxa in regard to trophic magnification. Other PFAS with consistent signals across taxa include PFOS (potential for trophic magnification) and PFOA and PFHxS (potential for trophic dilution). In conclusion, exposure models focused on a plant to mammal path or an invertebrate to amphibian will have different foci for PFAS outside PFOS, which appears to have potential for trophic magnification using this kinetic-based approach.

1.02.P-Mo-020 Uncovering the PFAS Complexity: A Powerful IMS-QTOF Workflow for Biota Analysis Combining Targeted and Non-target Approaches

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With their widespread presence in the environment and organisms, and their persistent, bioaccumulative, and toxic (PBT) properties, Per- and Polyfluoroalkyl Substances (PFAS) pose a significant challenge as organic micropollutants. The vast array of commercially available PFAS compounds and their transformation products necessitate tight monitoring within environmental compartments. This study aims to the development of a combined targeted and non-target workflow for the thorough characterization of PFAS in complex environmental matrices, particularly biota, by incorporating trapped ion mobility spectrometry (TIMS) into LC-HRMS. Biota extracts were prepared by a generic protocol for the simultaneous extraction of 56 PFAS compounds from various sub-groups. RPLC was coupled to a novel heated electrospray source (HESI) for analyzing the extracts in negative ion mode on a timsTOF Pro 2 (Bruker). The targeted workflow utilized bbCID (broadband collision-induced dissociation) in data-independent acquisition (DIA), while the non-target workflow employed PASEF (parallel accumulation and serial fragmentation) in efficient data-dependent acquisition (DDA). The CCS-aware target analysis incorporated information for a list of 60 PFAS compounds, including elemental composition, retention time, MS1 and MS2 qualifier ions, and ion mobility-derived collision cross section (CCS) values. The results of the targeted screening demonstrated improved sensitivity and lower PFAS detection limits due to HESI, along with higher quality for the full-scan MS and bbCID MS/MS spectra supported by the ion mobility filtering. Adding CCS values enhanced the identification confidence. In the non-target workflow, raw data was transformed into a comprehensive feature table, and the detected features were filtered by Kendrick mass analysis. A PFAS suspect list with 5,000 compounds was employed for further annotation. In-silico prediction of MS/MS spectra and CCS prediction aided the identification of suspected compounds. Notable examples highlight the identification of suspected features as PFAS-related compounds, supported by the extensive MS2 coverage provided by PASEF.
This combined approach contributes to a better understanding of the chemical universe of PFAS in the environment and plays a crucial role in safeguarding the environment, wildlife, and human health in a One Health approach.

1.02.P-Mo-021 Uptake and Bioaccumulation of Per and polyfluoroalkyl Substances (PFAS) in Lower Trophic Levels of Marine Food Webs

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Per- and polyfluoroalkyl substances (PFAS) are a group of highly stable man-made compounds ubiquitously distributed in the environment. Several PFAS compounds have been found to bioaccumulate and cause adverse effects in humans and biota. Understanding human exposure pathways to short chain and long chain PFAS is of large interest. The bioaccumulation of long chain PFAS in both wildlife and humans drives the need for reliable detection and understanding of the bioaccumulation patterns of PFAS. Consumption of fish and shellfish was associated with elevated PFAS levels in blood in a representative sampling of Americans. Infiltration from highly contaminated sites into waterways and discharges from wastewater treatment plants are known point sources of PFAS to aquatic environments, leading to potentially large amounts entering the estuarine and coastal systems via leaching and discharges. In the aquatic environment, PFAS get assimilated by the base of the food web, namely phyto- and zooplankton. Estuaries make up the largest proportion of nursery grounds for fish, leading the further trophic magnification of PFAS along the food web. Measurements along off the North Atlantic shelf implied that long-chained PFAS and precursor compounds might be taken up by plankton preferentially. In a controlled study of PFAS uptake by phytoplankton species, *Heterosigma akashiwo* and *Skeletonema marnioi*, we found long-chain PFAS (C>8) being preferentially taken up. This finding is in agreement with the environmental data. We determined that four days of exposure is enough to reach partitioning equilibrium. Perfluorotetradecanoic acid (PFTeDA) detection was the highest among the tested 16 perfluoroalkyl acids, in *Heterosigma Spp*. (6000ng/g d.w. ± 1785) and in *Skeletonema Spp*. (2500ng/g d.w. ± 473). Perfluorooctanesulfonic acid (PFOS) accumulated the second most, in *Heterosigma Spp*. (4000ng/g d.w. ±158) while in *Skeletonema Spp*. (840ng/g d.w. ± 96). We presume the difference in the uptake potential between the two species is related to their morphological, presence of an exoskeleton shell in *Skeletonema Spp*. affecting PFAS active uptake by plankton.

1.02.P-Mo-022 Bioaccumulation Assessment of Six Siloxanes Using In Vitro Trout S9 Biotransformation Assays

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Hydrophobic chemicals have proven to be difficult to test using Organisation of Economic and Cooperation and Development (OECD) 305 water-exposure protocols, and while the OECD have produced guidelines to use a dietary exposure there is no guidance on how that data should be interpreted to produce a measurement that is accepted by regulatory agencies. Additionally, these exposure studies can be very costly and time consuming for a laboratory to run which has helped lead to a backlog of chemicals needing to undergo risk assessment. To help expedite the detection of bioaccumulative chemicals in the environment, the use of in vitro to in vivo extrapolation (IVIVE) methods have been proposed to help with evaluation. The present study aims to assess the bioaccumulation potential using in vitro depletion data of 6 different volatile methylsiloxane (VMS) compounds which are difficult to test using standard bioconcentration experiments due to their hydrophobicity and volatility. Depletion data from 3 linear siloxanes - octamethyltrisiloxane (L3), decamethyltetrasiloxane (L4), and dodecamethylpentasiloxane (L5), and 3 cyclic siloxanes – octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclopentasiloxane (D6) were used to assess the bioaccumulation potential of these substances using these IVIVE approaches. The objectives of this study are 1) determine the BCF and BMF of 6 different siloxanes using a novel IVIVE model that has been modified to fit the physiological parameters of trout; 2) compare outputs to reported values; and 3) assess the application of the IVIVE model for determining the bioaccumulation behavior of hydrophobic (log $K_{OW} > 5$) and volatile compounds. BCFs are reported on a wet weight total, with 5% lipid normalization with linear BCFs calculated at 1099 (134 SE), 4195 (277 SE) and 3013 (149 SE) L kg-wet wt^{-1} for L3, L4 and L5, respectively. Cyclic BCFs were calculated at 7664 (1006 SE), 6942 (1763 SE) and 3513 (337 SE) L kg-wet wt^{-1} for D4, D5 and D6, respectively. Our results here are within the range of both aqueous and dietary exposure studies, meaning, that the IVIVE method developed for hydrophobic compounds could be a useful tool for estimated BCF values in compounds that would otherwise prove difficult to measure. The present study provides a novel approach using IVIVE modeling to assess the bioaccumulative potential for hydrophobic compounds.

1.03.A.T Advances in Environmental Quality Guidelines, Criteria, Objectives and Benchmarks

1.03.A.T-01 Development of Tributyltin Sediment Quality Guidelines Using Established and Novel Approaches

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Tributyltin (TBT) is a synthetic organotin compound that has historically been used as an aquatic pesticide and antifouling agent for the protection of boats and ships, and in industrial processes. TBT is known to impact aquatic organisms in sediments. The use of TBT-based pesticides in Canada has been prohibited since its phase-out in 2010 under the *Pest Control Products Act*. Despite these actions, TBT and its degradation products are still present in Canadian sediments in part due to its persistence, which increases under anaerobic conditions. The Canadian Council of Ministers of the Environment has a 1999 protocol for the development of sediment quality guidelines (SQGs), from a time when there were very little sediment toxicity data available. Therefore, Canadian SQGs have mainly been derived following the National Status and Trends Program (NSTP) approach as outlined in the CCME protocol, which uses field study (including co-occurrence) and sediment toxicity data, and sediment values calculated using equilibrium partitioning from aquatic toxicity data. The NSTP approach calculates a threshold effect level (TEL) and probable effect level (PEL) based on the geometric means of specific percentiles of the no-effect and effect concentration data. Sediment toxicity data is more readily available now and newer approaches to developing environmental quality guidelines, such as the use of species sensitivity distributions (SSDs), have been adopted for other media (i.e., water). Environment and Climate Change Canada is developing a novel approach using SSDs and no-to-low effects data derived from spiked sediment toxicity and aquatic studies (via equilibrium partitioning) to derive a Federal SQGs for TBT. This novel approach is described and a comparison of the SQGs derived using the NSTP approach from 1999 with those derived following the novel method is presented.

1.03.A.T-02 Incorporation of New Approach Methods into Species Sensitivity Distributions for Ecological Risk Assessment and Environmental Quality Guidelines

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New approach methods (NAMs) have the potential to inform the ecological risk assessment of chemicals and environmental quality guidelines but first require uptake into regulatory frameworks. NAMs are approaches that contribute to replacing, reducing, and/or refining the use of vertebrates in toxicity testing and include endpoints such as gene expression, enzyme activity, and endpoints generated using *in vitro* testing. The objective of this case study was to evaluate an

approach for using NAMs in environmental quality guideline development and ecological risk assessment in a way that looks and feels like traditional test data. Species sensitivity distributions (SSDs) can be used to estimate hazard concentrations impacting 5% of organisms (HC5) and considered equivalent to a predicted no effect concentration (PNEC). To date, SSDs have typically included traditional apical endpoints (i.e., survival, growth, and reproduction). In this case study, we compared HC5s derived from SSDs that included 1) only traditional endpoints, 2) only NAMs and 3) both traditional and NAM endpoints. The hypothesis was that SSDs containing NAM data would be sensitive and could estimate an HC5 that is protective of chronic endpoints. An initial list of 63 chemicals that included the list of chemicals selected to validate OECD TG 249 (Fish Cell Line Acute Toxicity), reflecting a broad coverage of physical and chemical properties and toxicity levels was selected to generate SSDs. Using both traditional and NAM data contained within the US EPA's ECOTOX knowledgebase, we generated SSDs for our list of chemicals. However, limited data availability resulted in only 4 chemicals for which it was possible to generate all three types of SSDs. The entire list of chemicals contained within the ECOTOX knowledgebase (i.e., 10,082 chemicals) was subsequently filtered and SSDs for chemicals having sufficient data available were retained. This resulted in 18 chemicals for which sufficient data was available to meet the minimum data requirement of an SSD.
The effects on the HC5 and the potential benefits of incorporating NAM data into SSDs used in regulatory ecological risk assessment will be discussed. The approach developed in this case study offers an immediate potential application for the use of NAM data in environmental quality guideline development and ecological risk assessment. *The contents of this abstract neither constitute, nor necessarily reflect U. S. Environmental Protection Agency policy.*

1.03.A.T-03 A Novel Approach to Developing Water Quality Guidelines for Polycyclic Aromatic Hydrocarbons (PAHs)

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Polycyclic aromatic hydrocarbons (PAHs) are a complex group of organic substances that are ubiquitous in the environment, including in surface waters, with thousands of individual non-substituted (parent) and alkylated PAHs possible. Due to their sources, PAHs do not occur as individual substances but rather as a mixture of PAHs in the environment. In addition, many PAHs demonstrate increased toxicity in the presence of ultraviolet light, a process called phototoxicity. Current Canadian Council of Ministers of the Environment (CCME) water quality guidelines are outdated, limited to eleven non-substituted PAHs, do not provide a means to consider the overall toxicity of PAHs as a mixture and have limited consideration of their phototoxicity. Therefore, CCME has initiated a project to update or derive new water quality guidelines for PAHs for a large number of parent and alkylated PAHs which will include consideration of phototoxicity and a means to derive guidelines for a mixture of PAHs. A novel approach using two models, the narcotic target lipid model (NTLM) and the phototoxic target lipid model (PTLM), will be trialed as a method to address current challenges to PAH guideline development, including overcoming data limitations and consideration of phototoxicity. This presentation describes the proposed approach for deriving water quality guidelines for both low and high ultraviolet light conditions as well as how to apply them to mixtures.

1.03.A.T-04 Practical Actions to Help Advance the Use of New Tools and Approaches in Ecological Risk Assessment

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Tools are constantly being developed that offer great promise in helping ecological risk assessors address issues of concern across the Ecological Risk Assessment paradigm. Some tools may be in use and undergoing enhancement, others may have undergone successful demonstrations, and some may have passed a proof of concept and be ready for use in case studies. Tools face significant challenges if they are to be incorporated into mainstream decision-making processes. This presentation takes a broad look at challenges that occur between tool development and incorporation into management decisions. Solutions and best practices to help new tools and approaches become successful are discussed. Topics covered include: Lessons learned from the example of New Approach Methodologies to reduce animal testing, highlighting (1) key elements to obtain broader acceptance of the new approaches and application to higher tier regulatory decisions and (2) key roles for government, industry, the regulatory sector, academia, and journals. The value of a framework for the trajectory of tool development that can bridge from case studies to decision-making. Actions to advance the use of new tools and approaches such as: (1) Unlocking obstacles by understanding decision contexts, building confidence, and involving stakeholders; (2) Creating compelling arguments for decision makers; (3) Providing support such as technical and regulatory guidance, practical assistance during adoption, and training, so tools can be successful; (4) Reviewing examples where new tools and approaches have been successful such as new technologies (e.g., eDNA, real time sensors) and have cross-walked with existing methods.

1.03.A.T-05 Weight of Evidence for Water Quality Criteria and Other Benchmarks

***Glenn W. Suter II**, Retired*

I believe that species sensitivity distributions have been a great success as a standard approach to deriving benchmark values, but I also believe that they need to be augmented. In particular, weight of evidence (WoE) is essential to inference in complex assessments. WoE is based on the premise that, in our highly complex world, there is never enough evidence, and we should use all available relevant and reliable evidence. The need for WoE is illustrated by failures of standard laboratory tests and SSDs used alone. Examples include the sensitivity of unionid mussels and pulmonate snails to ammonia, sensitivity of some aquatic insects to major ions (specific conductivity), deformities of fish due to dietary selenium, deformities of oysters due to tributyl tin, and the effects of aqueous speciation of copper on its toxicity. These failures were identified by field studies. Weight of evidence may be employed in three ways. First, when deriving a criterion, all available information may be assembled and weighed to determine the criterion that is best supported by the evidence. Second, the available information may be used to determine what critical evidence is needed to identify a criterion. Third, a standard set of evidence may be defined and generated for each criterion and weighed using interpretive guidelines. The evidence might include laboratory toxicity tests, field biological surveys, and mechanistic evidence. Concepts and tools are available for weight of evidence. In particular, the U.S. EPA has guidelines for WoE and WoE-based causal assessment methods for effects in the field. Australia and New Zealand have well developed water quality management methods that deeply incorporate WoE.

1.03.A.T-06 Development of a Chloride Water Quality Guideline Based on Hardness and Consideration for Cation Toxicity

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Aquatic life can be exposed to multiple chemical stressors where toxicological response can be modified by stressor interaction. An extensive literature database is available on chloride toxicity as a function of water hardness. The majority of data is for acute exposure durations and relatively limited for sensitive species (*e.g.*, certain Bivalvia, Ephemeroptera, and Daphnia) under a chronic exposure duration and/or broad hardness range conditions. Chloride is a relatively widespread contaminant in North America, sourced from oil and gas produced water sites, naturally saline groundwater discharges, salt storage yards, and major roadways where road salt is applied. Given toxicity may vary acutely with hardness level, should this also occur for sensitive species under chronic exposures, the availability of a chronic guideline that incorporates toxic response as a function of hardness level could notably improve the management of salt use and application across the continent. Surface water hardness is known to vary considerably within North America. Research was commissioned to toxicology laboratories in Canada and the United States to evaluate the relationship between water hardness and chronic chloride toxicity for key species including several demonstrating a high level of sensitivity towards chloride exposure (*Ceriodaphnia dubia, Raphidocelis (P. subcapitata), Anodonta subangulata, Lampsilis fasciola, Lampsilis siliquoidea, Lithobates pipiens,* and *Neocloeon (Centroptilum) triangulifer*). A subset of studies were conducted using different cations to assess the influence of cation composition on chloride toxicity. Results from these studies demonstrate a relationship between increasing water hardness and amelioration of chloride toxicity under a chronic exposure duration, for sensitive species. Data including published literature were compiled and processed via a species sensitivity distribution (SSD) approach for guideline development. Relationships between chloride exposure and water hardness were generated and subsequently used to derive a family of SSD chloride chronic toxicity curves as a function of water hardness. A mathematical algorithm was determined that could be used by various groups including regulatory agencies for the provision of chloride guidelines as a function of water hardness, for more optimal management of salt use and application. Aspects of cation composition were addressed as toxicity can vary as a function of the paired cation.

1.03.B.T Advances in Environmental Quality Guidelines, Criteria, Objectives and Benchmarks

1.03.B.T-01 Risks of Major Geochemical Ions to Aquatic Communities - Lessons Learned from Laboratory Investigations of Ion Toxicity to Several Aquatic Species

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Anthropogenic exposures of major geochemical ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, HCO_3^-/CO_3^{2-}) inherently involve complex mixtures for which relative ion concentrations can vary greatly. Laboratory investigations of ion toxicity have demonstrated multiple mechanisms of toxicity whose expression and potency depend on these relative ion concentrations and on the receptor organism. These mechanisms include osmotic impacts of total ions; toxicities specific to individual ions; and non-specific toxicity correlated with total ions but not associated with osmotic effects; in addition, the potency of some salts can depend on attributes of the exposure water such as Ca concentration. This complicates the assessment of risk of major ions to aquatic communities, necessitating consideration of multiple factors regarding both the relative concentrations of the various ions and the composition of the aquatic community. Practical considerations for addressing these complexities will be discussed, including the use of total ion concentration to address many exposure scenarios. *This abstract does not necessarily reflect U.S. Environmental Protection Agency policy.*

1.03.B.T-02 Development of a Draft Aquatic Life Criterion for Mercury in the State of Idaho

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The US Environmental Protection Agency (USEPA) has recently developed and published a draft chronic water quality criterion for the protection of aquatic life from the harmful effects of mercury in the State of Idaho. This assessment provides a critical review of all available data quantifying the toxicity of mercury to aquatic life and provides the basis for a tissue- and water-based criterion that will assure the protection of populations of fish, aquatic invertebrates, and aquatic life stages of amphibians in Idaho. Studies considered for possible inclusion for derivation of the whole-body and muscle tissue criterion elements utilized chronic dietary exposures of mercury (mostly as methylmercury), consistent with available data. USEPA used the tissue-based chronic toxicity data to derive muscle and whole-body criterion elements based on tissue from fish and aquatic invertebrates. USEPA then used the chronic tissue-based toxicity values based on all available aquatic taxa in conjunction with paired monitoring data for mercury in fish, crayfish and amphibian tissues and water to derive species- and trophic magnitude-specific bioaccumulation factors (BAFs) enabling the translation of tissue-based chronic toxicity values to water based chronic values. This approach considers the inherent sensitivity of the aquatic taxa to mercury via dietary exposure, as well as their bioaccumulation potential based on knowledge of trophic ecology and using Idaho-specific BAFs. Both the tissue and water column criterion elements were derived based on regression analysis using the 1985 Guidelines approach (Stephan et al, 1985). The proposed criterion element(s) of 225 ng total mercury/gram wet weight (ng THg/g ww) for muscle tissue and 162 ng THg/g ww for whole-body tissue are paired with the water column criterion element for Idaho waters of 2.1 ng/L THg in whole water will be implemented as a tiered criterion consisting of multiple elements, similar in structure to the national selenium criterion (USEPA, 2016, 2021).

1.03.B.T-03 You're killing me: Cautions for Developing Environmental Criteria in Terms of Fish Tissue Concentrations

***Chris Mebane**, U.S. Geological Survey*

For bioaccumulative substances, expressing aquatic life criteria in terms of fish tissue concentrations is attractive because this sidesteps problems of speciation, bioavailability and food web mediated trophic transfer from water to the organism. Also, toxicity can only occur once the substance enters the organism’s body, so expressing criteria in units closest to the mode of action more closely links the criteria to toxicity. However, the logic of killing fish to protect fish may be opposed by the fishing public. Depending how criteria are structured and implemented, assessing criteria compliance and trends through lethal sampling can result in large

numbers of killed fish. This conundrum is illustrated with the example of selenium (Se), for which an elaborate, intellectually elegant, fish-tissue based aquatic life criterion has been developed. Complications include nonscience-based implementation constraints, mobility of fish, seasonality of pre-spawning condition, debates over representativeness of ovary tissues, the need for overkill to obtain target numbers of gravid fish, and administrative assessment units that multiply data requirements for a water body. While these problems are exacerbated for the Se criteria because of its reliance on ovary/egg tissue, some of these issues are also pertinent to other substances with muscle tissue-based criteria. Dietary thresholds could be further considered in criteria development, as they capture major steps in the enrichment and tropic transfer from water to fish.

1.03.B.T-04 A Critique of Score-Based Data Quality Evaluation

***Dave Kuo**, National Taiwan University, Taipei, Taiwan (China)*

Data quality (DQ) evaluation is important for the benchmarking, modeling, and macro-level understanding of environmental and ecotoxicological data. It is commonly performed by scoring or screening experimental data against shortlisted DQ criteria. Such approach is appealing as it is quantitative and allows apparently effective and unbiased differentiation of poor-quality data from acceptable ones. Despite its widespread application in recent studies, the scientific validity of DQ scoring/screening has never been thoroughly examined. This study offers analytical evidence and theoretical arguments that seriously challenge the objectivity of score-based DQ assessment. It is argued and demonstrated using environmental and ecotoxicological datasets that: (i) the evaluation protocol as well as the selection of DQ criteria and their weights are inherently arbitrary, and they cannot be determined objectively, (ii) multiple DQ evaluation outcomes are possible for a given dataset since different choices of protocol, criteria, and weights can be made, (iii) DQ criteria change with data applications, so one-size-fit-all evaluation is not possible. These can be illustrated by general abstract considerations as well as practical DQ evaluation examples on environmental or ecotoxicological datasets. These three consequences imply that an objective DQ evaluation before application is known is not possible, and that any such evaluation requires case-by-case justification. Furthermore, biases intrinsic in preferential recording or omission of data attributes cannot be eliminated completely though they may be reduced with the inclusion of more attributes. This also means that the availability of experimental conditions or parameters and other relevant characterizations is, in itself, a good indicator of data quality. High dimensionality data enables more complex and thorough examination of the phenomenon in question by supporting in-depth and condition-specific analysis of the data. This work illustrates the limitations of score-based assessment methodology and advocates for: (i) neutral but comprehensive documentation of relevant measurement conditions / properties without DQ judgment, (ii) application-specific DQ evaluation and justifications, and (iii) development of next generation DQ paradigm and an evaluation protocol that is objective and rigorous.

1.03.B.T-05 Behavior of Different Target Attainment Frequencies for Aquatic Life Criteria: Quantifying the Overall Level of Effect Allowed by the EU-UK Annual Mean Versus USA Upper-Tail Targets

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Because ambient toxicant concentrations at any site exhibit time variability (a bell-curve distribution), a decision must be made about how much of the distribution should be below the criterion. The EU and UK target their aquatic life chronic criteria to be attained by the annual mean concentration. In contrast, the USA targets an upper-tail concentration, although in real-world regulatory practice its exceedance frequency is not uniformly definable because criteria are usually applied to hydrologic critical design events, thereby obscuring long-term performance. The work described here compares the behavior of the EU-UK and USA attainment frequency goals by quantifying their level of toxicity. Their difference depends on the degree of time-variability. Lesser time variability yields lesser differences between central tendency and upper-tail concentrations. For typical degrees of time variability, assuming lognormal distribution statistics, this difference in attainment goals might be calculated to be equivalent to a criterion concentration difference of roughly 2X. Such a difference appears to be dwarfed by common order-of magnitude differences in criteria concentrations for pollutants for which both the USA and EU or UK have tabulated values, with EU-UK values usually lower. Nevertheless, the work described here is less interested in comparing stringency than in comparing how their protectiveness responds to different degrees of time variability. The approach integrates the effect level across an entire distribution of concentrations – that is, it multiplies the likelihood of each concentration occurring (in the lognormal bell curve), times the effect level associated with that concentration (from the concentration-response S-curve). This was done for the 5th percentile sensitive species (having effect threshold assumed equal to the criterion), and for all species in a Species Sensitivity Distribution. Calculated in this manner, the overall level of effect produced by either attainment target appears to be small, although the USA target yields less effect. These calculations also indicated that the overall level of effect of an upper-tail target strongly depends on the degree of time variability, being most protective with high variability. In contrast, an arithmetic mean target (since its lognormal distribution percentile depends on the degree of variability) tends to yield a more uniform level of protection, irrespective of the degree of variability.

1.03.B.T-06 Water Quality Benchmarks for Human Pharmaceuticals in Freshwater: A US FDA Perspective

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Under the National Environmental Policy Act of 1969 (NEPA), the US Food and Drug Administration (FDA) is required, as a part of its regulatory process, to consider the environmental impacts of its actions such as approving drug applications, per 21 CFR part 25. With the increasing usage of FDA-approved medicines and the growing concerns over the impacts of contaminants of emerging concern (CECs) including pharmaceuticals on water safety and quality, there is an increasingly urgent need for establishing pharmaceutical water quality benchmarks (WQBs) for aquatic life in freshwater. Recently, the FDA Center for Drug Evaluation and Research (CDER) initiated a human pharmaceutical WQB program—similar to one initiated for aquaculture drugs previously by the FDA Center for Veterinary Medicine (CVM), and to some states—in partnership with the EPA national pollutant discharge elimination system (NPDES) on development of WQBs for human pharmaceuticals, to provide regulatory mitigation guidance values designed to protect aquatic life in freshwater. Within the prioritized categories of human pharmaceuticals, hormonal drugs are of particular concern as these drugs may cause detrimental effects on fish reproduction at trace levels that can be several orders of magnitude lower than FDA's current categorical exclusion (from an environmental assessment) level of 1 μg/L. Here we present a case study of a hormonal drug, the synthetic progestin levonorgestrel, for which we developed an interim benchmark in freshwater following a modified species sensitive distribution (SSD) approach. We also predicted the concentration distributions of levonorgestrel in surface water across the US by using iSTREEM, which is an environmental modeling tool for predicting the river concentrations of chemicals from consumer products disposed or otherwise released down the drain. Then we integrated these data and conducted a conservative environmental assessment of levonorgestrel in surface water in the US. Our assessment results indicate there might be potential ecological effects from levonorgestrel in the limited arid and semi-arid and high population locations. Thus far, however, FDA is not aware of any reported concentrations of levonorgestrel in US waters. The limitation of analytical method, together with other knowledge gaps such as the fate and transformation of levonorgestrel in the environment, need to be addressed in future studies.

1.03.P-Tu Advances in Environmental Quality Guidelines, Criteria, Objectives and Benchmarks

1.03.P-Tu-007 Addressing Fundamental Questions in Estimating Species Sensitivity Distributions

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Estimating species sensitivity distributions (SSDs) and the resulting hazardous concentrations for 5% of species is essential to derive defensible 'safe' concentrations, such as water quality benchmarks. There are several fundamental questions in estimating SSDs: Which statistical distribution to choose for deriving a species sensitivity distribution? Is model-averaging useful to accurately estimate an HC5? Can we predict chronic (or saltwater) SSDs from acute (or freshwater) SSDs? How much of an assessment factor should be applied? In this presentation, we present the results of a series of research to address these questions (Yanagihara et al. 2024 in Ecotox & Environ Safety; Hiki & Iwasaki 2020 in ES&T; Yanagihara et al. 2022 in ET&C; and Kamo et al. 2022 in Ecotox & Environ Safety).

1.03.P-Tu-008 Evaluating the Alignment Between Proposed Risk Assessments for Microplastics and Ecosystem-Based Mesocosm Experiments

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Approaches to derive ecological risk thresholds for microplastic pollution in aquatic environments have recently been proposed in the scientific literature; however, governments worldwide have yet to widely adopt environmental quality guidelines for microplastics. In 2022, the International Joint Commission's (IJC) Great Lakes Science Advisory Board (SAB) convened a work group to provide guidance on advancing frameworks for monitoring as well as assessing and managing the ecological risk of microplastic pollution in the Laurentian Great Lakes Basin. In 2023 and 2024, the IJC SAB Work Group hosted workshops with regional scientists and environmental managers to elicit recommendations on adapting existing frameworks—namely those used and developed by work groups convened, coordinated, and co-led by the Southern California Coastal Water Research Project Authority—for applicability to the Great Lakes region. Regarding risk assessment and management, recommendations included considerations of ecological relevance, jurisdictional standards for deriving environmental quality guidelines, and regional governance programs and structures. Ultimately, the IJC SAB Work Group used the Toxicity of Microplastics Explorer 2.0 to construct species sensitivity distributions (SSDs) and derive risk thresholds for exposure to microplastics in ambient water and sediment. This presentation will provide (1) more details on the approach used and factors considered by the IJC SAB Work Group to produce these outputs and (2) an assessment of their alignment with effects data produced from large-scale mesocosm experiments conducted at the IISD-Experimental Lakes Area. In 2021 and 2022, as part of a large collaborative project to better understand the fate and effects of microplastics in freshwater ecosystems, called the pELAstic Project, we conducted a series of in-lake mesocosm experiments in the pelagic and littoral zones of a freshwater lake. All mesocosms included relevant biotic communities, and microplastics were added at varying concentrations to mimic environmentally relevant exposures. Exposure and effects were measured throughout the experiments, which ran for about ten weeks. Because the concentrations tested overlap with those included in the SSDs constructed by the IJC SAB Work Group, and thus also the derived risk thresholds, the pELAstic Project provides a unique opportunity to assess the environmental relevance of the IJC SAB Work Group outputs.

1.03.P-Tu-009 Using Transcriptomic Point-of-Departures to Compare Sensitivity Across Fish Species

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Chemical regulatory assessors require hazard data from multiple species spanning a range of sensitivities as the foundation for developing environmental quality guidelines. Due to lengthy exposures, high costs, and ethical concerns, chronic chemical hazard data for diverse species are currently lacking. New Approach Methods (NAMs) have been touted as potential alternatives that could provide toxicity data quickly and more ethically. Our group is currently developing a NAM that uses early life stage fish exposures to obtain transcriptomic points-of-departure (tPODs). Previous studies support the possibility of substituting chronic apical PODs (aPOD) with tPODs generated from short-term exposures given their high degree of correlation. The overall objective of our project is to validate tPODs as suitable toxicity data for the derivation of environmental quality guidelines. As a first step, the aim of this study is to assess the reliability of tPODs to predict sensitivity differences across fish species. The oxidative product of 6PPD, 6PPD-Quinone (6PPD-Q), was chosen for this study because it is known to cause variable toxicity in different species of fish with a larger impact on salmonids. Correspondingly, a range of fish species such as salmonids, listed native species and model lab species were included in our study. Eleutheroembryos (0 to 24 hours post-hatch) were exposed for 24 hours to 6PPD-Q of 12 different concentrations (10^{-8} to 10 mg/L). The concentration range spanned across 9 orders of magnitude to cover the transcriptomic response and to potentially include the apical response of lethality. RNA was isolated from pooled eleutheroembryos, followed by RNA sequencing. Further processing was then done to generate tPODs using ExpressAnalyst. Our current results show that a difference of sensitivity of at least 3 orders of magnitude was observed among species. Similarly, a difference of 3 orders of magnitude was observed between acute LC50 and tPODs for at least one species. More exposures are currently underway to obtain acute LC50 and tPODs data, which will be presented and discussed. Future work will focus on comparing tPODs across fish species relative to chronic apical endpoints, paving the way for the potential use of tPODs in guideline development for chemicals of concern.

1.03.P-Tu-010 Aquatic Life Ambient Water Quality Benchmarks for Data-Limited PFAS Chemicals Using New Approach Methods

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The 1985 *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses* (1985 *Guidelines*) recommends that test data be available for a minimum of eight families to fulfill taxonomic minimum data requirements (MDRs) in order for US EPA to calculate aquatic life criteria values. Although direct test data are available and have been used by EPA to derive aquatic life criteria for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), direct test data are more limited and do not fulfill MDRs for most other per- and polyfluoroalkyl substances (PFAS). Accordingly, EPA recently developed acute freshwater benchmarks for selected PFAS using a New Approach Methods (NAMS) process, through the application of Interspecies Correlation Estimation (ICE) models. Benchmarks were developed for the selected PFAS using ICE model predictions in conjunction with available direct test data to support filling missing MDRs for each substance and allow the derivation of acute freshwater benchmark recommendations for the protection of aquatic life. Although not a criterion based on 1985 *Guidelines* specifications, derivation of benchmarks using this approach is consistent with the 1985 *Guidelines* "good science" clause, and with EPA's interest in providing relevant information to states and tribes regarding protective values for aquatic life. Additionally, development of the PFAS benchmarks reflects goals in EPA's PFAS Strategic Roadmap and aligns with the Agency's intention to reduce the use of additional animal testing through the application of NAMS.

1.03.P-Tu-011 Derivation of Environmental Quality Guidelines for Perfluorooctanoic Acid (PFOA)

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The Canadian Council of Ministers of the Environment (CCME) is the federal, provincial and territorial interjurisdictional forum responsible for the development of Canadian environmental quality guidelines. Currently, the CCME is deriving water, soil and groundwater environmental quality guidelines for perfluorooctanoic acid (PFOA). PFOA is an anthropogenic substance that falls under the broader class of chemicals known as per- and polyfluoroalkyl substances (PFAS) and is highly persistent and able to accumulate in organisms. The Government of Canada previously concluded that PFOA, its salts and its precursors are toxic to the environment. As such, tools are needed to help assess and manage the risks of PFOA to environmental receptors in the ambient environment and at contaminated sites. One such set of tools is environmental quality guidelines, which are science-based benchmarks for environmental protection based on hazard. Environmental concentrations can be compared against the guideline values to inform site screening, risk assessment and/or risk management; aid in interpreting monitoring data; or provide a scientific basis for control measures. These guidelines represent concentration thresholds below which there is low likelihood of adverse impacts from PFOA on ecological organisms or, in the case of soil, below which important ecological functions of the land will be maintained. The derivation process will be discussed and available draft guidelines will be presented.

1.03.P-Tu-012 Challenges in Estimating Appropriate Screening Values for Potential Direct Toxicity of Trivalent Chromium in Soil

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Current methods of estimating trivalent chromium toxicity in soil may greatly underestimate toxicity thresholds compared to field-contaminated soils, presenting challenges for assessing risks to plants and soil invertebrates. Comparing published studies of chromium geochemistry in soil with soil ecotoxicology studies, an apparent contradiction is evident: trivalent chromium is considered relatively nontoxic, yet soil ecotoxicity thresholds for this chromium species are relatively low. Trivalent chromium is typically added to soils as soluble chromium chloride in laboratory soil toxicity tests, which reacts with water in the soil to form chromium hydroxide precipitate, liberating protons and substantially increasing soil acidity. The decrease in pH can itself cause adverse effects and can also artificially increase chromium bioavailability. Further, the addition of trivalent chromium in an initially soluble form, combined with vigorous mixing, can promote artificially high oxidation by manganese oxides yielding hexavalent chromium, which is much more toxic than the trivalent form. Collectively, these factors result in unrealistically high toxicity of trivalent chromium in laboratory soil toxicity tests. By comparison, in environments with pH above 5.5, trivalent chromium almost fully precipitates; as such, trivalent chromium bioavailability is expected to be very low in historically contaminated soils with circumneutral pH conditions. Further research should focus on conducting spiked soil toxicity tests with soluble and insoluble forms of trivalent chromium to inform more environmentally relevant toxicity thresholds. Based on pH-dependent chromium solubility, risk assessors could consider using a narrative, site-specific approach when considering ecotoxicological effects, as is currently applied for iron and aluminum.

1.03.P-Tu-013 Development of U.S. Environmental Protection Agency Recommended Ambient Water Quality Criteria for Human Health for Per- and Polyfluorinated Substances (PFAS)

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Per- and polyfluoroalkyl substances (PFAS) are ubiquitous chemicals which may pose human

health risks. PFAS are found in nearly all environmental media (i.e., water, air, soil, etc.), and a broad range of cancer and noncancer health effects have been reported in animal toxicology and human epidemiology studies. One of the commitments in the U.S. Environmental Protection Agency's (EPA's) PFAS Strategic Roadmap is the development of human health criteria (HHC) for ambient water quality for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), as well as additional PFAS for which final EPA toxicity assessments are available. Under the Clean Water Act section 304(a), HHC represent specific levels of PFAS in ambient water at or below which adverse effects are not expected when humans are exposed via drinking water and/or fish and shellfish consumption. National recommended HHC provide states and authorized Tribes information to consider when adopting water quality standards to protect applicable designated uses. The EPA is developing HHC based on the best available scientific information for four PFAS: PFOA, PFOS, perfluorobutane sulfonate (PFBS), and hexafluoropropylene oxide dimer acid (HFPO-DA, "GenX chemicals"). This presentation will provide an overview of the HHC development process and summarize key HHC inputs, including toxicity values (reference doses, cancer slope factors), bioaccumulation factors (BAFs), relative source contributions (RSCs), and exposure factors (body weight, fish consumption rate, and drinking water ingestion rate) for four PFAS. *The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

1.03.P-Tu-014 Sensitivity Analysis of Human Health Sediment Quality Objectives at a Small PCB Impacted Site

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Regional Water Quality Control Boards in California are currently requiring Human Health (HH) Sediment Quality Objective (SQO) assessments to evaluate the potential for surface sediment bioaccumulatives to cause unacceptable risks to human seafood consumers. As detailed in the 2018 State of California's Water Quality Control Plan for Bays in Estuaries, the HH SQO assessment involves integration of chemical exposure and site linkage. Chemical exposure is measured by evaluating chemical concentrations in sport fish to determine if concentrations are associated with unacceptable exposure to human consumers based on exceedances of tissue advisory thresholds, and site sediment linkage, or the contribution of sediment contamination to seafood contamination levels, is estimated using a pre-established food web model. When applied to small sites (<1 km^2), there is a high degree of uncertainty due to sediment-fish linkage uncertainties (i.e., uncertain model bias, potential over-estimate of linkage due to unconfirmed home range size of fish), and chemical exposure uncertainties (i.e., the assessment assumes all seafood caught and consumed are from the site of interest and only sport fish required in the HH SQO are consumed). Thus, we evaluated a small Site in a Southern California embayment (0.1 km^2) to determine whether it meets the HH SQO and if not, what exposure inputs must change for the site to meet the HH SQO. Results revealed that there are no human health risks associated with chlordane, dieldrin and DDx at the Site. However, the HH SQO indicated a risk to human health from PCBs. Chemical exposure and sediment linkages were categorized as "very high" and "high", respectively, resulting in an overall assessment of "clearly impacted," a classification that indicates potentially unacceptable PCB risks from sediment. SQO model sensitivity analyses were conducted to simulate a reduction in sediment exposure, fish tissue concentrations, and aqueous PCB concentrations to determine what model input assumptions are necessary to achieve HH SQO compliance. One scenario in which the HH SQO can be met, is if sediment, fish tissue, and water concentrations drop by a factor of 7 to approximately 90 µg/kg dw, 20 µg/kg ww and 480 pg/L, respectively. In this case, the chemical exposure (HH fish consumption risk) has dropped from the very high to the low category. Numerous additional trials are underway as part of this sensitivity analysis, and will be presented.

1.03.P-Tu-015 How Well Does Score-Based Data Quality Evaluation Performs on BCF Data?

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Increasingly rigorous data quality (DQ) evaluations and/or screening practices are being adopted into modeling and meta-analysis studies over last two decades. This shift in attention to data quality reflects the growing awareness of how the accuracy in prediction and understanding relies on the quality of observations. Currently, data quality of environmental and ecotoxicological phenomena is typically examined by scoring given data against a set of preselected DQ criteria (Klimisch *et al.*, 1997). The score-based DQ methodology is also widely used in various non-environmental disciplines due to its generally intuitive and sensible nature. This study examined the effectiveness of score-based DQ evaluation using fish bioconcentration factor (BCF) (Arnot and Gobas, 2006) as an example. The overall goal was to see if DQ screening can support statistically meaningful differentiation of log *BCF* values. This was achieved by examining how log *BCF* differed with the overall DQ evaluation and specific data quality tags provided with the original data. Interactive effects among DQ criteria and hierarchical dependence of deviation in log *BCF* were also examined. Insignificant difference in log *BCF* between low-quality (LQ) and acceptable-quality (AQ) measurements was found for over 90% of the chemicals. Individually, the selected DQ criteria did not offer statistically meaningful differentiation in log *BCF*, with at least half of the criteria showing only minor influence on log *BCF* values. Tree analysis of deviation in log *BCF* revealed the absence of common structural dependence in the criteria violated. Analysis also revealed that violation of more criteria did not necessarily lead to greater deviation in log BCF. Collectively, these results suggested that the applied data quality assessment was not as effective as supposed. Finally, simple averaging of all measurements produced statistically identical log *BCF* as those associated exclusively with HQ high-quality ones. The surprising results from this work did not negate the need for DQ evaluation in general; however, the usefulness of score-based approach for DQ evaluation was challenged. The implications of this study with respect to growing numbers of environmental and ecotoxicological measurements and databases will be discussed.

1.03.P-Tu-016 Evidence for a Combined Ion Mechanism of Major Ion Toxicity to the Mayfly *Neocloeon triangulifer*

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Field assessments and laboratory studies have demonstrated that mayflies (Ephemeroptera) are relatively sensitive to elevated concentrations of major ions. Previous studies have noted some differences in the responses of the parthenogenetic mayfly *Neocloeon triangulifer* to various major ion salts relative to crustaceans and fish, but much remains to be learned about potential mechanisms of toxicity. In the present study, we sought to determine whether solution osmolarity is sufficient to predict the toxic effects of major ions to *Neocloeon triangulifer,* or if specific ions or aggregate ion toxicity better explained effects observed. We attempted to address these questions using organic molecules to manipulate solution osmolarity in the absence either of any specific ions, or of anions. In addition, we tested the responses of the mayfly to binary or ternary mixtures of major ion salts to assess whether ion toxicities were additive or independently acting. We observed that major ion salts caused toxicity at external osmolarities much lower than did the organic molecule mannitol (presumed to have only an osmotic effect), which led to the conclusion that a general osmotic effect is not responsible for major ion toxicity in this species. Several other general trends emerged from the data. First, increasing calcium concentrations in dilution water is associated with a decrease in major ion toxicity, as has been reported elsewhere for other species. Second, in contrast to what was observed for other species, we saw no indication that chloride salts of magnesium, calcium, or potassium were any more toxic than sodium chloride when compared on the chloride concentration basis, suggesting a possible common toxicity mechanism for all the chloride salts in the mayfly. Third, while sulfate salts appear more toxic than chloride salts when compared on the basis of total ion concentrations, accounting for the reduction in calcium activity caused by sulfate better aligned their toxicities. Finally, our mixture evaluations indicated that all salt pairs had at least some degree of additivity across diverse salts/ions, supporting the idea of an aggregate ion toxicity. Thus far, the best explanation of the mayfly responses to major ions is an aggregate or combined ion effect, with no strong evidence of greater toxicity from a single ion.

1.04.P-Th Behavioral Endpoints and Methods as a Line of Evidence in Regulatory Toxicity Testing

1.04.P-Th-001 Examining the Role of Particulate Matter Exposure on Autism Spectrum Disorder-Like Behaviors in Developing Zebrafish

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Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders that can impact communication and behavior to varying degrees. The causes of ASD are thought to be a combination of genetic and environmental factors. Recent evidence suggests that air pollution may play a role in ASD. Particulate matter (PM) is the solid and liquid portion of air pollution that has been associated with various human health impacts, including ASD. However, PM is comprised of complex, variable mixtures so much is unknown about the role PM plays on the development of ASD-like behaviors. Therefore, we aimed to assess various measures of sociality and anxiety in zebrafish exposed to PM compared to ASD-induced zebrafish. Zebrafish (n=33/group) were exposed to valproic acid (VPA), a known inducer of ASD for 5 days during development, beginning at the embryonic stage. The exposures occurred individually and in combination with a PM standard reference material and melatonin (a natural product hypothesized as treatment of ASD-related symptoms). Behavioral assays that may be indicative of ASD-related changes, including larval photomotor response (LPR), light/dark preference, shoaling activity, mirror attack, and social contact, were performed on subsequent days after exposure to compare sociality, aggression, and anxiety between treatment groups. In preliminary exposures, survival was decreased by 25% or more and hatching was delayed in larvae exposed to PM alone and in combination with VPA or melatonin. In the LPR, which measures movement over light and dark conditions, swim distance was decreased by approximately 50% or more in larvae exposed to PM or VPA, with combination treatment of PM and VPA eliciting a potentially additive or synergistic effect. Interestingly, melatonin treatment did not appear to ameliorate the impacts of the exposures, as expected, but it is possible that melatonin treatment will be able to exert a therapeutic effect on the other tested ASD-like behaviors. Biological replication in addition to video and statistical analyses of other behavioral assays are currently in progress. Overall, this work will elucidate the impact of PM and melatonin on the development and progression of behaviors related to autism spectrum disorder in developing larval zebrafish to ultimately expand knowledge of PM related health impacts beyond the cardiovascular and respiratory systems.

1.04.T Behavioral Endpoints and Methods as a Line of Evidence in Regulatory Toxicity Testing

1.04.T-01 EthoCRED: A Framework to Guide Reporting and Evaluation of the Relevance and Reliability of Behavioural Ecotoxicity Studies

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Behavioural analysis has been attracting significant attention as a broad indicator of sub-lethal toxicity, and has secured a place as an important sub-discipline in ecotoxicology. One of the most notable characteristics of behavioural research, compared to other established approaches in sub-lethal ecotoxicology (e.g. reproductive and developmental bioassays), is the wide range of study designs being used and the diversity of endpoints considered. At the same time, environmental hazard and risk assessment, which underpins regulatory decisions to protect the environment from potentially harmful chemicals, often recommends that ecotoxicological data be produced following accepted and validated test guidelines. These guidelines typically do not address behavioural changes, meaning that these, often sensitive, effects are not represented in hazard and risk assessments. In this presentation, we propose a new tool, the EthoCRED evaluation method, for assessing the relevance and reliability of behavioural ecotoxicity data, which considers the unique requirements and challenges encountered in this field. This method, and accompanying reporting recommendations, are designed to serve as an extension of the 'Criteria for Reporting and Evaluating Ecotoxicity Data (CRED)' project. As such, EthoCRED can both accommodate the wide array of experimental design approaches seen in behavioural ecotoxicology, and is able to be readily implemented into regulatory frameworks in different jurisdictions to allow better integration of knowledge gained from behavioural testing into environmental protection. Furthermore, through our reporting recommendations, we aim to improve the reporting of behavioural studies in the peer-reviewed literature, and thereby increase their usefulness in chemicals regulation.

1.04.T-02 Towards a "Behavioral Best Practice": Engineering and Experimental Considerations in Developing Behavioral Assays, an Example Using Hypoxic Response in the Pond Snail

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In recent years, there has been greater integration of behavioral changes in toxicology studies and discussions for the inclusion of behavioral endpoints in regulatory frameworks. The reliability of behavioral data is highly dependent upon the design of the behavioral assay used. Various recommendations and considerations have been put forth in an effort to improve the reliability and relevance of behavioral assays but remain in their infancy. Putting these recommendations into practice, we outline the engineering and experimental considerations involved in designing a behavioral assay to assess the greater pond snail, *Lymnaea stagnalis*, response to hypoxia. *Lymnaea stagnalis* exhibit bimodal breathing – having the ability to respire across the somatic epidermis and via the lung – the relative proportions of which are determined by the oxygen content in the water. The respiratory physiology of this species has been exploited in studying hypoxic response; however, the methods used do not align with the best practices in behavioral assessments and the potential for confounding factors in these assays are substantial. The establishment of this assay considered aspects such as acclimation, gradual change, sociality, and researcher interference, the learnings of which can be applied to future instances of behavioral assay design and development.

1.04.T-03 A Zebrafish-Based Platform for Dissecting the Etiologies of Chemical-Induced Stress Behavioral Alterations

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Zebrafish behavioral assays have emerged as effective tools for identifying contaminant-induced developmental sub-lethal toxicities due to their ease, sensitivity, and high throughput capacity. These assays quantify zebrafish behavioral responses to acute stimuli and observe how these responses are altered following contaminant exposures. However, the recorded behavioral motor responses rely on multiple systems, such as the nervous system, sensory organs, and musculoskeletal system, making it challenging to pinpoint contaminant-induced deficits in a particular organ or system. To address these challenges, we optimized a high-throughput zebrafish-based platform that incorporates three behavioral motor response assays: a visual motor response (VMR) assay, an acoustic motor response (AMR) assay, and a peripheral motor response (PMR) assay. The VMR assay relies on visual input, neuronal processing, and a locomotor response, with contaminant-induced alterations in any of these systems potentially affecting the associated behavioral stress response. To pinpoint changes related to visual deficits, we developed the AMR assay, which uses acute acoustic stimuli to elicit a behavioral motor response. Additionally, we created an assay that assesses the locomotor capacity of larval zebrafish through chemical irritants that elicit a peripherally driven motor response. Using this platform, we identified various patterns and etiologies associated with contaminant-induced stress behavioral alterations, making it very useful in mechanistic investigations focusing on the origins of disease.

1.04.T-05 Utilizing Multiple Behavioral Endpoints to Identify Negative Control Chemicals in a Larval Zebrafish Behavior Assay

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Identifying reliable positive and negative control compounds is integral to determining the sensitivity and specificity of behavioral assays. Our laboratory screens chemicals for developmental neurotoxicity potential using behavioral assays that assess larval zebrafish locomotor activity in response to visual stimuli. There are established positive controls for these assays; however, well-characterized negative controls are lacking. Using nine chemicals that have no evidence of developmental neurotoxicity in mammals (PMID: 35908584; Martin *et al.*, 2022), we performed a range-finding study to assess lethality and teratogenic potential of each chemical (≤ 100 μM). Next, using non-toxic concentrations, the locomotor activity profile was assessed at 6 days post fertilization using a light/dark transition assay. Behavioral data were analyzed using two methods: (1) the traditional method which compared the average distance moved in the light period and in the dark period using a repeated measures analysis of variance (ANOVA), and (2) a novel approach which analyzed 13 aspects of the locomotor profile (*e.g.*, average speed, habituation, maximum/minimum activity, aspects of the startle response, activity range, and area under the curve) and constructed a benchmark concentration for each endpoint if there was a concentration-related change. Fluoxetine, the positive control for developmental neurotoxicity, was also evaluated (≤ 4 μM) using the two analysis methods: the traditional method showed an overall effect of concentration ($p < 0.0001$), and the novel method showed concentration-related changes in 9 out of the 13 endpoints, thus confirming its developmentally neurotoxic effects in zebrafish larvae. With the remaining chemicals, the traditional method showed no effect from the nine putative negative chemicals (*i.e.*, D-mannitol, glycerol, ibuprofen, L-ascorbic acid, metformin hydrochloride, omeprazole, saccharin, selegiline hydrochloride, or sodium benzoate); however, the novel method uncovered a concentration-related change in average speed in the light for selegiline hydrochloride. Therefore, the novel analysis approach may be more sensitive to behavioral changes than the traditional method. Standardizing a set of negative control chemicals is essential for researchers to properly evaluate the behavioral alterations precipitated by unknown chemicals and will assist with screening new chemicals. *This abstract does not reflect the official policy of the U.S. Environmental Protection Agency.*

1.04.T-06 Effects of Methylmercury Exposure on Complex Behaviors in Fathead Minnows (*Pimephales promelas*)

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Adverse effects on the neurobehaviors of fishes due to environmental toxicants can have a serious impact. Complex behaviors such as learning, social behaviors, and aggression are crucial for survival and fitness of the species population. Methylmercury is a neurotoxicant that can bioaccumulate in aquatic organisms including fishes and is known to affect several crucial behaviors in various species. This study aimed to determine the impacts of dietary methylmercury exposure on aggression, social and learning behaviors in adult male and female fathead minnows (*Pimephales promelas*). Fish were exposed to two diets: control and methylmercury (1ppm) for 30 days. The impact of methylmercury on the complex behaviors of aggression, social preference and learning was compared in control and exposed fish using a mirror test, a dual-choice sociability test and T Maze assay respectively. Overall activity and swimming behavior was analyzed using the Novel Tank Dive test. Methylmercury exposure lead to a significant increase in aggression in male fish shown by an increase in the number of attacks on the mirror, increased time spent attacking the mirror, and increased time spent in the mirror zone. There was a significant reduction in overall swimming activity in female fish shown by increased time spent in the bottom zone, reduced time spent in the top zone, reduced distance traveled in the novel tank dive test. Similar results were seen in male fish in the novel tank dive test with increased time spent in the bottom zone, reduced time spent in the top zone, reduced distance traveled and reduced active time. There was decreased sociability in female fish, shown by reduced visits and time spent in social zone, increased visits and time spent in asocial zone. We also expect to see changes in the learning ability in methylmercury exposed male and female fish. The end points that will be measured in the T Maze assay includes, time taken to reach positive zone, percentage of visits to positive zone, average time spent in positive and negative zone and percentage of fish entering positive or negative zone first. We will then examine physiological and biochemical changes underlying these neurobehavioral impairments. This research has both ecological as well as human health relevance and can help in predicting the long-term impacts of methylmercury.

1.05.P-Tu Bioconcentration and Biological Magnification of Emerging Contaminants: Synergism and Antagonism

1.05.P-Tu-018 Cladistic Profiling of Biosurfactant Producing Bacteria Species and the Comparative Functional Activity of the Product on Hydrocarbon Degradation

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Biodegradation by biological surfactants from natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environments. In this study, fifty-two (52) bacterial isolates were isolated from nine (9) environmental samples and screened for biosurfactant production ability. The twenty-three (23) bacteria isolates (44%) that showed positive β hemolysis were identified by 16S rRNA sequencing. Sixteen (16) bacteria genera identified were *Comamonas testosteroni* (3), *Enterobacter ludwigii* (1), *Bacillus cereus* (1), *Uncultured Pseudomonas* sp. clone SCA_201L (1), Uncultured bacterium clone JX981747 (2), *Burkholderia caledonica* (1), *Pseudomonas tolaasii* (1), *Pseudomonas putida* (2), *Pseudomonas aeruginosa* (2), *Stenotrophomonas maltophilia* (1), *Pseudomonas plecoglossicida* (1), *Pseudomonas aeruginosa* strain R873 (1), *Enterobacter* sp. PD-P37 (1), *Arcobacter trophiarum* (1), *Odoribacter splanchnicus* DSM 20712 (1) and uncultured *Paenibacillus* sp (2). The cladistic analysis of these bacteria genera, using the phylogenetic tree drawn with the neighbour joining method, depict 5 clusters with varying sub-clusters of isolates habouring genes of negligible differences. Biosurfactants from *Odoribacter splanchnicus* DSM20712 and unidentified bacterium clone JX981747 had the highest Emulsification index (E_{24}) value. Comparatively, the functional ability of the test organic surfactant and chemical surfactant (sodium lauryl sulphate [CHS]) to lower the Total Petroleum Hydrocarbon (TPH) content of polluted soil was evaluated by Gas Chromatography using seven treatments set up analyzed at week 0, week 4 and week 16. Although the CHS recorded slightly higher diesel engine oil degrading efficiency than *O. splanchnicus,* the later degraded crude oil more at faster rate.

1.05.P-Tu-019 Influence of Salinity and Temperature on PFAS Accumulation and Depuration in Bluegills

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Concerns are growing over the bioaccumulation of per- and polyfluoroalkyl substances (PFAS) in fish. The uptake of PFAS in fish can be influenced by many factors, including species, location, PFAS compounds, and water quality parameters. In regions where de-icing salts are used on roads, major ions like sodium are introduced into freshwater systems, potentially impacting fish physiology and their ability to uptake contaminants. Additionally, temperature can affect PFAS accumulation in fish by influencing metabolism and subsequent enzyme activity. This study evaluated the uptake of PFAS in Bluegill (*Lepomis macrochirus*) under different temperature and salinity conditions. Two separate experiments were conducted at 20 and 25 °C, while maintaining salinity levels of 300, 600, and 1200 µS/cm. The PFAS mixture contained four highly relevant PFAS compounds: 1,000 ng/L Perfluorooctanesulfonate (PFOS), 600 ng/L Perfluorohexanesulfonic acid (PFHxS), 250 ng/L Perfluorohexanoic Acid (PFHxA), and 170 ng/L Perfluorooctanoic Acid (PFOA). The fish were exposed to the PFAS mixture for 32 days, followed by a depuration period of 32 days in dechlorinated tap water (without PFAS). A control treatment of 300 S/cm salinity was also monitored under the same conditions and sampling schedule. Liver and water samples were collected on days 2, 4, 8, 16, 24, and 32 for analysis of PFAS concentrations during both uptake and depuration phases. This research aimed to enhance our understanding of how environmental factors such as temperature and salinity influence the bioaccumulation of PFAS mixtures in fish.

1.05.P-Tu-020 Studies on the Biodegradation of Low-Density Polyethylene by Bacillus Spp.

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Polyethylene (PE) products have short-term applications, resulting to the daily release of enormous quantities of wastes into the environment with their attendant public and environmental health threats of global concern. Developing an eco-friendly management method to abate the menace prompted this research work. Three (3) Bacillus species were isolated from different dumpsites in Owerri Metropolis, and identified using morphological, biochemical and molecular characteristics as *B.pumilus*, *B.siamensis* and *B.coagulans*. The ability of the microbial isolates to degrade polyethylene (table (pure) water sachets) was determined using microbial growth measurement in liquid medium (Optical Density (OD600)), disappearance and introduction of functional groups (Fourier Transform Infrared spectroscopy (FTIR) and weight loss analysis after, measured sixty (60) days of incubation in mineral salt vitamin media. Microbial concentrations showed a gradual and progressive increase: *B. siamensis* (0.120-0.835), *B. pumilus* (0.110-0.669) and *B. coagulans* (0.118-0.608), as against the control medium which remained unchanged (0.108 and 0.108). Similarly, FTIR spectra showed additional functional groups (carboxylic acids, ethers, esters, amines, methylene, alcohols, and alkenes in the polyethylene materials exposed to the test isolates: *B. siamensis* (12 peaks), *B. pumilus* (12 peaks) and *B. coagulans* (13 peaks), but control (6 peaks). There were substantial reduction in the residual weights of the PE materials: *B. siamensis* (52%), *B. pumilus* (26%) and *B. coagulans* (18%). The observations showed that these Bacillus spp. indigenous to Owerri Metropolis have the ability to degrade PE and utilize it as a carbon source, and it was concluded that PE which was regarded to be inert can be biodegraded when exposed to the right microorganisms.

1.05.P-Tu-022 Mixture Effects of Per- and Polyfluoroalkyl Substances on Embryonic and Larval Sheepshead Minnows (*Cyprinodon variegatus*)

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants originating from many everyday products. Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are two PFAS that are particularly pervasive in aquatic environments. Both chemicals are toxic to fish and have complex and largely uncharacterized mixture effects. However, limited information is available on saltwater species. In this study, embryonic and larval sheepshead minnows (*Cyprinodon variegatus*) were exposed to several PFAS mixtures to assess lethal and sublethal effects. PFOS alone was acutely toxic to larvae, with a 96 h LC_{50} of 1.97 mg/L (1.64–2.16). PFOS + PFOA resulted in a larval LC_{50} of 3.10 (2.62–3.79) mg/L, suggesting an antagonistic effect. These observations were supported by significant reductions in malondialdehyde (105% ± 3.25) and increases in reduced glutathione concentrations (43.8% ± 1.78) in PFOS + PFOA exposures compared to PFOS-only treatments, indicating reduced oxidative stress. While PFOA reduced PFOS-induced mortality (97.0% ± 3.03), perfluorohexanoic acid (PFHxA) and perfluorobutanoic acid (PFBA) did not. PFOS alone did not affect expression of peroxisome proliferator-activated receptor alpha (pparα) but significantly upregulated apolipoprotein A4 (apoa4) (112.4% ± 17.8), a downstream product of pparα, while none of the other PFAS affected apoa4 expression. These findings suggest that there are antagonistic interactions between PFOA and PFOS that may reduce mixture toxicity in larval sheepshead minnows through reduced oxidative stress.

1.06.P-Th Case Studies Using Molecular Tools and New Approach Methodologies for Assessing Toxicity in Non-Model Species

1.06.P-Th-002 Updating Algal Models in US EPA's Web-based Interspecies Correlation Estimation (Web-ICE) Toxicity Extrapolation Tool

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The United States Environmental Protection Agency's Web-based Interspecies Correlation Estimation tool, Web-ICE, is a New Approach Methodology which uses existing standard test data to extrapolate and predict toxicity to diverse taxa. This publicly accessible application allows users to estimate acute chemical toxicity to a diversity of not only wildlife, but also fresh and saltwater invertebrates, fish, and plants (algae) that may have limited toxicity data. While the recently updated ICE aquatic animals database is robust, the lack of available algae data resulted in only 104 algae ICE models in Web-ICE v3.3 (updated in 2016). Efforts are underway to expand upon the existing ICE algal database. In addition to database and model expansion,

uncertainty analyses like those conducted on ICE aquatic animal models are limited for algae due to small original datasets. As a result, user guidance and model selection criteria have been based on animal models. Here, the expansion of the ICE algae database and model set aims to improve the availability of ICE models for diverse algae species, reduce uncertainty, confirm user guidance for these taxa, and further strengthen the applicability of Web-ICE for risk assessors and environmental managers for ecological risk assessment of chemicals. *The views expressed in this abstract are those of the authors and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency. Any mention of trade names, manufacturers or products does not imply an endorsement by the United States Government or the U.S. Environmental Protection Agency.*

1.06.P-Th-003 The Mysid Shrimp as a Model for Endocrine Disruption Screening: Identification of Transcriptomic Biomarkers

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The mysid shrimp (*Americamysis bahia*) is a commonly-employed model organism for the routine assessment of effluents discharged into marine environments. Though this species is primarily used to evaluate acute and chronic toxicity, the results of previous studies provide information indicating its potential as an invertebrate model for endocrine disruption screening. The number of endpoints available for detecting ecdysteroid signaling disruption in this species is limited; therefore, the aim of this work was to assess the impacts of ponasterone A (a model ecdysteroid), 17b-estradiol, and trenbolone on mysid growth and global gene expression profiles in an effort to identify biomarkers indicative of endocrine disruption. To accomplish this, newly-hatched mysids were exposed to three concentrations of each chemical for 14 d. Growth was assessed in all groups at 7 and 14 d, while transcriptomic profiles were determined for a subset of groups. Ponasterone A exposure significantly increased length on day 7, but did not alter growth parameters after 14 d of exposure. Transcriptomic analysis identified a total of 253 downregulated and 172 upregulated genes between control mysids and those exposed to the highest concentration of ponasterone A. The nature of these differentially-expressed genes will be discussed, along with the concentration-dependent transcriptional responses to 17b-estradiol and trenbolone. Overall, the results of this study provide key insights into the molecular responses of mysid shrimp to endocrine disruptors and aid in biomarker identification.

1.06.P-Th-004 Effects of Mixtures on Gene Expression and Enzymatic Activity on the Freshwater Gastropod *Physella acuta*

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Aquatic ecosystems are increasingly threatened by pollution. These are affected not only by a single pollutant but also by anthropogenic compounds such as microplastics, pesticides, and heavy metals. The presence of multiple chemicals results in complex interactions between them that we generally do not consider. As the environmental conditions are intricate, standard toxicity tests can underestimate the effects and the interactions between the chemicals, which could affect the development and survival of aquatic invertebrates, menacing their populations. Therefore, as a first approximation to mimic environmental conditions, we exposed the freshwater gastropod *Physella acuta* to three known contaminants with different chemical natures. The aim is to obtain data regarding the response over time and identify biomarkers that can help standardize the gene expression analysis using a low-cost approach compared to omics technology. The exposure to Bisphenol A (BPA), Endosulfan, and Cadmium was performed using a ray approach (individually, in binary and ternary mixtures). The animals were collected for gene expression and enzyme activity analysis at 24h, 7, and 21 days. The effects were evaluated using retrotranscription and Real-Time PCR with genes related to stress response, endocrine system, and detoxification mechanisms. Additionally, enzymatic activity was also analyzed to complete the gene expression profile. The mixture analysis provides a closer picture of the invertebrates' actual exposure. Finally, the data obtained can be helpful for other species since the main obstacle is the lack of proper identification of non-model invertebrate sequences in the database. In conclusion, this work can help to develop a simple standard procedure by using a set of genes common to different invertebrate groups to evaluate the impact at the molecular level of the chemicals. By doing this, more species could be analyzed, and the management of the ecosystems subject to pollution could be improved.

1.06.P-Th-005 Modeling Synthetic Progestin Binding to Fathead Minnow Steroid Hormone Receptors

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Pharmaceuticals in the environment have been extensively researched over the past 20 years. Several of these compounds have been extensively researched due to their potential impacts to the endocrine system of aquatic organisms. The focus of endocrine disruption research has centered on how estrogenic and androgenic compounds interact with the endocrine system to elicit reproductive effects. Synthetic progestins have only recently been investigated in which these compounds display negative reproductive consequences in teleost species. This research utilizes CB-Dock 2, a computational tool that provides accurate protein-ligand blind docking to explore binding sites of receptors. Progesterone and several synthetic progestins are docked to the human progesterone receptor (hPR) to confirm ligand interactions with amino acids within the ligand binding domain (LBD). Next, the synthetic progestins are blindly docked with the LBD of the fathead minnow progesterone receptor (fhmPR). Finally, the fathead minnow androgen and estrogen receptors docked with synthetic progestins to investigate potential interactions with other steroid hormone receptors. This is the first research to demonstrate the receptor-ligand interactions of synthetic progestins and fathead minnow steroid hormone receptors. Results from this study will be utilized to design receptor-ligand binding studies to investigate the affinity of synthetic progestins and fish steroid hormone receptors.

1.06.P-Th-006 The Multispecies Ovary Tissue Histology Electronic Repository (MOTHER): A Resource for Evaluating Adverse Effects

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Multiple reproductive strategies exist among animal species with each species having its own "normal" processes and range of interindividual variability. To identify adverse effects of toxicants on fertility and reproduction, we first need to understand what is normal, and in the case of ecological endpoints this spans a wide range of species. The Multispecies Ovary Tissue Histology Electronic Repository (MOTHER) is a web-accessible repository of ovary tissue histology digital images and metadata for non-human species (see mother-db.org). MOTHER facilitates the sharing of digitized ovary histology images from (i) untreated and treated animals, (ii) across species, and (iii) across life stages. MOTHER provides a Web-based application ezEML+MOTHER for contributing slide images and metadata, a database for storing the metadata that is searchable via a Web interface, and a process for curating contributions into MOTHER. As part of the image metadata collection, contributors are required to specify intellectual rights for use of the image, which facilitates the reuse of images for research studies and education. In addition, the MOTHER project team maintains written protocols that ensure consistent data collection and proper provenance of the images. If needed, the MOTHER project team can assist contributors with digitizing histology slides. Currently, MOTHER contains histology slides and their metadata from over 300 individual macaques, mice, and other species that have been submitted by MOTHER team members and our collaborators. With these images, we have tested our data quality protocols, data storage and curation pipeline. MOTHER provides curated data that enables the development of models and tools to advance the understanding of ovarian (dys)function. Shared images and metadata can lead to: (i) the development of spatially realistic cell-/mechanism-based computational models to study ovarian development and toxicant-induced adverse effects; (ii) comparative studies across species; (iii) use in artificial intelligence and machine learning algorithms; and (iv) educational applications. Further, the design of MOTHER can be adapted for other tissues. We invite scientists to collaborate with MOTHER by sharing their ovary histology slides from any non-human species and to explore the images in MOTHER for developing new approaches to evaluate female reproductive toxicity.

1.06.P-Th-007 Rapid and Predictive Assessment of Developmental and Reproductive Toxicity Using *C. elegans* as a New Approach Methodology

Sudip Mondal, *Adam Laing, Amber Shen, Evan Hegarty and Adela Ben-Yakar, vivoVerse*

DART assessment (Developmental and Reproductive Toxicity) is an important part of product safety assessment and is traditionally performed with vertebrate models due to the need to study a complete life cycle and reproductive system. *C. elegans* has been of great interest to the toxicology community for years as a promising *in vivo* alternative to vertebrate animal testing. It has a short life cycle, high genetic homology with humans, shares many toxicology-relevant cellular pathways, has multiple wild-type genetic backgrounds cataloged, and can be cultured rapidly. However, the lack of high throughput analysis methods and limited endpoints have been major obstacles to its usefulness. We have developed a microfluidic-based imaging platform that enables multiparametric analysis of a range of sublethal phenotypes relevant to DART-related endpoints in *C. elegans* at high throughputs. We have shown that *in utero* counting and classification of embryonic developmental stages enabled by 4D imaging of the *C. elegans* germline is a more sensitive readout (~4×) of reproductive toxicity than total embryo number. Rank correlation of toxicity between our C. elegans-based method and reported rat data is 0.83. In addition, we have developed fully automated assays for several endpoints relevant to developmental and systemic toxicity using ML-assisted image analysis. Body volume, motility, and stress-induced autofluorescence can all be quantified in the same assay along with the embryonic phenotypes. The combination of multiple endpoints increases assay sensitivity and predictiveness and can inform further mode of action studies. We have validated our assay by performing multiparametric toxicity analysis of several agricultural chemicals relevant to environmental toxicology (mesotrione, propiconazole, glyphosate). These results demonstrate the utility of our assay as a New Approach Methodology (NAM) for DART, providing rapid predictive ethical in vivo toxicology testing without using vertebrate animals, at a fraction of the cost and time of mammalian studies.

1.06.T Case Studies Using Molecular Tools and New Approach Methodologies for Assessing Toxicity in Non-Model Species

1.06.T-01 Transcriptomic Responses of Zebrafish Following Dietary Exposure to the Short Chain PFAS Perfluorohexanoic Acid (PFHxA)

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Per- and polyfluoroalkyl substances (PFAS) have garnered tremendous concern due to the widespread contamination and environmental persistence as PFAS and their heavy use in commercial products. While the properties of PFAS are useful for various consumer goods, the same properties result in high persistence of PFAS in the environment as well as an increased risk of exposure and deleterious effects for humans and ecosystems. Historically, research has focused on perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), but there are more than 9,000 PFAS which can potentially impact human and ecological health in different ways. To address data gaps for less studied PFAS, the use of molecular tools is essential to identify novel toxicity pathways that help to reduce additional animal testing for this large chemical class. In our current study, we exposed a model species (zebrafish) to an emerging short chain PFAS, perfluorohexanoic acid (PFHxA), via the diet, which is an understudied exposure route in fish. Fish were exposed over 28 days to an environmentally-relevant dietary dose (100 ng/g) as well as a higher dose (1000 ng/g). At the end of the exposure, fish were euthanized, weighed, and measured to examine effects on growth, hepatosomatic index, and gonadosomatic index. Liver tissues were processed for mRNA and libraries were prepared for RNA-seq analysis. Sequencing results have been received and will analyzed for differential expression in zebrafish to examine novel toxicity pathways as well as similarities and differences across a model species. In addition to the transcriptome, qPCR will be performed to further assess changes in gene expression due to PFHxA dietary exposure. Analyses from these exposures are currently underway and will be presented. This study will be the first to examine sub-chronic dietary exposures to PFHxA and the resulting impacts on the transcriptome. We anticipate that these results will drive the development of new approach methodologies and assays that can help to screen the 1000s of PFAS with unknown toxicity profiles, which are needed to make accurate estimates of the potential risk these chemicals pose.

1.06.T-02 Multi-Omics Integration for In silico Biomarker Discovery and In Vivo Verification Using a Zebrafish (*Danio rerio*) Stoichiometric Metabolic Model

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We describe the use of an integrated multi-omics systems toxicological approach to identify physiologically relevant biomarkers of perfluorooctane sulfonate (PFOS) toxicity in fish. An *in silico* stoichiometric metabolism model of zebrafish was used to integrate available metabolomics and transcriptomics datasets from *in vivo* toxicological studies with 5 days post fertilized embryo-larval zebrafish. The experimentally derived omics datasets were used as constraints to parameterize an in silico stoichiometric model of zebrafish metabolism. *In silico* simulations using flux balance analysis (FBA) showed prominent effects of PFOS exposure on the carnitine shuttle and fatty acid oxidation. Further analysis of impacted metabolites indicated carnitine to be the most highly represented cofactor metabolite. Taken together, our integrative *in silico* results showed dyslipidemia effects under PFOS exposure and uniquely identified carnitine as a candidate metabolite biomarker. Subsequently, verification of this prediction was sought through an *in vivo* environmental monitoring study which showed carnitine to be a modal biomarker of PFOS exposure in wild-caught fish and marine mammals sampled from the northern Gulf of Mexico. Therefore, we highlight the efficacy of FBA to integrate multi-omics datasets to study the properties of large-scale metabolic networks and identify biomarkers of exposure and likely adverse effects.

1.06.T-03 Developing a Crustacean Embryo Toxicity Assay for Screening Environmental Contaminants

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Environmental pollutants can disrupt normal developmental processes leading to neurobehavioral and developmental abnormalities. Toxicity tests are effective monitoring tools in evaluating environmental pollutants, however, standard toxicity tests are time and cost-intensive, and typically require many vertebrate animals. Due to these factors, there is a need to reduce reliance on vertebrate testing and develop New Approach Methodologies (NAMs) that can achieve similar results that are cost-effective and efficient. This research aims to design a screening assay for evaluating developmental toxicity using an invertebrate species, *Hyalella azteca. H. azteca* is a freshwater amphipod that is an existing model organism for sediment toxicity in ecotoxicology; however, little has been studied using *H. azteca* embryos. This study utilizes *H. azteca* embryos to develop the Crustacean Embryo Toxicity (CET) assay. The goal of the present study is to further optimize the CET assay using well-known teratogens. Three teratogens; ethanol, warfarin, and lead were chosen for optimizing assay design and endpoints: morphology, gene expression, and neurobehavior. Morphological impacts from the three teratogens were identified through broad phenotypic changes after hatching and include impacts such as incomplete limb and antenna development, and abnormalities in the digestive system from ethanol exposure, an enlarged abdominal area from lead exposure, and a thick abdomen in warfarin exposures. Gene expression evaluated several conserved genes associated with embryonic development, including the orthodenticle (otd), engrailed (en), and antennapedia (antp) genes, to establish baseline expression and identify differences in expression from embryos exposed to teratogens. Neurobehavior assessments evaluated un-exposed *H. azteca* to establish a behavioral baseline, and *H. azteca* exposed to the three teratogens to identify alternations to neurobehavior. At the end of this study, I will have developed a standard operating procedure for the CET assay that will be used to evaluate the developmental toxicity of air pollution samples from Vieques, PR.

1.06.T-04 Mechanisms Underlying Differential Species Sensitivity to Polycyclic Aromatic Hydrocarbons in Birds

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Polycyclic aromatic hydrocarbons (PAHs) are chemical stressors that are ubiquitous in the environment. Although birds are receptors of concern for PAHs, the mechanisms underlying differential species sensitivity to PAH exposure are poorly understood. Previous results from our group showed a 30-fold difference in sensitivity (LD50) between model (Japanese quail; *Coturnix japonica*) and free-ranging/non-model (double-crested cormorant; *Nannopterum auritum*) bird species following exposure to benzo(a)pyrene (BaP) via egg injection. This contrasts with their expected sensitivity based on their shared Aryl Hydrocarbon Receptor 1 (AHR1) genotype and their similar responses in an *in vitro* transactivation assay that assessed the binding efficiencies of various PAHs. Here, we investigated the roles that toxicodynamics and toxicokinetics play in influencing the differences in avian species sensitivity to BaP by pairing embryonic air cell injections with transcriptional endpoints in Japanese quail and double-crested cormorant. We used Seq2Fun (seq2fun.ca), an RNA sequencing pipeline for species without available reference genomes, to analyze whole transcriptome data for these two species at mid-incubation following early-life stage air cell injections with BaP. The rate of metabolism of BaP in developing embryos was addressed for both species by a time-course assay that monitored phase I biotransformation gene (CYP1A4/5) expression patterns using quantitative polymerase chain reaction (qPCR) and the depletion of the injected parent chemical using Gas-Chromatography/Mass Spectrometry (GC-MS). Our results show discordant transcriptional profiles at mid-incubation between both species, and comparatively rapid metabolic responses initiated early in embryonic development. Collectively, our results show promise in the application of molecular tools and new approach methodologies for understanding toxicity mechanisms in non-model species like double-crested cormorant. The findings also highlight the need for additional toxicity data for specific PAH congeners in birds, along with a more coherent understanding of species' life history traits' impact in predicting differential species sensitivity to contaminants.

1.06.T-05 Cross-Species Molecular Docking Method to Support Predictions of Species Susceptibility to Chemical Effects

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The Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool was recently updated to incorporate protein structural prediction capabilities. The new features generate

species-specific structures from protein sequences. This update enables SeqAPASS to generate an additional line of evidence for predicting species susceptibility to chemical effects based on protein structural similarities. Through the novel integration of various open-source computational tools, we developed a method that makes further use of these predicted structures from SeqAPASS through application of molecular docking simulations to enhance the structure-based predictions of species susceptibility. This method was demonstrated using the androgen receptor, a pertinent modulator of endocrine function, and its interaction with two different chemicals that are known modulators. The approach was used to screen 268 species. Overall, this computational method offers a means of generating additional lines of evidence to support species-specific susceptibility predictions using multiple metrics for understanding protein structural similarity across species and holds promise for improved ecosystem-specific hazard evaluations. By leveraging available protein structural data through SeqAPASS and molecular docking, these methods contextualize species susceptibility within a functional framework and helps to integrate molecular docking into the repertoire of New Approach Methodologies (NAMs) that support the Next-Generation Risk Assessment paradigm. Moreover, the chemical-protein models generated through this docking approach can be further scrutinized via molecular dynamics simulations, which considers the underlying physics of each atom of the molecular system that could reveal species-specific differences in the chemical's molecular initiating event. *The views expressed in this work are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency*

1.06.T-06 Bio-QSARs Unlock a New Level of Predictive Power for Machine Learning-Based Ecotoxicity Predictions by Exploiting Chemical and Biological Information

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Practical, legal, and ethical considerations drive the imperative need to develop alternative methods to replace animal experiments, leading to the emergence of new approach methodologies (NAMs). Among these, machine learning (ML)-based quantitative structure-activity relationship (QSAR) models have been widely recognized as a crucial component. Recently, we introduced the Bio-QSAR concept for multispecies aquatic toxicity regression tasks, seeking to overcome limitations of traditional ML QSAR approaches in toxicity prediction. In this study, the Bio-QSAR models were significantly enhanced through a series of key advancements. Firstly, the training datasets were expanded by approximately 20-fold, encompassing the entire chemical landscape and a broader spectrum of aquatic species, while also accommodating flexible test durations. Notably, the utilization of Gaussian Process Boosting, an ML algorithm capable of handling fixed and random effects, contributed to the improved data foundation of the Bio-QSARs. Furthermore, the incorporation of additional biological information, such as taxonomic distances, genus-specific invertebrate trait information, and the mode of action of chemicals, was found to enhance the predictive power of the models. This comprehensive approach enabled the development of second-generation Bio-QSARs with unprecedented predictive powers, achieving high test R-squared values of approximately 0.89-0.92, which surpass the capabilities of previous multi-species models for acute aquatic toxicity prediction. Moreover, the improved Bio-QSAR models were made fully explainable by addressing multicollinearity in the datasets, allowing for unbiased estimates of feature importance based on the state-of-the-art SHAP (SHapley Additive exPlanations) values.

Additionally, new methods for constructing applicability domains (ADs) were developed, considering feature importance and testing their effectiveness in restricting predictions to samples suitable for the models. In conclusion, the second-generation Bio-QSAR models not only demonstrate the capability for flexible cross-chemical and cross-species predictions beyond the levels present in the training data but also meet the criteria for consideration in regulatory contexts. This advanced NAM approach holds significant promise for applications in environmental risk assessment, as well as in the research and development of chemicals.

1.07.A.T Cell-Based Approaches for Ecotoxicity Assessments

1.07.A.T-01 An In Vitro Transcriptomic Point of Departure (tPOD) Approach to Characterize 19 Pesticides in Fish and Human Cell Lines

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In vitro transcriptomics is emerging as an efficient strategy to assess chemicals, especially when performed in a high-throughput manner. In addition, studies from human health are beginning to demonstrate that quantitative transcriptomic point of departure (tPOD) values derived from short-term in vitro studies are often lower than apical POD concentrations derived from long term animal bioassays. The objectives of this study were to: a) establish an in vitro workflow in rainbow trout gill cells (RTgill-W1) and human liver (HepG2) and intestinal cells (Caco-2) to derive tPODs; b) apply this workflow to evaluate 19 pesticides in three cell lines; and c) compare the resulting tPOD data to existing data (e.g., in vivo) on these same compounds. We evaluated one fungicide (chlorothalonil), ten herbicides (atrazine, glyphosate, imazethapyr, metolachlor, diquat, s-metolachlor, AMPA, dicamba, dimethenamid-P, metribuzin), and eight insecticides (chlorpyrifos, diazinon, permethrin, carbaryl, clothianidin, imidacloprid, thiamethoxam, chlorantraniliprole). We also modeled the nominal exposure concentrations using In Vitro Mass Balance Model Equilibrium Partitioning (IV-MBM EQP) Version 2.1. Focusing specifically on the RTgill-W1 results, tPOD(mode) values were derived for each pesticide with values ranging from 0.3 to 147.4µM. Even in eight cases where cytotoxicity was not measured, discrete tPOD data were obtained thus highlighting the sensitive nature of this approach. In most cases the tPOD values (based on model-predicted exposure values) were within 10-fold of corresponding in vivo LC50 values from fish acute studies, and the relationship between the two measures was significant (0.92x, r2=0.6, p<0.001). Enrichment analysis of differentially expressed genes was also performed to derive biological pathway benchmark doses for some pesticides (ranged from 0.1 to 55µM), with the most commonly affected pathways being neuroactive ligand-receptor activation, ribosome, and TNF signaling. Similar tPOD data are available for the two human cell lines. Ongoing analysis aims to integrate the three datasets. This study highlights the benefits of in vitro high-throughput tPOD methods to screen chemical libraries, yield quantitative PODs that are comparable to PODs from animal bioassays, provide deeper insights into a pesticide mechanism of action, and achieve all of these in a relatively cost-effective and ethical manner.

1.07.A.T-02 In Vitro Models for Evaluating the Toxicity of 6PPD-Quinone and Other Tire Wear Particles

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Tire wear particles are prominent stormwater pollutants in urbanized areas that can have harmful effects on aquatic life. One such chemical, 6PPD-quinone (6PPDQ), a transformation product of the car tire additive 6PPD, has been identified as the causal toxicant inducing mass pre-spawn mortality events in adult coho salmon in the Pacific Northwest. *In vivo* studies utilizing species sensitive to 6PPDQ (coho salmon, brook trout, white-spotted char) are hindered by logistical constraints associated with species availability, cost, and facility requirements. Thus, *in vitro* cell lines models are an attractive alternative for assessing the potential for cytotoxic effects of 6PPDQ and other tire wear particles. Immortalized cell lines show promise in recapitulating species-specific toxicity of 6PPDQ, including metabolic and cytotoxic effects in cells derived from a coho salmon embryo. Similar effects are not elicited in Chinook or sockeye salmon cell lines derived similarly, matching species sensitivity observed in *in vivo* toxicity studies. These data indicate that the mechanisms underlying 6PPDQ toxicity are conserved in CSE-119 coho salmon cells and can be a valuable resource for studies on the mechanisms of toxicity. Further, whole-transcriptome sequencing during exposure has indicated alterations epithelial and endothelial processes that are consistent with whole-organismal responses observed *in vivo*. Other assays are also currently being developed to examine subcellular effects that may be involved in toxicity. Beyond 6PPDQ, these salmonid cell lines are also being utilized to assess the toxicity of other tire wear particles, including alternative antiozonants being considered as replacements for 6PPD in vehicle tires.

1.07.A.T-03 Metabolic Disruption and Mechanisms of Toxicity Caused by Bisphenol Analogs in Human In Vitro Cell Models

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Bisphenols, extensively employed in production of plastics, coatings, and resins, are under scrutiny for potential endocrine disruption. Despite banning Bisphenol A (BPA), its perceived safer alternatives (bisphenol analogs) may still pose health risks, urging thorough studies on their toxicity mechanisms. This study investigated the cellular toxicity of the top seven most commonly used BPs, bisphenol S (BPS), bisphenol F (BPF), bisphenol AF (BPAF), bisphenol P (BPP), bisphenol AP (BPAP), bisphenol B (BPB), bisphenol E (BPE) in eight different relevant human *in vitro* cell models: liver (HepaRG), intestinal (CaCo-2), breast (T47D), brain (HMC-3), lungs (MRC-5), kidney (HEK293), endothelial (HMEC-1), and skin (HEK-001) cell lines. BPE manifested the highest cytotoxicity in CaCo-2 cells, presenting an EC50 value of roughly 0.2 µM (95% confidence interval). In contrast, HEK293 and HepaRG cells demonstrated significant resilience to BPS (EC50 >1000 µM). BPAF, BPP, and BPAP had consistently low EC_{50} values across cell lines (6-27.9 µM, 0.6-134.7 µM, and 3.6-178.8 µM), indicating elevated toxicity. After 24 hours, all bisphenols adhered to nominal concentrations except BPAF, BPP, and BPS. BPP's concentration notably decreased (30.82 ± 5.53% of nominal value). Results suggest varied effects of bisphenol analogs on different cell types, contributing to evidence of their health impacts. Based on the cytotoxicity study, we are also conducting assays to determine the effects of the most toxic bisphenol analogs on the generation of radical oxygen species (ROS) and

mitochondrial membrane potential changes to elucidate the mechanisms of toxicity further. These additional tests will help clarify the oxidative stress and mitochondrial dysfunction induced by the most cytotoxic bisphenol analogs, providing a more comprehensive understanding of their cellular impacts. Understanding these mechanisms is crucial for assessing the health risks associated with bisphenol exposure.

1.07.A.T-04 Using Macrophage Cells as a Bioindicator to Evaluate Oil Sands Process Water (OSPW) Toxicity: Contribution of Naphthenic Acids to the Toxic Effects

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Mining operations in Alberta's oil sands yield large quantities of oil sands process water (OSPW), which contains a complex mixture of organic and inorganic substances, leading to acute and chronic toxicity in aquatic and terrestrial species. It has been established that naphthenic acids (NAs), a group of organic compounds comprising aliphatic and alicyclic carboxylic acids, are mainly responsible for the toxic properties of OSPW. NAs naturally occur in the oil sands of Alberta and other oil reserves, raising environmental concerns due to their potential harm. In addition, their resistance to natural degradation and surfactant properties make NAs challenging to remove from OSPW. Therefore, recent studies have focused on methods to eliminate NAs, including advanced oxidation processes (AOPs). In this study, we developed immune cell-based bioassays to assess the impact of untreated and treated OSPW sample exposures on mammalian macrophage cells, including mouse macrophages (RAW 264.7) and the human macrophage-like cells (THP-1). Since NAs are a major contributor to OSPW toxicity, we investigated the immunomodulatory properties of commercial NAs and OSPW-extracted NAs on macrophage cells. Cytokine multiplex assays were conducted after a 24-hour exposure to measure inflammatory cytokines. Additionally, synchronous fluorescence spectroscopy (SFS) and ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTof-MS) analyses were performed to measure NAs in OSPW samples before and after treatment. Our results indicate that untreated OSPW samples triggered higher secretion levels of pro-inflammatory cytokines compared to treated OSPW samples. The reduced inflammatory response observed in treated OSPW samples corresponded to the targeted removal of NAs from OSPW, suggesting that NAs may possess inflammatory properties that activate macrophage cells. Furthermore, both commercial and OSPW-extracted NAs induced pro-inflammatory cytokine secretion, confirming the ability of macrophages to sense NAs. Interestingly, our research also suggests that toll-like receptor (TLR)-4 may be the primary immunoregulatory receptor for sensing the presence of NAs in OSPW. In conclusion, our study demonstrates that an immune cell-based bioassay can serve as a sensitive bioindicator for evaluating OSPW inflammatory components, such as NAs, and for further assessing ongoing remediation strategies.

1.07.A.T-05 Can Cell Lines be Used to Screen Candidate Chemical Lampricides? Exploring the Use of Gill Cell Lines from Rainbow Trout (RTgill-W1) and Lake Sturgeon (LSTGill3)

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The use of cell lines in ecotoxicology is advancing the field towards the goal of replacing, reducing, and refining animal use in research (3Rs). In general, studies with fish cell lines have explored their ability to rank environmental toxins, assay environmental samples, study interactions between toxicants and environmental conditions, or develop molecular biomarkers. Typically, these studies have been conducted using a single, commercially available cell line from a model fish, such as RTgill-W1 from rainbow trout (*Oncorhynchus mykiss*). In contrast, our work seeks to establish multiple novel fish cell lines to serve as in vitro tools for developing new pesticides to target the invasive sea lamprey (*Petromyzon marinus*). This work is motivated by concerns over the potential development of resistance by the sea lamprey to the selective pesticide (lampricide) 4-nitro-3-(trifluoromethyl)phenol or TFM, which has been used for > 60 years to control sea lamprey populations in the Laurentian Great Lakes, Lake Champlain, and the Finger Lakes of North America. Lampricides must be highly toxic towards the sea lamprey while causing minimal harm to native fish, such as lake sturgeon (*Acipenser fulvescens*) and rainbow trout, which reside in streams where lampricides are applied. Towards this end, our project seeks to develop fish cell lines and cytotoxicity assays to screen the efficacy and specificity of candidate lampricides so that only the most promising are advanced to in vivo testing.

We have established a novel cell line from lake sturgeon gill tissue (LSTGill3). Characterization of LSTGill3 has included assessing senescence-associated β-galactosidase activity, response to cryopreservation/thawing, effects of temperature and media fetal bovine serum percentage on growth, routine mycoplasma testing, and performing species authentication by cytochrome c oxidase subunit 1 (COX1) gene barcoding. Furthermore, we have used LSTGill3 and RTgill-W1 to assess the cytotoxicity of TFM with alamarBlue, CFDA-AM, and neutral red using an approach modified from the Organization for Economic Development and Cooperation (OECD) Test Guideline No. 249 Fish Cell Line Acute Toxicity: The RTgill-W1 cell line assay. Our characterization results and experimentally measured half-maximal effective concentrations (EC_{50}) will be reported and compared to in vivo LC_{50} values to assess the ability of these cell lines and assays to serve as effective lampricide screening tools.

1.07.A.T-06 Screening Pesticides for Estrogenic and (Anti)Androgenic Activity in Support of Endocrine Disruptor Screening Program (EDSP) Revival

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EPA's Endocrine Disruptor Screening Program (EDSP) was established in 1998 to identify pesticides and other chemicals that disrupt human endocrine system signaling. EPA plans to use EDSP's tiered testing framework to support the risk assessment of pesticides under FIFRA, the Federal Insecticide, Fungicide, and Rodenticide Act. Out of 403 pesticides undergoing FIFRA registration review in 2023, 317 lacked adequate reproductive toxicity data, including 30 pesticides with potential estrogenic (human estrogen receptor; ER) and/or anti-androgenic activity (human androgen receptor; AR) identified by ToxCast high-throughput screening (HTS). We propose using sensitive, validated *in vitro* cell-based bioassays to supplement ToxCast data for these 30 pesticides and compare purported AR antagonist activity with concurrent cytotoxicity assessment. To measure ER agonist activity, we used the T47D-kbluc cell line, which endogenously expresses ER-alpha and was stably transfected with an ER-sensitive luciferase reporter gene. Two out of four suspected ER agonists, fenhexamid (*in vitro* EC50: 16.84 µM) and flumetralin (*in vitro* EC50: 2.43 µM), showed significant estrogenic activity in our assay when compared to 17-beta estradiol positive control, while hydramethylnon and pyridaben were ER-inactive and exhibited cytotoxicity at high concentrations. AR antagonist activity was measured using a CV1 cell line virally transduced with an AR gene (chAR) and AR-sensitive luciferase reporter gene. To ensure that any apparent AR antagonist effects were receptor-mediated, rather than cytotoxic in nature, this transcriptional activation assay was run alongside the MTT rapid colorimetric cytotoxicity assay. These Tier 1-type *in vitro* assays enable us to efficiently and accurately screen for pesticides that pose the greatest risk to human and environmental health and support appropriate resource allocation for Tier 2 *in vivo* testing. Furthermore, the *in vitro* screening approach used to assess the ER and anti-AR activity of these pesticides provides a model for validating HTS results and the continued expansion of tiered screening programs like EDSP. *The views expressed in this abstract are those of the author(s) and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

1.07.B.T Cell-Based Approaches for Ecotoxicity Assessments

1.07.B.T-01 Dysregulation of Key Hormones Induced by Individual and Mixed PPCPs in Rat Pituitary Cells

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Emerging contaminants (ECs), including pharmaceuticals, personal care products, and other industrial chemicals, are increasingly detected in the environment. Despite their prevalence, limited studies have explored their endocrine-disrupting effects, particularly at environmentally relevant concentrations. This study investigated the effects of these contaminants on key hormones and hormone signaling molecules in rat pituitary cells. Pituitary cells function as primary regulators of the endocrine system, controlling numerous physiological processes throughout the body. Their central role makes them a prime model for investigating endocrine-disrupting effects of ECs. Our findings reveal intricate, time-dependent impacts of ECs on cell proliferation and cytotoxicity, emphasizing the importance of considering both exposure duration and compound-specific responses. ECs demonstrated variable impacts on key hormone levels within rat pituitary cells, with adrenocorticotropic hormone (ACTH) exhibiting dose- and time-dependent dysregulation. Additionally, significant dysregulation of cyclic adenosine monophosphate (cAMP) levels was observed, particularly in studies involving 17α-Ethynylestradiol and testosterone. These results highlight the complex interactions between ECs and hormonal regulation, emphasizing the need for comprehensive evaluation of individual and combined chemical exposures on endocrine health.

1.07.B.T-02 Sensitivity of Fishes to Polycyclic Aromatic Hydrocarbons

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Polycyclic aromatic hydrocarbons (PAHs) are naturally occurring chemicals that are ubiquitous in the environment. PAHs can be alkylated, and alkyl PAHs are more abundant in certain environments. PAHs can exert toxicity to fishes by activating the aryl hydrocarbon receptor (AhR). Although there is evidence that alkylation could increase potency of PAHs, these effects are not well characterized. Most research has focused on a small number of species which likely do not reflect interspecies variation in sensitivity. However, toxicity testing across numerous species is impractical due to pragmatic limitations. As a step towards characterizing how alkylation affects potency of PAHs, the potency for activation of the zebrafish (*Danio rerio*) AhR by benz[a]anthracene and three alkyl homologues was quantified using a standardized cell-based assay. Alkylation had position-dependent effects on potency. The most potent alkyl PAH, 8-methylbenz[a]anthracene, was 6.9-fold more potent than benz[a]anthracene, 4-methylbenz[a]anthracene was 1.4-fold more potent, and 7,12-dimethylbenz[a]anthracene was 0.8-fold as potent. Next, the potency for early life-stage mortality was quantified by injecting zebrafish embryos with each PAH to determine whether the cell-based assay was representative of *in vivo* exposure. Relative potencies *in vivo* were similar to those observed *in vitro*. To assess interspecies differences in sensitivity, the cell-based assay was performed for additional phylogenetically diverse species. Results showed that alkylation had position-dependent effects on the range of species sensitivity with sensitivity ranging up to 561-fold. To determine whether differences in sensitivity to AhR activation correspond with differences in sensitivity to early life-stage mortality embryos of fathead minnow (*Pimephales promelas*), Japanese medaka (*Oryzias latipes*), and brook trout (*Salvelinus fontinalis*) were exposed to PAHs by microinjection to determine lethality. There is a significant relationship between sensitivity to AhR activation and sensitivity to early life-stage mortality ($p = 0.007$, $R2 = 0.86$). Due to the abundance of PAHs in the environment and differences in sensitivity across species, developing *in vitro* tools to assess these toxicities more efficiently could be essential for more objective ecological risk assessment of these chemicals.

1.07.B.T-03 Invasive and Native Fish Species Gill Cell Line Authentication

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The development of new pesticides has historically required testing on numerous animals. Recently however, *in vitro* cytotoxicity tests that can reduce the number of test animals and support high throughput chemical screening are becoming more common. An increasing number of studies are utilizing fish cell lines instead of whole organisms to assess the toxicity of chemicals from agricultural, industrial, pharmaceutical, and personal care products. While a few commercially available cell lines can be purchased, the development of novel fish cell lines is a rapidly growing field of research. Fish cell line characterization is essential to identify the domain of applicability and the uncertainty of their use during screening assays and *in vivo* toxicity extrapolation. Therefore, the present study will describe the characterization conducted for new cell lines developed for screening candidate pesticides for invasive fish removals. Freshwater gill osmoregulation gene expression levels from freshly dissected fish tissue and established fish gill cell lines will be compared for each target species (e.g., Grass Carp, Silver Carp) and non-target species (e.g., Lake Sturgeon, Bluegill, Rainbow Trout) of interest. Gene expression results will be used to verify the retention of whole organism gill cell osmoregulatory genes throughout each corresponding fish gill cell line after continuous culture. Overall, these results will be used to characterize the physiological similarity and appropriateness of novel fish cell lines to screen pesticide candidates for developing invasive species controls.

1.07.B.T-04 Assessing the Cytotoxic and Proliferative Effects of Indole Compounds Synthesized by Bacteria on Six Human Cell Lines

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In recent years, there has been a notable surge in research interest regarding the intricate interplay among diet, gut microbiota, and their impact on the host organism. Emerging evidence suggests that the microbiota in the gut metabolizes tryptophan, giving rise to a class of compounds known as indoles. While previous studies have predominantly focused on the actions of indole and indoxyl sulfate, our investigation delves into the toxic effects of five distinct indole derivatives: indole-3-carboxylic acid (I3CA), indole-3-aldehyde (I3A), indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), and 3-methylindole (also referred to as skatole or 3-MI). We conducted our study across six human cell lines, each representing different tissues: adipose-derived mesenchymal stem cells (MSC), lung fibroblasts (MRC-5), hepatic progenitors (HepaRG), hepatic carcinoma (HepG2), colorectal carcinoma (Caco-2), and breast cancer (T47D). MRC-5 was sensitive to all indole derivatives (EC_{50} 0.5-50 µM) while all derivatives except IAA were cytotoxic to MSC (EC_{50} 0.3-2 µM). Similarly, all derivatives except IPA were cytotoxic to HepaRG (EC_{50} 2-65 µM). IAA showed cytotoxic properties in Caco-2 and T47D (EC_{50} 0.52 ± 0.12, 1.68 ± 0.46 µM, respectively). None of the other derivatives indicated cytotoxicity in Caco-2 nor T47D. Furthermore, HepG2 was not sensitive to any of the indole derivatives. All EC_{50} for indole exposure were greater than 100 µM. Activation of the aryl hydrocarbon receptor (AhR) pathway was significantly induced by I3A, IPA, and I3CA (EC_{50} = 0.04 (0.03-0.05)), 0.11 (0.10-0.13), 0.88 (0.80-0.95) µM respectively) using the gene reporter cells HepG2-AhR-Lucia cells. Proliferation and migration assessments revealed that I3CA elicited a significant reduction in proliferation in colorectal carcinoma (Caco-2) cells, while paradoxically promoting proliferation in breast cancer (T47D) cells. Our study underscores the potential cytotoxic impact of indole derivatives on specific cell types, suggesting a nuanced interplay influenced by both the specific indole derivative and the cellular context. These findings offer valuable insights into the intricate mechanisms underlying the biological effects of indoles, shedding light on their diverse roles in cellular physiology and pathophysiology.

1.07.B.T-05 Inclusion of Marine Fishes in a Cross-Species Comparison of Relative Sensitivities to Dibenzo-p-dioxins, Dibenzofurans, Polychlorinated Biphenyls and Polycyclic Aromatic Hydrocarbons In Vitro

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Evaluations of the sensitivity of fishes when exposed to ubiquitous environmental contaminants such as dioxin-like compounds (DLCs) and polycyclic aromatic hydrocarbons (PAHs) have historically utilized freshwater species. However, previous studies have shown that differences in sensitivity among freshwater species can range by over 400-fold, highlighting the significant variability within this taxon. Thus, the objective of the present study was to expand on previous research by evaluating the sensitivity to a suite of DLCs and PAHs for five marine laboratory model species, namely the gulf killifish (*Fundulus grandis*), Atlantic killifish (*Fundulus heteroclitus*), inland silverside (*Menidia beryllina*), Atlantic silverside (*Menidia menidia*), and sheepshead minnow (*Cyprinodon variegatus*) relative to the freshwater laboratory model, zebrafish (*Danio rerio*). The sensitivity of each species was evaluated using a standardized in-vitro aryl hydrocarbon receptor (AHR) transactivation assay of COS-7 cells transfected with an AHR2 cloned from the species of interest. The sensitivity of the AHR2 from each species was determined for 4 DLCs, namely a dibenzo-*p*-dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), a dibenzofuran (2,3,7,8-tetrachlorodibenzofuran), and a non-ortho and a mono-ortho polychlorinated biphenyl (PCB; PCB 77 and PCB118, respectively). The sensitivity of each species to a 5 ring (Benzo[a]pyrene), 4 ring (Benzo[a]anthracene), 3 ring (anthracene) and alkylated (8-Methylbenzo[a]anthracene) PAH were also determined. From this assay, the 50% effective concentrations (EC50) and effective concentration thresholds (ECThreshold) were determined. These values were then compared to the corresponding EC50s and ECThresholds for zebrafish AHR2. Results indicate that the sensitivity to the 4 PAHs was similar among zebrafish and the 5 marine species. However, there was a notable difference between zebrafish and all 5 marine species for DLCs, with some EC50 values differing by more than 50-fold. This illuminates that assessing the impact of DLCs on marine species using data from freshwater species could underestimate risks, meanwhile freshwater species appear to be suitable surrogates for estimating risks to PAHs. Results of this study improve our understanding of differences in relative species sensitivity to DLCs and PAHs between freshwater and marine laboratory model species.

1.07.P-Tu Cell-Based Approaches for Ecotoxicity Assessments

1.07.P-Tu-024 Assessing New-Age Pesticide Chemistries with the RTgill-W1 Cell Line: Correlation to In Vivo Fish Toxicity

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Reduction of animal use as part of the Replacement, Reduction and Refinement paradigm is a key global goal for research sustainability. This goal often intersects with New Approach Methodologies (NAMs), particularly when considering novel *in vitro* assays. One such assay, the RTgill-W1 cell line assay (OECD Test Guideline No. 249), can be actively leveraged as pre-screening tool to rapidly evaluate chemicals in a ranking method for predicting fish acute toxicity, or possibly a range-finding tool to aid in dose-setting decisions prior to conducting more in-depth assays such as a Fish Short Term Reproduction Assessment or Amphibian Metamorphosis Assay. Importantly, because of the more-high throughput nature of the RTgill-W1 assay compared to an *in vivo* assay, this screen can rapidly generate a wealth of toxicity information that can be leveraged by models associated with Quantitative Structure Activity/Toxicity Relationships (QSA/TR) and other Integrated Testing Strategies. The ability to accurately correlate *in vitro* toxicity to *in vivo* toxicity is an important aspect of this assay but has demonstrated to be difficult to do with compounds common to the crop protection chemical industry, often due to the physical properties associated with pesticides. Here, we investigate the cell line's ability to perform when exposed to a variety of herbicides, fungicides, and insecticides from our discovery pipeline that span physiochemical properties and modes of action and compare the cytotoxicity IC_{50} values to *in vivo* LC_{50} values derived from a standard OECD Test No. 203: Fish Acute Toxicity Assay. This work will help to demonstrate the ability of the RTgill-

W1 cell line to be leveraged using new-age crop protection chemicals, ultimately contributing to evidence in support of protocols that allow for rapid data gathering using animal alternatives for toxicology studies.

1.07.P-Tu-025 Comparative Analysis of Molecular Impacts of Short-Chain PFAS Exposure on ABCG2/BCRP Transporter Expression and Localization in Various Human Cell Models

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Both short- and long-chain PFAS are widespread in the environment, persistently accumulating due to their resistance to degradation. With ongoing accumulation and new data on alternative PFAS, human exposure is increasingly significant. Numerous health effects and cellular changes are linked to PFAS exposure in humans and animals, yet the detailed mechanistic pathways and specific protein-level changes remain largely unexplored. ATP Binding Cassette Protein G2 (ABCG2), a transport protein crucial for its protective mechanisms and transport functions for endogenous chemicals and pharmaceuticals throughout the body is just one of many critical transport proteins within the ABC superfamily. While previous work has demonstrated the ability of PFAS exposure to alter ABCG2 expression in a human model, the impacts on its localization in cells or the mechanism of this regulation are unknown. Developing a model to quantify ABC protein expression and localization in a relevant human context is essential to addressing this knowledge gap. Differentiated hepatocytes (HepaRG) and kidney cells (HEK293) were exposed to various short-chain PFAS. Imaging and quantification showed increased ABCG2 protein expression in response to PFAS exposure, along with variations in cellular protein localization and differences in protein expression between cell types. These results align with previous data demonstrating elevated RNA expression of ABCG2 after treatment with perfluorohexanoic acid (PFHxA), perfluorohexanesulphonic acid (PFHxS), and 6:2 fluorotelomer alcohol (6:2 FTOH) at concentrations of 1µM and 1nM. Additionally, ABCG2 was absent in HEK293 cells, while in differentiated HepaRG cells, the protein was specific to hepatocytes and not present in biliary cells. These findings provide proof of concept for this model, reveal cell line-specific ABCG2 expression, and guide future research into the regulatory mechanisms behind these protein changes.

1.07.P-Tu-027 Using Cell Painting Methodology to Assess Acute Fish Toxicity in a Fish Gill Cell Line, RTgill-W1

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Regulatory agencies seek alternatives to the use of animals in toxicity testing to demonstrate environmental safety, particularly for products meant for agricultural use with inherent environmental exposures. To assess safety to aquatic vertebrates like fish, current test guidelines necessitate the use of live animals, which requires significant time and resources, and which raises ethical concerns. Thus, alternative *in vitro* assays are sought to screen the thousands of new compounds created to discover a new and safe agricultural product. Cell painting is a novel imaging-based approach that uses organelle-specific fluorescent stains to determine how cell morphology is affected by chemical exposure. Here, we apply cell painting to the rainbow trout (*Oncorhynchus mykiss*) gill cell line, RTgill-W1, to show how this approach can be leveraged to

estimate acute fish toxicity. Cells from gill tissue are advantageous for this purpose because this organ is naturally in contact with the water column, so *in vitro* assays represent realistic exposure scenarios. We show that the cell painting methodology produces reliable and reproducible estimates of acute fish toxicity for chemicals across a suite of modes of action.

1.07.P-Tu-028 Exploring the Applicability of the OECD TG 249 Fish Cell Line Acute Toxicity Assay in Environmental Hazard and Risk Assessment of Cosmetic and Personal Care Product Ingredients

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Globally, significant efforts are being made to advance the adoption of animal free safety assessments. However, acute fish toxicity data are currently a component of environmental risk assessments and often a requirement in global chemical regulations. Thus, to meet the goal of minimizing animal use while ensuring the environmental safety of chemicals and products, it is necessary to consider alternative methods for assessing acute fish toxicity that will provide for robust and regulatory compliant approaches. One such alternative that has shown utility for assessing acute fish toxicity is the OECD TG 249, a rainbow trout gill cytotoxicity assay. Although this assay has shown good correlation with historical standard acute fish toxicity (e.g., OECD TG 203) test data, there have been calls for further data generation with TG 249 to better define chemical applicability domain and identify potential assay limitations before full implementation within regulatory schemes. To further explore the suitability of this assay and identify potential chemical-specific limitations, a project is underway by the not-for-profit science organization ICCS (International Collaboration on Cosmetics Safety) to evaluate the OECD TG 249 against a diverse set of cosmetic and personal care product ingredients. This presentation will serve as an overview of the planned multi-phase approach for the project, an update on the current status, and an opportunity for stakeholders to provide feedback or share relevant findings in either testing or conducting environmental safety assessments using OECD TG 249 data. Ideally, this will ensure efficient, relevant, and strategic use of resources in building a high-quality dataset to support best use of this assay in NAM (new approach methodology) frameworks for environmental risk assessment and will help expedite broader regulatory acceptance of approaches leveraging this assay.

1.07.P-Tu-030 Alternative Approaches to Animal Testing for Ecotoxicity Assessments: Validating the OECD319 regulations

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Fish bioconcentration studies are carried out to determine whether substances have the potential to bioaccumulate and have potential to bio-magnify throughout different trophic levels. As the requirements to conduct these studies on a broad variety of industrial chemicals, pharmaceuticals and crop protection products increase across the globe, so does the numbers of fish needed to fulfil these investigations. Prior to the revision to the OECD Test Guideline (OECD 305: 2012), the preferred method of testing requires three doses of chemical (including a control) with up to 100 fish per dose. Latterly, there is now the option to test on one concentration and the control only, providing there is scientific justification. This has the potential to decrease the numbers of fish used in these tests by one third (100 fish per study). There are opportunities to reduce animals further by following new in vitro alternatives such as the OECD 319 study (Determination of in vitro intrinsic clearance using cryopreserved rainbow trout S9/hepatocytes). Determination of the liver intrinsic clearance in vitro provides a direct input to physiologically based toxicokinetic (PBTK) models for fish bioaccumulation. Alternatively, this value may be extrapolated to a whole-body (in vivo) biotransformation rate constant using an appropriate in vitro to in vivo extrapolation (IVIVE) model. The in vivo biotransformation rate can be included into in silico models for prediction of bioconcentration factors (BCF). Although novel validated methods exist to conduct in vitro fish bioaccumulation studies, regulators are not yet confident to accept these studies in isolation without supporting in vivo data. This is because they have not seen enough studies of this type submitted and do not have confidence in the direct in vitro/in vivo extrapolation. Without an accepted in vitro approach, businesses will continue to go directly to the in vivo studies to save time and money yet increasing the need for more and more fish. Therefore, without the direct comparison of data generated both in vivo and in vitro on the same chemicals, we will never gain regulatory acceptance. Here we present our approach to determining the intrinsic clearance in vitro and results we have obtained in our laboratories comparing predicted BCF values obtained in vitro to those conducted in vivo.

1.07.P-Tu-031 Evaluating Practicability and Accuracy of OECD 249 Fish Gill Cell Assay by Comparison with In Vivo Acute Fish Toxicity Data for Difficult-to-Test Industrial Chemicals

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Alternative methods are critically needed to reduce reliance on live vertebrate animal testing for estimating chemical toxicity. We evaluated an *in vitro* OECD 249-aligned rainbow trout gill cell assay for concordance with traditional acute fish toxicity results and its usefulness as an alternative for industrial chemical testing. Seven industrial chemicals with varying physicochemical properties were tested, including anionic surfactants and chemicals with very low water solubility. Poor water solubility posed an initial challenge under the *in vitro* testing conditions and was accounted for in determinations of optimal concentration ranges. Cytotoxicity estimates from pretesting showed good correlation with expected toxicity values, with EC/LC50 values closely matching available *in vivo* acute fish lethality data. Further definitive testing across a full concentration-response curve will evaluate reproducibility of the pretesting results. Overall, under standardized and optimized conditions, the gill cell assay shows strong potential for accurate toxicity screening of industrial chemicals, especially for water soluble chemicals. Ongoing work expands the chemical test set to include further difficult-to-test substances like UVCBs, metal-containing substances, ionizable chemicals and polymers to continue characterizing assay performance across diverse chemical classes and solubility profiles. This will better define the applicability domain, limitations, and opportunities for optimization of the method. With adequate optimization, this assay could provide a practicable animal alternative for rapid, inexpensive environmental toxicity estimation of a wide range of industrial chemicals and reduce reliance on fish acute toxicity testing.

1.07.P-Tu-033 Exposure to "Alternative" Flame Retardants Alters Rat Aortic Smooth Muscle Cell Function In Vitro and Decreases Heart Rate In Ovo

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Flame retardants are routinely incorporated into common household items, including furniture, car seats, and crib mattresses, to enhance fire safety. In 2004, concerns regarding the toxicity of traditional flame retardants, such as polybrominated diphenyl ethers (PBDEs), led to their replacement with 'alternative' flame retardants. However, the potential toxicity of these alternatives remains largely unknown. A particular concern is the risk of maternal transfer to the developing embryo, as these flame retardants have been detected in breast milk and placental fluid of pregnant women. In utero exposure to alternative flame retardants may lead to congenital defects, especially if exposure occurs during early embryonic development, which involves sensitive processes. This study focused on the potential effects of two alternative flame retardants, Triphenyl phosphate (TPP) and Tris 2-chloroethyl phosphate (TCEP), on arterial wall development. We employed in vitro tests, including wound scrape migration assays and microscopy, to assess cell migration and proliferation. To better understand adverse outcomes on cardiac function, we conducted in ovo experiments with chicken embryos to examine the effects of TPP and TCEP on embryonic heart rate and organ mass. Our findings suggest that TPP and TCEP may inhibit aortic smooth muscle cell function, which could potentially impact proper heart development and function. These results underscore the importance of further investigation into the safety of alternative flame retardants in household products.

1.07.P-Tu-034 Concentration-Response Modeling to Predict Assimilative Capacity of Wastewater Treatment Plant Effluent Estrogenicity

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While wastewater treatment plants (WWTPs) have dramatically reduced pollution and improved water quality globally, wastewater effluents continue to represent significant point sources of contaminant loading to aquatic ecosystems. The main goal of this study was to demonstrate a bioassay-based technique for estimating the concentration and downstream extent of wastewater effluent that may lead to adverse effects on the aquatic environment. Effluent samples were collected from four municipal WWTPs in Massachusetts, along with upstream and downstream surface water samples along reaches of the Taunton and Assabet rivers. Effluent was diluted using deionized water (DIW) or upstream surface water to generate 7 sample dilutions per series (100, 85, 70, 55, 30, 14, and 0% WWTP effluent). All dilutions were concentrated to 1000X in DMSO using a solid phase extraction method and subsequently used to determine *in vitro* estrogenic activity using a human estrogen receptor (ER) assay. Additionally, 24 h *in vivo* high-throughput assays were conducted to evaluate global gene expression in larval fathead minnows

(*P. promelas*) and effects on algal (*R. subcapitata*) growth and photosynthesis with exposure to the effluent extract dilutions. Results from the *in vitro* ER assays indicated estrogenic activity ranging from non-detectable to approximately 14 ng/L 17β-estradiol equivalents (E2-EQs) across the four effluents. Similar responses were observed for dilutions using either DIW or upstream water, indicating little contribution of estrogenicity from the upstream sites. Concentration-response modeling of estrogenic activity as a function of percent effluent was employed to estimate the percentage of effluent that would exceed screening level bioactivity benchmarks. Modeling results suggest that effluent percentages that would be required to exceed 2 ng/L E2-EQ were 11, 17, 196, and 685%, depending on the WWTP. Neither the effluent nor surface water samples significantly impacted *R. subcapitata* growth or photosynthesis-related endpoints. When paired with hydrologic modeling, including wastewater discharge capacities, this type of concentration-response modeling can help identify both specific river reaches where exceedance of assimilative capacity may be expected, as well as the periods of year when impacts are most likely. *The contents of this presentation neither constitute, nor necessarily reflect, U.S. Environmental Protection Agency policy.*

1.07.P-Tu-035 Effects of Charge, Concentration, Exposure Duration, and Size of Polymethyl Methacrylate Micro/Nanoplastics on Human Liver Cells

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Micro/nanoplastics (MNP) are ubiquitous environmental contaminants that are detected in urban and remote airs, waters, soils, and seas. Human exposure to MNP has been demonstrated by the presence of MNP in blood, feces, milk, urine, and placenta amongst other tissues. Polymethyl methacrylate (PMMA) MNP have frequently been isolated from biota, food, environmental matrices, and humans, possibly due to large quantities of historical production and broad arrays of use. However, information about the cellular effects of PMMA MNP is scant. To this end, the effects of size, surface function (aminated, carboxyl or non-functionalized), concentration, and exposure duration of PMMA particles on HepG2 human liver cells were studied. Aminated and carboxylated PMMA MNP ranging from 50 - 5000 nm were not toxic at concentrations of 0.1 - 100 µg/mL for up to 24 h. The majority of non-functionalized PMMA MNP size-concentration-duration combinations were not cytotoxic, with the exception of the following: 50 nm, 10 µg/mL, at 1hr duration; 1000 nm, 10 µg/mL, at 6 h duration; and 1000 nm, 0.1 µg/mL, at 24 h duration. Production of the pro-inflammatory interleukin-8 (IL-8) increased > 2x with an increase in exposure from 4 to 24 h regardless of particle size (50 - 5000 nm) or surface function studied. Confocal microscopy images revealed that PMMA MNP 50, 100, and 1000 nm particles were taken up by HepG2 cells irrespective of surface function. Less than 10% of cells underwent apoptosis at 24 and 48 h for 50 nm and 1000 nm beads at 100 µg/mL. From 72 - 120 h, apoptosis increased in a time dependent manner for cells treated 50 nm beads of all surface functions, with amine beads causing highest apoptosis at 120 h (36 %). While the percentage apoptosis was similar between 50 and 1000 nm for amine beads, the percentage apoptosis increase was less uniform for cells treated with 1000 nm carboxyl or 1000 nm non-functionalized beads. Collectively, PMMA MNP were able to translocate into HepG2 cells, elicit an IL-8 response, and induce apoptosis, but were not cytotoxic at the concentrations tested.

1.08.P-Tu Comprehensive Exploration of Immunotoxicity, Disease Susceptibility, and Immunology Across Organisms

1.08.P-Tu-035 Development of a High-Throughput Phagocytosis Assay for Testing Immunotoxicity of Environmental Chemicals

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Phagocytosis is essential for an organism's innate immune defense. When exposed to immunosuppressive chemicals, phagocytosis may be inhibited, potentially increasing susceptibility to pathogens. To assess the immunosuppressive potential of a chemical library, a high-throughput assay that can measure and reliably quantify phagocytic function is necessary. Hence, we hypothesized that, through high-content imaging of a neutrophil-like cell line, we would be able to quantify phagocytosis after exposure to xenobiotics in a high-throughput manner. To achieve this, we utilized neutrophil-like HL-60 (dHL-60) cells that were challenged with fluorescent pH-sensitive *E. coli*-derived particles (pHrodo™ BioParticles™; Invitrogen) and imaged on the Opera Phenix Plus high-content imaging system (PerkinElmer). Five chemicals suspected of phagocytic inhibition were selected for testing: wortmannin (0.01 μM), SB 203580 (10 μM), bisindolylmaleimide I (10 μM), perfluorohexanoic acid (PFHxA; 100 μM), and phenanthrene (150 μM). Cytochalasin D (10 μM) was used as a known positive control for phagocytosis inhibition, and D-mannitol (10 μM) was selected as an expected negative control. After thirty minutes of exposure to individual test chemicals, opsonized pHrodo BioParticles were incubated with dHL-60 cells for three hours. Intracellular fluorescence of phagocytosed pHrodo BioParticles was then quantified on the Opera Phenix Plus. A one-way analysis of variance test was performed, followed by a Dunnett's *post-hoc* to test for pairwise comparisons between each chemical treatment and the untreated control cells. As expected, cytochalasin D inhibited phagocytosis, and D-mannitol did not inhibit phagocytosis. When compared to untreated control cells, phagocytosis was significantly decreased following exposure to SB 203580 (92.4%), phenanthrene (83.9%), and PFHxA (66.0%). Bisindolylmaleimide I and wortmannin did not significantly alter phagocytosis. These results show that high-content imaging of neutrophil-like cells can quantify phagocytosis in a high-throughput manner. Decreases in phagocytic function may weaken an organism's overall immune response. Therefore, it is important to identify chemicals that can inhibit phagocytosis. The development of this *in vitro*, high-throughput assay will facilitate the screening of many environmental chemicals, including PFAS, for their immunosuppressive potential via neutrophil phagocytosis inhibition. This abstract does not reflect U.S. Environmental Protection Agency policy.

1.08.P-Tu-036 Per- and Polyfluoroalkyl Substance Impact on Smallmouth Bass Kidney Leukocytes: Immune Function & Transcriptomics

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Per- and polyfluoroalkyl substances (PFAS) are ubiquitous environmental contaminants known for their persistence and potential adverse effects on wildlife and human health. These substances have been detected in various tissues of adult wild smallmouth bass (*Micropterus dolomieu*),

including blood plasma, whole blood, liver, gonad, and muscle, across different sampling sites. Previous work has shown that PFAS exposure is linked to altered immune responses, possibly affecting the ability of organisms to fight off pathogens. For instance, research on wild smallmouth bass revealed that higher levels of total PFAS in blood plasma were associated with decreased immune responses, particularly in background lymphocyte mitogenesis. This study investigates the impact of PFAS at environmentally relevant concentrations on the immune function of anterior kidney leukocytes isolated from adult laboratory-reared smallmouth bass (over one year old). The leukocytes were exposed to two commonly found PFAS in wild fish blood plasma, perfluorodecanoic acid (PFDA) and perfluorooctanesulfonic acid (PFOS). Immune responses were assessed through tests measuring respiratory burst activity and lymphocyte mitogenesis. Additionally, transcriptomic analysis was performed to evaluate changes in gene expression related to immune function and to help suggest potential mechanisms of PFAS-induced immunomodulation. In this talk, we will present the study design and preliminary results from our PFAS exposure experiments. Our goal is to improve understanding of how PFAS affects the immune systems of smallmouth bass raised in a controlled environment, allowing for comparison with our studies on wild fish health. The study also highlights the importance of examining both immune responses and genetic changes to more fully understand the immunomodulatory effects of PFAS on fish health.

1.08.P-Tu-037 Susceptibility of PFAS-Exposure During Critical Windows for RAG+ T-Lymphocyte Maturation

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T-lymphocyte maturation is a critical developmental phase for an intact immune response and the generation of immune competence in vertebrates. The *recombination activating gene 1* (*RAG1*) is a crucial lymphocyte marker responsible for T-cell receptor and immunoglobulin diversity, which are essential for an adaptive immune response. Dysfunction of *RAG1* has been associated with increased oxidative stress, defect lymphocyte maturation and immune disorders in vertebrates. Notwithstanding in vivo assessment of T-lymphocyte maturation during the embryonic stage are scarce and difficult to obtain in mammals. Observation of the *RAG1:gfp* transgenic medaka (*Oryzias latipes*) line via fluorescence microscopy revealed critical windows of T-lymphocyte maturation during progenitor migration and thymus colonization between 2 to 5 days post fertilization. As previously reported, anterior to thymus and posterior to thymus *RAG1*-positive progenitor cell migration was observed. Impairment of this critical window through endocrine disrupting chemicals, like flame retardants (PFAS), demonstrated a modified progenitor migration and delayed onset of *RAG1* expression. Differences in the response are reported between legacy and replacement PFAS, indicating that PFAS exposure during development may severely affect the T-lymphocyte maturation and functionality of the immune system.

1.08.P-Tu-038 Investigating the Immunotoxicity of Perfluoro-3,6,-Dioxa-4-Methyl-7-Octensulfonic Acid (Nafion Byproduct 1)

***Macon Carroll**[1]*, *Tracey Woodlief[2] and Jamie DeWitt[2], (1)East Carolina University, Greenville, United States, (2)East Carolina University*

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic compounds with a wide range of applications, spanning consumer goods and industrial products. Two members of this class, perfluorooctanoic acid and perfluorooctane sulfonic acid, have been well-studied and are known to induce immunotoxicity in experimental models and humans, which raises questions of the ability of other PFAS to induce immunotoxicity, especially those that are toxicologically data poor. Many of these understudied compounds can be found in surface water and drinking water across the country. One of these understudied compounds, found in North Carolina surface water, is perfluoro-3,6,-dioxa-4-methyl-7-octensulfonic acid (nafion byproduct 1 or NBP1; CAS 29311-67-9). Despite this compound being found in North Carolina surface waters, its toxicity in living organisms has only been studied minimally. Considering previous studies from our laboratory that indicate that exposure to individual PFAS can result in immune system alterations, this study aimed to investigate potential immunotoxicity of NBP1 in an experimental mouse model. Adult male and female C57BL/6 mice (6-8 weeks old) were exposed by gavage once/day for 30 days with NBP1 at 0, 0.05, 0.5, or 5 mg/kg. Endpoints collected include organ weights, spleen and thymus cellularity, and basic immunophenotype of lymphoid organs. Analysis demonstrated increased relative weight of spleens and thymuses in females, compared to males, across all dose groups. There appeared to be no sex-related differences for cellularity or immunophenotype. Of concern, NBP1 is only one of several understudied PFAS found in surface waters in North Carolina and the additive effect of PFAS mixtures is possible. Further investigation is necessary to confirm these findings, investigate additional immunotoxicity markers, and evaluate the potential dose-additive effects of PFAS mixtures on such markers.

1.08.P-Tu-039 Validation of a Small Fish Model for Immunotoxicity Assessments: Bridging the Gap Between Transcriptional Responses and Organismal Health

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The presence of contaminants that adversely impact the health of fish poses a threat to aquatic ecosystems. The impacts of such contaminants on reproductive and developmental processes have been well studied; however, their immunotoxic potential has received less attention. Thus, there is a need to develop approaches for screening immunotoxic compounds. The goal of this study was to validate the fathead minnow (FHM) as a model for immunotoxicity testing. The specific aims of this work were to evaluate the impacts of dexamethasone (DEX) exposure on: 1) pathogen resistance and 2) pathogen-stimulated molecular immune responses. To accomplish this, FHMs were subjected to 28-d exposures to DEX at concentrations of 150 or 600 mg/L, alongside of a corresponding solvent control (SC) group. At 14-d, FHMs from each group were infected with *Yersinia ruckeri*. Of these fish, kidneys were collected from a subset of fish for transcriptomic analysis to identify pathogen-stimulated molecular immune responses; whereas, the remaining fish were monitored for survival to evaluate pathogen resistance. Significant reductions in pathogen resistance were noted in males exposed to 150 and 600 mg/L relative to the SC, and in females exposed to 600 mg/L. Transcriptomic analysis revealed between 95 and 621 differentially-expressed genes (DEGs), and the number of DEGs was dependent upon DEX concentration and sex. These findings validate the FHM as an immunotoxicity model by confirming the impacts of a known immunosuppressant and identify potential biomarkers for immunotoxicity assessments.

1.08.T Comprehensive Exploration of Immunotoxicity, Disease Susceptibility, and Immunology Across Organisms

1.08.T-01 Altered Immune Function and Apical Endpoints in Colonial Waterbirds Exposed to Persistent Organic Pollutants

***Keith Grasman**, Great Lakes Ecotoxicology and Risk Assessment Laboratory, Calvin University*

The immunotoxicity of polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins, and dibenzofurans (PCDD/Fs) has been well-studied in biomedical and wildlife toxicology. One of the first studies of PCB-induced immunosuppression and increased susceptibility to viral infection was conducted in mallard ducklings. New data on immunotoxic mechanisms of PCDD/DFs and PCBs continues to emerge after 5 decades of research, with immunomodulatory effects often mediated by the aryl hydrocarbon receptor (AhR), a regulator of gene expression and cell cycling. This presentation will focus on immunological endpoints in colonial waterbirds exposed to PCBs, PCDD/Fs in the Great Lakes and elsewhere, including two populations of whose breeding numbers have declined significantly in areas with high PCBs: >95% decline in herring gulls in the River Raisin Area of Concern (AOC) and 42% decline in Caspian terns, a state-threatened species, in the Saginaw Bay and River AOC. These two populations frequently show poor chick productivity, an apical measure of egg and chick survival. T cell-mediated immunity was assessed using the phytohemagglutinin (PHA) skin response. The initial wing web thickness of 3 week gulls and terns and 2 week herons was measured with pressure-sensitive calipers. PHA, a T cell mitogen, in phosphate buffered saline (PBS) was injected into one wing web and a control injection of PBS into the other. The PHA stimulation index was calculated as the difference between the change in the PHA wing web and the change in the PBS wing web when re-measured 24h later. The PHA skin response was suppressed >50% in River Raisin gull and Saginaw Bay tern chicks, and in other populations with significant exposure to PCBs and PCDD/Fs. Antibody-mediated immunity was assessed using the anti-sheep red blood cell (SRBC) immunization test. SRBCs were injected into 3 week chicks to stimulate antibody production. Antibody titers were measured in plasma of 3 week old chicks by serial dilution of plasma and incubation with SRBCs. IgG (or avian IgY) antibody response was assessed by adding 2-mercaptoethanol to denature IgM antibodies prior to addition of SRBCs. Antibody titers in gull chicks in the River Raisin AOC were 2X lower than reference for total antibodies and 2.5X lower for IgG. Consistent with population declines observed in this study, other studies in avian immunoecology have shown that birds with lower immune responses using these types of assays have lower survival rates.

1.08.T-02 Assessing the Effects of Environmental Stressors on Orca Health - An In Vitro Approach

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Puget Sound contains a rapidly growing human population where contaminants have and will continue to uniquely impact aquatic animal health relative to other regions in the state of Washington. Environmental stressors can be lethal to aquatic species as well as contribute to sublethal effects including immunomodulation. Notably, the Southern Resident killer whale population is critically endangered such that recent studies have predicted the potential collapse of orca populations due in part to climate change, viral infection, and chemicals of concern possibly through compromised skin health. The skin is the first line of defense against infection where epithelial cells (epidermis) and dermal fibroblasts work in cooperation with skin microbiota to defend against pathogens and to maintain tissue integrity. Through this joint agency partnership, we developed dermal fibroblast cultures from a Bigg's killer whale skin biopsy to assess cellular responses to environmental stressors that might affect orca health. We first assessed the immune competency of the dermal fibroblasts as a tool for studying orca antiviral responses. Upon stimulation with the viral mimic poly(I:C), orca dermal fibroblasts mount a robust antiviral response highlighted by significant ($p>0.001$) induction of type 1 interferon (*ifna*), interferon stimulated genes (e.g., *mx1, isg15*) as well as proinflammatory genes including interleukin 8 (*il8*). We then exposed these cells to either 4-n-nonlyphenol or PFOS at environmental levels with and without poly(I:C) to investigate the effects of these chemicals of concern on cetacean antiviral immunity. Through this joint agency effort, we hope to learn more about the potential effects of legacy and emerging chemicals of concern on orca skin health that can be used for risk assessment and management of orca populations.

1.08.T-03 Marine Medaka (*Oryzias melastigma*) as a Developmental Immunotoxicity Model for PFAS (Per- and Polyfluoroalkyl Substances) Exposure

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Production and use of legacy per- and polyfluoroalkyl substances (PFAS) is heavily regulated due to their immunotoxicy. Still, it remains unclear if replacement PFAS pose a similar risk. To evaluate potential immunotoxic effects of PFAS during critical windows of immune system development, marine medaka (*Oryzias melastigma*) were exposed to PFAS levels equivalent to prenatal/postnatal human blood concentrations. Legacy PFAS (PFOS, PFOA) and replacements (PFHxS, PFBS, PFHxA, GenX) were assessed for immunotoxic impacts using a bacterial challenge and transcriptomics. Exposure windows encompassed lymphocyte progenitor cell migration and thymus colonization (7-11 days post fertilization (dpf)) and the establishment of immune competence in the thymus (12-19 days post hatching (dph)). PFHxA exposure at 7-11dpf reduced immune competence, while exposure during 12-19dph revealed diminished immune competence in response to PFHxS, PFBS, PFOA, and GenX. The transcriptome indicated changes in expression profiles of PFAS-exposed larvae (12-19 dph). Similar numbers of genes were differentially regulated for PFOA (919) and its replacement GenX (964). Comparatively, fewer genes were differently expressed after exposure to PFOS (492) and its replacement PFHxS (666). However, the shortest PFOS replacement, PFBS, resulted in the highest deregulation with 1697 differentially expressed genes. Modified genes were found to be associated with Gene Ontologies (GOs) related to immune function including thymus development, B-cell and T-cell differentiation, and myeloid and lymphoid progenitors. The data demonstrate variability in mechanisms of PFAS-induced immunotoxicity. Thus, specific critical windows may be more susceptible to certain PFAS which is important to consider for risk assessment.

1.08.T-04 Development of a Rapid Immunotoxicity Screening Assay: Identification of Molecular Biomarkers of Innate Immune System Dysfunction

Katie Solomons *and Marlo K Sellin Jeffries, Texas Christian University, Fort Worth, United States*

Environmental contaminants can adversely impact fish health by altering immune system function. Despite the potential population-level impacts of immunomodulation, a standardized immunotoxicity screening method has yet to be developed leaving a gap in the hazard assessment toolbox. Here, we explore the refinement of a neutrophil migration assay for use as an immunotoxicity screening assay. Neutrophil migration assays have been primarily used to study immune system development and function in zebrafish. In these assays, the tail of a larval fish is injured and the number of neutrophils that move to the injury site are counted. This approach is valuable as it provides insights into innate immune system (IIS) function; however, counting neutrophils is time-intensive and the assay requires a large number of fish due to high individual variation. Thus, we sought to transform this assay into an immunotoxicity screening method using fathead minnows (FHM) as a model species. Specifically, we explored the utility of gene expression endpoints as indicators of immune cell migration and function following tail injuries in larval FHM. To accomplish this, differentially expressed genes were identified using transcriptomic analysis of injured tails collected from 7-day-old FHM tails relative to uninjured tails to identify potential biomarkers of IIS function. This analysis revealed differential expression in 6125 genes and of these, several were identified as potential biomarkers of IIS function (e.g., *CL-12, Cav1, A2M, etc.*). To validate the use of these potential biomarker genes for immunotoxicity evaluations, we exposed newly hatched larval FHM to dexamethasone (a known immunosuppressant) or estradiol for 7 days, subjected them to a tail injury, and then measured the expression of several potential biomarker genes. The results of this study, which will be presented in detail, demonstrate the potential utility of a larval FHM IIS assay for immunotoxicity evaluations.

1.08.T-05 Transcriptional Analysis of the Role of Immune Response Gene (IRG) in the Suppressive Effects of Tert-butylhydroquinone (TBHQ) on Macrophage Activation by LPS and Poly I:C

Alyssa M. Whisel[1] *and Charles D. Rice*[2]*, (1)Biological Sciences, Clemson University, (2)Clemson University*

One of the most pervasive food additives and environmental contaminants is the synthetic antioxidant tert-butylhydroquinone (TBHQ). This compound is also considered a classical inducer of oxidative stress mediated through Nrf2 activation and downstream expression of antioxidant proteins, though at fairly high levels. In this study, we demonstrate that similarly high levels are cytotoxic to RAW264.7 murine macrophages. However, at low levels (1 and 5 μM) for 24 hr, macrophage activation stimulated by both lipopolysaccharide (LPS) and polyinosinic:polycytidylic acid (poly I:C) as determined by the induced expression induction of IRG, iNOS and COX-2 proteins, and proinflammatory cytokines, as well as phagocytic activity, is suppressed. Of note, IRG is both an antimicrobial protein and a key regulator of inflammation by itaconate-associated acetylation of key components of cellular respiration (anerobic glycolysis), inhibition of oxidative stress, and down regulation of inflammatory processes. Moreover, elevated nitric oxide following activation is now known to also nitrosylate key components of cellular respiration, and thus is also involved in regulating inflammatory processes. Together, these events are involved in macrophage polarization between M1 and M2 phenotypes. In additional studies using wildtype RAW264.7 and IRG mutants (KO), we treated cells with 5 μM TBHQ vs control in the presence or absence of LPS and poly I:C for 12 hr for transcriptome analysis. Differential gene expression (DGE) and KEGG/GO analysis show that the two lines differ the most in terms of basal cytokine-cytokine receptor interactions, cellular respiration pathways, monocyte-macrophage differentiation, and histone modification pathways. In the presence of LPS, the KO cells have reduced TNF, IL-17, and ABC transporter pathways compared to wildtype cells. Of note, compared to wildtype cells stimulated with LPS, KO cells stimulated with poly I:C exhibited suppressed JAK-Stat, PI3K-AKT, and cellular adhesion signaling pathways. DGE and pathway analysis show that TBHQ is associated with NFκ-B pathway suppression, chemokine-chemokine receptor interactions, and all genes associated with "malaria" (KEGG mmu05144) in KO cells, indicating a strong role of ACOD1/IRG in the susceptibility to TBHQ immunotoxicity. Under the conditions of our studies, TBHQ treatments were associated mostly with NFκ-B pathway suppression, and not oxidative stress.

1.08.T-06 Spatiotemporal Monitoring of Demonstration Pit Lake Samples Using Cell B ased Bioassays

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Oil sands process affected waters (OSPWs) are generated as a by-product during bitumen extraction, and this effluent has been shown to exert toxic effects in several animal models such as fish and invertebrates. As OSPWs vary significantly in complexity and composition, monitoring these waters require quickly employed approaches that can sensitively respond to these dynamic mixtures. *In vitro* bioassays are rapid, sensitive, and high throughput screens that can be used to help identify environmental perturbations or remediation efficacy. Our lab has developed several bioactivity (i.e., measured biological responses) based tests of water using prokaryotic and eukaryotic cells. Specifically, immune cells (e.g., mammalian macrophages) were used to evaluate OSPW immunotoxicity via a pro-inflammatory cytokine secretion assay. We have also incorporated bacterial cytotoxicity screens using the standardized MicroTox test and an adapted Microbial Inhibitory Concentration (MIC) assay. These analyses were chosen to encompass various biological outcomes, as the exposure time spans 30 minutes to 24 hours and range in lethal and sublethal concentrations. In this study, OSPW samples were subjected to an advanced oxidation process (AOP) treatment that focuses on detoxification of organics, such as naphthenic acids (NAs), which are thought to be major toxicants in OSPW. Synchronous fluorescence spectroscopy of the samples showed a reduction in organic compounds after a 2-hour treatment, correlating with a significant reduction of the pro-inflammatory cytokines interleukin (IL)-6 and IL-1β post treatment. These results are supported by MicroTox data, with the untreated (UT) sample inhibiting more bioluminescence (37.5%) compared to after treatment (27.7%). MIC results also show lower cell viability (1.73 log reduction) in the UT exposure that was ameliorated post treatment (0.6 log reduction). This *in vitro* framework was then used to survey water from a demonstration pit lake (DPL) that is being investigated as a potential tailings remediation approach. DPL samples overall have significantly lower immunotoxicity and maintain higher bacterial viability and bioluminescence when compared to the UT OSPW source. Altogether, these data show how cell-based bioassays can sensitively detect changes in treatments that focus on the reduction of organic toxicants such as NAs and how they can also be used for the spatiotemporal monitoring of a DPL.

1.09.P-We Distinguishing Mode-of-Action-Specific Toxicity from Non-Specific Effects: An Endocrine Disruption Conundrum

1.09.P-We-002 The RADAR and REACTIV Assays as Tools for Shedding Light on Steroidogenic Mechanisms

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A large number of environmentally relevant chemicals have been found to interfere with either the expression or activity of steroidogenic enzymes. In order to correctly identify endocrine active chemicals, it is necessary to identify the mechanism by which endocrine targets might be affected. Recently, the OECD Test Guidelines (TGs) of two new screening assays were adopted for the Rapid Androgen Disruption Activity Reporter (RADAR) assay (OECD TG 251), and the Rapid Estrogen ACTivity *In Vivo* (REACTIV) assay (TG publication expected in June 2024). The two assays make use of transgenic eleutheroembryos of Japanese medaka (*Oryzias latipes*), which do not fall under the legislation on protected life-stages of laboratory animals in the European Union. The two assays detect alterations in androgen and estrogen axis signaling, respectively. The RADAR and REACTIV assays are also capable of detecting the impact of chemicals interfering with downstream steps of steroidogenesis. Overall, the combination of the two assays covers estrogenic, androgenic and, at least in part, steroidogenic (EAS) modalities. We hypothesized that by comparing the results of both assays for the same chemicals that are known to act at different steps of the steroidogenesis, evidence could be obtained to aid in specifying the type of steroidogenic mode of action (MoA). With this in mind we set out to test the hypothesis using data from the OECD validation of both assays and by performing novel tests with substrates of steroidogenic enzymes and reference pharmaceuticals targeting steroidogenic enzymes. The results of these studies showed that test chemicals with different MoA showed differing response profiles in the two assays. Chemicals with the same MoA showed similar response profiles across the two assays. We also demonstrated that by performing the REACTIV assay in the presence and absence of both an aromatisable androgen and an estrogen, inhibitory effects on the estrogen axis can be attributed to either estrogen receptor antagonism or aromatase inhibition. We conclude that in cases where both assays are performed, additional information can be gleaned allowing more precise conclusions on the MoA, should it be linked to the androgen or estrogen axis, or to steroidogenesis. This provides additional information on EAS-mediated endocrine activity to be included in a weight of evidence approach.

1.09.P-We-003 Thyroid Hormone Disruption of Di-Isononyl Phthalate (DiNP), Bis(2-ethylhexyl) Terephthalate (DEHTP), and Trioctyl Trimellitate (TOTM) in Embryolarval and Adult Zebrafish (*Danio rerio*)

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As conventional plasticizers such as di(2-ethylhexyl) phthalate (DEHP) face restrictions due to their adverse effects, the use of alternative plasticizers has been on the rise. The detection and concentration of conventional phthalates have been decreasing; however, exposure to alternatives like di-isononyl phthalate (DiNP), bis(2-ethylhexyl) terephthalate (DEHTP), and trioctyl trimellitate (TOTM) has been increasing, leading to concerns about their environmental

health consequences. However, only a limited number of toxicological studies have been conducted, with even fewer addressing their potential as endocrine disruptors. In this study, zebrafish embryos were exposed to various concentrations of DEHP (0.1, 0.3, 1 mg/L), DiNP (1, 3, 6 mg/L), DEHTP (0.1, 0.3, 1 mg/L), and TOTM (1, 3, 10 mg/L) from 4 hours post-fertilization (hpf) to 120 hpf to assess alterations in thyroid hormones and changes in the transcription of thyroid-regulating genes. The 5-day exposure to DEHP, DiNP, DEHTP, and TOTM resulted in significant increases in whole-body thyroid hormone levels, triiodothyronine (T3), and thyroxine (T4). The lowest concentrations that led to a significant increase in T3 were observed for DEHP (0.3 mg/L), followed by DEHTP (1 mg/L), TOTM (3 mg/L), and DiNP (6 mg/L). While the hormonal alterations were similar for all four chemicals, transcription profiles of related genes differed. DEHP and DiNP led to a significant increase in most of the thyroid-regulating genes, while DEHTP and TOTM led to a decrease in most of the genes. Adult male zebrafish were exposed for 21 days to DEHTP (0.01, 0.1, 1 mg/L) and TOTM (0.1, 1 mg/L) to evaluate thyroid outcomes. Exposure to DEHTP led to a significant decrease in T3 with an increase in thyroid stimulating hormone (TSH), whereas exposure to TOTM led to a decrease in T3 without any changes in other thyroid hormones. These results suggest that in the early life stage of zebrafish, all four plasticizers lead to similar hormonal effects, though the mechanisms that lead to changes might differ among chemicals. Also, thyroid disruption by DEHTP and TOTM varies by life stage, as DEHTP and TOTM exhibited similar effects in early stages but divergent effects in the adult stage. Nonetheless, current observations show that the thyroid hormone disruption potential of these major alternative plasticizers is concerning, thus calling for further studies.

1.09.P-We-004 Effects on Zebrafish Length and Pigmentation as an Indicator of Depleted Uranium Impact on Thyroid Hormone Disruption

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Depleted uranium is a dangerous heavy metal that many vulnerable communities have been and continue to be exposed to. Depleted uranium (DU) exposure is linked to several cancers, but its capacity to disrupt hormone function has only recently received attention. Thyroid hormone is critical to appropriate development of melanocytes, pigmentation cells, and length. This project aims to determine whether DU affects the development of cells involved in pigmentation and length through disruption of thyroid hormone function. To evaluate the effect of DU on development of these cells, we used the larval zebrafish, *Danio rerio,* because they are a model organism for ecotoxicology. We hypothesize that early exposure to waterborne depleted uranium will disrupt thyroid hormone-related development in zebrafish, leading to morphological abnormalities, such as decreased length and a decrease in pigmentation across the head and body. Fish were exposed to PTU (a thyroid hormone disruptor), as a positive control, and increasing, and environmentally relevant concentrations of DU. We measured fish length from top of the head to tip of the tail by using ImageJ and finding a mean length. We measured melanization by using a pigmentation score between 0 and 2, 0 being no pigmentation and 2 being fully pigmented. It was found that at 300 ppb of DU, there was a significant decrease in length. The PTU and T4 reintroduction groups suggest this effect is not thyroid hormone dependent. This project will aid groups at risk such as the United States military, Native Americans, humanitarian organizations in war zones, and local populations where water, dust and/or mining operations can lead to DU exposure.

1.09.P-We-005 Development of Short-Term Screening Assay System for Detecting Anti-Juvenile Hormone (JH) Activity: JH Receptor Antagonists and JH Biosynthesis Inhibitor

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Juvenile hormones (JHs) play a significant role in growth, metamorphosis, reproduction, and sexual determination in arthropods. The freshwater crustacean genus *Daphnia* generally reproduces through parthenogenesis, but male offspring production is induced not only by environmental stimuli (e.g., short day length, low temperature, and crowding). but also by exposure to JH and JH-active chemicals (e.g. insect growth regulators) through stimulating JH biosynthesis and/or JH receptor activation. Thus, using male offspring induction as an endpoint, the short-term juvenile hormone activity screening assay (JHASA) using *Daphnia magna* has been developed to detect chemicals with JH agonistic activity, and as the Organization for Economic Co-operation and Development (OECD) test guideline this year. On the other hand, despite the ongoing development of novel insecticides that selectively inhibit JH receptor or JH biosynthesis pathways, the test methods to detect the effect of such anti-JH activity has not yet been established. It is expected that the anti-JH activity can be detected by suppression of male offspring production induced by JH-active chemicals. If co-exposure of a test chemicals with a JH receptor agonist or JH biosynthesis inducer results in a significant decrease in male ratio, the test chemical is presumed to be a JH receptor antagonist or a JH biosynthesis inhibitor, respectively. Additionally, for JH biosynthesis inducer/inhibitors, measurements of changes in endogenous JH titer in *Daphnia* in the presence or absence of JH-active chemicals can support disruption activity in JH biosynthesis. In this study, adult female *D. magna* was co-exposed to several candidate chemicals and JH-active chemicals and male ratio of offspring and JH titer were compared to JH-active chemical alone. Those candidate chemicals were selected based on literature review on insect JH studies and preliminary obtained findings from JHASA and reporter gene assay for detecting JH receptor activation. We also explored measurement methods for several candidate endogenous JHs in *Daphnia* using LC-MS/MS, and evaluated the changes in JH titer during reproductive cycle and compared the JH titer among each treatment and controls.

1.09.P-We-006 Cadmium as an Endocrine Disruptor on Freshwater Snail *Physella acuta*: Gene Expression Analysis of Hormonal Receptors

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The increasing pollution of freshwater ecosystems with Cadmium (Cd), resulting from its widespread industrial and agricultural use, has emerged as a global environmental problem of concern. The harmful impact of this heavy metal on water bodies has been widely documented, affecting the viability of aquatic invertebrates' communities. It has been described in vertebrates as an endocrine-disrupting chemical (EDC) that can mimic the action of estrogens by interacting with hormone receptors, being able to influence reproductive function and overall development. However, many unknowns exist concerning the mechanisms involved in the endocrine disruption. Mollusks are one of the most sensitive invertebrate phyla to anthropogenic inputs, and they are considered multidisciplinary models in environmental toxicology studies. Therefore, they are often used as excellent bioindicators to test ecosystem health. In this study, the freshwater pulmonate snail *Physella acuta* was used to evaluate the effects of Cd on the endocrine system and stress response in the Gastropoda. The snail was exposed to 0, 0.01, and 1 uM concentrations of $CdCl_2$. The effects were evaluated at the molecular level at 24h and 7 days of exposure, analyzing changes in the transcriptional activity of endocrine-related genes: *estrogen receptor* (*ER*), *retinoid X receptor* (*RXR*), *membrane Progestin Receptor* (*MPR*), *Hsd17b8*, and *galanin Receptor Type 2* (*GalR2*). Furthermore, the transcriptional activity of *Hsp90*, which is involved in hormonal receptor maturation, was analyzed. Transcriptional activity was quantified by retrotranscription and Real-Time PCR. This study provides information on the putative mechanisms that could work on gastropods in cadmium's presence. In addition, the results hint at the overall health of *P. acuta* at the molecular level. Using a sentinel organism for the analysis of cadmium mechanisms could be helpful in ecotoxicological studies and risk assessment.

1.09.T Distinguishing Mode-of-Action-Specific Toxicity from Non-Specific Effects: An Endocrine Disruption Conundrum

1.09.T-01 Physiologically and Biochemically Based Potency Thresholds are Essential for Distinguishing Endocrine Modes of Action

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Potency and physiological concentration determine whether a ligand produce physiological effects via a hormone receptor. We previously identified a Human-Relevant Potency Threshold (HRPT) of 1×10^{-4} relative to E2 as the minimum ligand potency required to produce clinically observable estrogenic agonist effects via the human estrogen receptor-alpha (ERα). Here we test the hypothesis that the HRPT for ERα arises from the receptor occupancy (RO) of the normal metabolic milieu of endogenous ERα ligands, composed of precursors to hormones, metabolites of hormones, and other normal products of metabolism. We conducted RO calculations to test the hypothesis that chemicals with potency equal to the HRPT can attain sufficient occupancy of ERα to alter estrogenic tone in an intact animal amidst normal levels of a subset of endogenous ERα-ligands that includes DHEA, DHEA-sulfate, androstenediol, E1, E2, and E3. Successful competition with the endogenous metabolic milieu was defined as an alteration of E2 RO greater than normal fluctuations amidst physiological concentration ranges of the endogenous metabolic milieu. Fractional RO calculations showed that at concentrations of 1 nM, ligands with potencies equal to the HRPT were unable to compete successfully for ERα against endogenous ERα-ligands present at minimal concentrations in human blood, or at 1μM against endogenous ERα-ligands present at mid-point concentrations in human blood. Even with affinity 10-fold greater than the HRPT, no physiologically-relevant change in RO would be produced by a ligand at 1 μM amidst mid-point plasma levels of the five endogenous ligands, or at 1 nM amidst minimal endogenous ligand concentrations. The endogenous metabolic milieu is responsible for the observed ERα agonist HRPT and provides a compelling mechanistic explanation for the HRPT that is grounded in basic principles of molecular kinetics using well characterized properties and concentrations of endogenous components of normal metabolism. The HRPT (10^{-4} relative to E2) proposed previously is quite conservative and should be considered strong evidence against the potential for disruption of the estrogenic pathway by agonists, partial agonists, or antagonists. For chemicals with potency 10^{-3} of E2, the potential for estrogenic endocrine disruption must be considered equivocal and subject to the presence of corroborative evidence. These thresholds must be exceeded for a chemical to act via an endocrine mode of action.

1.09.T-02 Optimising Concentration Setting for In Vivo Endocrine Screening Assays with Aquatic Vertebrates

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There is growing regulatory demand for fish and amphibian *in vivo* data to support identification of chemical-induced endocrine activity. To ensure that endocrine-specific effects are detected and aid data interpretation, improvements to concentration setting guidance are needed. Determining the most appropriate highest concentration – or maximum tolerated concentration (MTC) – allows for clear distinction of effects attributable to endocrine-related activity from those of systemic or secondary toxicity that is not directly endocrine mediated. This is challenging because biological effects may depend on the life-stage and species tested. Currently, the MTC is inconsistently defined within the different OECD test guidelines. This work aims to provide a consensus approach to MTC setting for fish and amphibian endocrine studies by developing a strategy for concentration setting using existing data. A database using results from regulatory fish acute and chronic toxicity studies and corresponding endocrine screening studies (fish short term reproduction and amphibian metamorphosis assays) was established for 32 pesticide active substances. The results of a meta-analysis performed to assess the effectiveness of using data from available acute and chronic studies in setting the MTC for the endocrine screening studies will be presented. This entails examining a) how MTCs derived using different approaches (; b) how MTCs derived from results of standard acute and chronic studies relate to the highest concentrations tested in endocrine screening studies; c) how the acute and chronic toxicity outcomes relate to the findings in the endocrine screening assays, *i.e.* whether overt toxicity was observed in the selected test concentrations and if the (remaining) concentrations were sufficient to allow for detection of endocrine activity. The results will be considered in development of a weight of evidence approach which can be utilised to optimise the setting of the MTC for aquatic vertebrate endocrine tests. Ultimately this work will support the interpretation of data from endocrine screening studies and reduce the instances where studies may need to be repeated, or higher tier *in vivo* tests are triggered unnecessarily. Reducing instances of overt toxicity will also result in the welfare benefit of reducing severity of effects experienced by animals in such assays.

1.09.T-03 Targeted Knockout of Deiodinases to Evaluate Thyroid Disruption

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Iodothyronine deiodinases regulate thyroid hormone (TH) action by activating and inactivating TH. Amphibian metamorphosis is mediated by TH, thus making *Xenopus tropicalis* an ideal model to investigate thyroid axis disruption. A challenge in evaluating thyroid disruption is linking exposure to the observed adverse effect; thus, in this work targeted gene knockout was utilized to elucidate the putative adverse outcome pathways (AOPs) of deiodinase type 2 and type 3 (DIO2 and DIO3). Single-cell *X. tropicalis* embryos were injected with guide RNA+Cas12 nuclease targeting either DIO2 or DIO3 to induce DNA strand breaks and mutations via imperfect DNA repair. Second generation (F2) offspring derived from the injected embryos were genotyped to identify wildtype (WT), heterozygous mutants (hets), and homozygous mutants. Homozygous mutations in the F2 generation included 26 and 102 base pair deletions in DIO2 and DIO3 knockout animals, respectively. The F2 tadpoles were reared through metamorphosis to evaluate the impact of non-functional DIO2 or DIO3. Endpoints observed included time to reach developmental stage, weight, and snout vent length (SVL). Blood plasma and thyroid samples for histological analysis were collected. Hind limbs and livers were sampled for DIO2 and DIO3 enzyme activity assays, respectively, to confirm loss of activity. As early as the F0 generation, asynchronous development (*e.g.*, unequal limb length) was observed in DIO2 mutants. Relative to WT, F2 mutants developed faster to stage 62 and then decelerated. Further, the mutants were smaller (weight and SVL) and had increased plasma T4 and decreased T3. DIO2 mutant thyroid glands were larger and had increased thyroid follicular cell hypertrophy and hyperplasia suggesting overstimulation. DIO3 F2 mutants developed faster and exhibited abnormalities (*e.g.*, smaller limbs, premature tail resorption) compared to WT. DIO3 mutants were also smaller (weight and SVL) and had reduced plasma T4 and T3. DIO3 mutant thyroid glands were smaller than WT, which is consistent with reduced stimulation of the gland. These results provide insight into endpoints that can be used to link inhibition of DIO2 or DIO3 to an adverse thyroid effect. Data from *in vivo* exposure assays could be compared to these results to discern whether chemical inhibition induces similar effects as DIO2 or DIO3 gene disruption. *The contents of this abstract neither constitute, nor necessarily reflect, U.S. Environmental Protection Agency policy.*

1.09.T-04 Combining Eleuthero-Embryo Assays to Identify the Mode of Action of Thyroid Disruptors

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For a substance to be identified as an Endocrine Disruptor, the way it induces adverse effects must pass through a well established endocrine mode of action (MoA). For thyroid disruption, two OECD guidelines are based on morphometric evaluations during amphibian metamorphosis. We developed the third guideline: the XETA (Xenopus Eleutheroembryonic Thyroid Assay) test. This test uses a xenopus transgenic line in which the expression of the GFP (green fluorescent protein) gene is placed under the control of a promoter responding to thyroid signaling. In the eleuthero-embryos, changes in fluorescence indicate that the tested substance is active on the thyroid axis. In addition to demonstrating endocrine activity, the XETA also provides clues about the MoA: the test is performed in the absence and presence of thyroid hormone (spiked and unspiked mode), and the directions of fluorescence variations in each mode depend on the substance's MoA. An agonist of the receptor induces an increase in fluorescence in both modes, whereas an inhibitor induces an increase only in the spiked mode. Interestingly, a decrease in unspiked mode reveals systemic toxicity or non-specific effects, offering a way to identify chemicals effecting the fluorescence level by a non-endocrine effect. We recently developed the TETHYS (Transgenic Eleutheroembryo ThYroid Specific test), based on a medaka transgenic line and on the same principle. In the genetic construct the Thyroglobulin promoter controls the expression of GFP. Treatment with thyroid hormone or with a TPO inhibitor induces, as expected, a decrease and an increase in fluorescence, respectively (thyroglobulin synthesis is influenced by the feedback loop of the thyroid axis and is induced when the hormone concentration decreases). As with the XETA test, the direction of fluorescence variation provides clues about the possible modes of action of the substance. MoAs not detected by the XETA are detected by the TETHYS test, such as iodine import inhibition. We took advantage of the complementarity of the two assays to apply a new method for identifying MoAs of thyroid active substances and systemic toxicity effects based on the combination of XETA and TETHYS tests. We will illustrate this methodology by presenting case studies using reference chemicals.

1.09.T-05 Early Life Stage Amphibian Thyroid Assay (ELSATA); A Thyroid-Focused Alternative to the Larval Amphibian Growth and Development Assay (LAGDA)

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The Larval Amphibian Growth and Development Assay (LAGDA) was designed as a higher tier *in vivo* assay to provide data on adverse effects of substances on thyroid-mediated endpoints (T modality). However, the second half of the LAGDA is focused on the estrogen, androgen and steroidogenic (EAS) modalities. Since triggering of a LAGDA is based on thyroid concerns, questions have been raised about the value of this additional 10-week study period for identifying additional effects related to potential (anti)thyroidal activity of substances. Also, the assessment of endpoints unrelated to the T modality requires additional animals. To focus on the core strength of the LAGDA, we developed a modified version: the ELSATA (Early Life Stage Amphibian Thyroid Assay), which only considers endpoints related to the T modality. Animal welfare is emphasized, as 320 instead of 480 individuals are used, without decreasing the amount of thyroid-specific data. The assay assesses not only effects on thyroid-mediated processes such as metamorphosis, but also on development and growth. The assay is initiated with Nieuwkoop-Faber (NF) stage 8-10 embryos. At test termination, NF stage 62 larvae are assessed for time to reach NF stage 62, snout-to-vent length (SVL), body weight, any external abnormalities, and thyroid histopathology. Terminating the study at NF stage 62 generates sufficient data for an assessment of thyroid-related endocrine effects. The ELSATA effectively limits the scope of the LAGDA to the T modality. The ELSATA also allows comparison to the Extended Amphibian Metamorphosis Assay (EAMA, covering NF stages 51 to 62). Importantly, the ELSATA reduces the number of animals required and thus provides benefits to animal welfare. Results from ELSATA studies on two thyroid-active substances, benzophenone-2 and perchlorate, are presented. The observed effects are discussed by considering both thyroid-mediated endocrine toxicity and developmental toxicity.

1.09.T-06 Approaches for Differentiating Between Non-Endocrine and Endocrine Modes of Action Causing Adrenocortical Pathologies and Potential Adversity

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The adrenal glands produce a diversity of hormones mainly from two discrete zones of the outer cortex. The innermost layer of the cortex, the *zona reticularis*, produces androgens that are converted to fully functional sex hormones in other organs, and the next layer, the *zona fasciculata*, produces the glucocorticoids cortisol and cortisone. The adrenal gland is one of the most common toxicological target organs within the endocrine system, with adrenal cortical vacuolization being a common observation following prolonged and high dose xenobiotic exposures. Vacuolization of the *zona reticularis* and *zona fasciculata* is often associated with vacuolization of neutral lipids and cholesterol caused directly by mitochondrial cytotoxicity and swelling to the smooth endoplasmic reticulum thus disrupting normal cellular function. Such vacuolization could be due to an inhibition of steroidogenesis as the rate limiting step in this pathway is import of cholesterol to the mitochondria. Cytotoxicity may also be observed in the adrenal gland at relatively high exposure levels for some xenobiotics due to the unique anatomical architecture and composition of the gland (i.e., high perfusion rates and high lipid content). The combination of high perfusion rates the hydrophilicity for some xenobiotics provides a mechanism to achieve tissue concentrations that lead to cytotoxicity. Therefore, it may not be evident if cortical vacuolization of the *zona reticularis* and *zona fasciculata* is caused by a direct effect on steroidogenesis (i.e., inhibition) or an indirect effect on the steroidogenic pathway by cytotoxicity. Currently, there is a lack of guidance on the interpretation and significance of adrenal cortical vacuolization, nor is there an established strategy for testing/performing a functional assessment of effects. This talk will present approaches to differentiate direct versus indirect effects to adrenal cortical steroidogenesis to help differentiate between endocrine and non-endocrine cytotoxic effects. In addition, the presentation will discuss approaches to assess the potential for adversity related to adrenal cortical pathologies.

1.10.P-Tu Emergent Environmental Issues and Perspectives in Latin America

1.10.P-Tu-040 Accumulation of Metals and Metalloids in Aquatic Organisms and Its Implications on Humans in Ecuadorian Mangroves

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The mangrove ecosystem of Ecuador is jeopardized from agricultural, industrial, urban and aquacultural practices, which together contribute diverse pollutants into these sensitive coastal forests. This study seeks to assess the concentration of toxic metals and metalloids in mangrove ecosystems as an indicator of overall pollution. We collected sediment, mussels, and clams from mangrove forests in two reserves: The Salado Mangroves Faunal Production Reserve and El Morro Mangrove Wildlife Refuge. Dietary intake surveys were also conducted with Puerto Hondo residents to assess potential exposures. We will use stable isotopes ratios of carbon and nitrogen to assess foodweb dynamics of metals and metalloids. Elements will be quantified with atomic absorption spectroscopy and inductively coupled plasma mass spectrometry. This project will highlight ecological risks associated with anthropogenic activities that impact mangrove forests. The findings will facilitate the prioritization of conservation measures and policy actions.

1.10.P-Tu-041 Emerging Pollutants in Yucatan's Karstic Aquifer: Pharmaceuticals and Personal Care Products in Cenotes (Sinkholes)

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A “cenote” is a natural sinkhole formed by the dissolution of limestone rock, exposing the groundwater underneath; part of the cenotes in Yucatan are geographically located forming a semicircle known as the Ring of Cenotes (RC). The karstic nature of the Yucatecan aquifer, which explains the formation of cenotes, also implies its high vulnerability to contamination. The quality of groundwater can be affected by different anthropogenic activities, including the growth of urban areas and tourist activities (poor treatment of wastewater and solid waste). Due to the ecological and socioeconomic importance of cenotes, this study aimed to evaluate the presence of highly prescribed pharmaceutical products (ibuprofen, naproxen and diclofenac); and frequently used personal care products: a bactericide (triclosan), an insect repellent (DEET) and a sunscreen (oxybenzone), the last two widely used in tourist activities that involve swimming and visiting areas with vegetation of mangrove and jungle and mosquitoes; caffeine in water was also determined as a chemical tracer of the presence of wastewater of human origin. Water samples were collected in 19 cenotes located in three different areas of RC: four sites in the northeast area; 10 cenotes in the south, and five points in the northwest. Samples were collected in different climatic seasons in 2023: May (dry season) and October (rainy season). Emerging pollutants and caffeine in water were determined by solid phase extraction (SPE) and gas chromatography coupled to mass spectrometry (GC-MS). All individual contaminants were detected in 100% of the cenotes in both the dry and rainy seasons, with statistically significant differences between climatic season for all compounds, except ibuprofen. The compound with the highest concentration was DEET, the active ingredient in commercial insect repellents; in the dry season, DEET presented an average concentration of 175.9 ± 89.0 ng/L; however, in the rainy season, average level of DEET increased two orders of magnitude and there was a high dispersion in the detected values (58577.0 ± 100284.6 ng/L). In general, the other pollutants and caffeine had higher concentrations in the dry season. Entry of untreated wastewater of human origin was verified through the detection of caffeine in all the cenotes studied. The presence of emerging contaminants means that there may be other compounds of emerging concern in the water of the cenotes that can affect humans and biota.

1.10.P-Tu-042 Tecolutla River Water Pollution Analysis Using Toxicity Identification evaluation (TIE)

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The risk of water pollution arises from multiple sources, including untreated wastewater overflows, animal waste runoff, the use of fertilizers, and the potential introduction of heavy metals. An example is the Tecolutla River in Mexico, where untreated wastewater and agricultural chemicals have influenced the river water quality for years. Additionally, the petroleum activity in the study area adds to this, potentially compromising the integrity of the surrounding aquatic life. Therefore, the objective of this study was to determine the water quality of a section of the Tecolutla River during two contrasting climatic seasons using the method of Toxicity Identification Evaluation (TIE) to establish the ecological risk posed by contaminants in the study area. Ten sampling stations were established, and in-situ measurements were taken for temperature, pH, dissolved oxygen, dissolved solids, transparency, salinity, electrical conductivity, and oxidation-reduction potential (ORP). Subsequent laboratory analysis included Biochemical Oxygen Demand (BOD_5), nitrites, and nitrates. Toxicity bioassays were conducted using wheat seeds (*Triticum aestivum*) at different concentrations: 100%, 85%, 70%, 55%, and 40%. Preliminary results indicated that the water quality was good during the dry season based on Biochemistry Oxygen Demand (BOD_5) values, averaging 2.9 mg/L. The average environmental conditions recorded were as follows: temperature averaged 28.5 °C, pH averaged 7.7, dissolved oxygen level was 5.8 mg/L, total dissolved solids averaged 1595 mg/L, and electrical conductivity was 3220.1 µS/cm. According to Mexican quality criteria for aquatic life protection, pH and dissolved oxygen levels met the minimum allowable values, falling within acceptable limits. However, applicable legislation does not provide information on the maximum allowable limits for parameters such as temperature, electrical conductivity, or total dissolved solids. Regarding nutrients, high levels of nitrites and nitrates were found, exceeding Mexican quality criteria, with values of 2.8 mg/L and 0.89 mg/L, respectively. Wheat seeds will be used for the TIE bioassays due to the salinity of some samples; the potassium dichromate LC50 (148 mg/L) will be used as a positive control, and the first sampling results will be presented.

1.10.P-Tu-043 Scientific Dissemination Strategies in Public Spaces in Latin America

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Scientific dissemination allows society to access complex and diverse knowledge generated in research centers and universities, thus advancing scientific literacy, and disseminating concepts that allow the community to broaden its horizons and awaken and motivate professionals in the biological and environmental sciences field. The main objective of this project is to publicize the results of a strategy of scientific dissemination in public spaces that has been implemented through the publication and distribution of magazines and participation in forums, festivals, and fairs in public spaces. The method used generally comprises four phases: the first is the design of the theme, the second is the selection of the target public, the third is the management of venues, the organizing committee, and the elaboration of the program, and the fourth is implementation and development. The results include 20 years of developing outreach experiences through various events involving society in general in public spaces such as schools, parks, and squares in rural and urban areas, holding forums, fairs, festivals, presentations of the magazine and interactive activities, highlighting motivation, creativity, innovation, and dissemination of scientific knowledge.

1.10.P-Tu-044 Environmental Education for Secondary Level in La Piedad, Michoacán

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The present project consists of five phases in which a combined environmental education plan (PEAM) was developed between non-formal environmental education and a formal education setting. The plan aims to raise awareness among secondary school students, which is the last average educational level reached in the region, to address some of the local environmental issues faced in the municipality of La Piedad, Michoacán. This plan was implemented in the main secondary schools where students from both the urban and rural areas congregate.

The main environmental problems identified are deforestation, inadequate solid waste management, pollution of the Lerma River, and stray dogs' overpopulation. Later, a diagnosis of environmental awareness among students was conducted, one of the main findings is that they did not associate environmental health with their well-being. Next, it was necessary to analyze the causes and consequences of the identified environmental problems to generate awareness milestones as guidelines for the plan's design, program content, selection of activities, and development of some teaching materials, and their implementation in the selected educational institutions. In the final phase of the project, the PEAM was evaluated by the students, and by a

SWOT analysis. The information obtained from the evaluation permitted identify the plan's scope, areas of improvement, and support strategies that could represent contributions to be added to the plan, as continuous improvement is essential.

1.10.P-Tu-045 Composition of Microplastics in Wild-Caught Mussels from a Mangrove Ecosystem in Ecuador

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Microplastic pollution has become a growing environmental concern in marine ecosystems due to its widespread distribution and potential impact on aquatic organisms. Microplastics (MP) can enter marine ecosystems via physical (sedimentation, accumulation), chemical (degradation and absorption) and biological (ingestion and biodegradation) pathways, where they can be ingested by a wide range of marine organisms. The goal of this study is to determine the composition of microplastics ingested by wild-caught mussels from a mangrove ecosystem in the Gulf of Guayaquil in Ecuador. For this purpose, we sampled mussels (*Mytella strigata*) from the study area to dissect their soft tissue, digest it, filter the remaining, and visualize the MPs present in the samples. Our results revealed that fibers and filaments were the most abundant types of MP, however the number of particles found in mussels through this study are low when compared to previous research. Future steps aim to better understand the potential sources of these contaminants, the extent of this pollution in the mangrove ecosystem, effects on other aquatic species and potential risks posed to human health to develop an effective mitigation strategy.

1.10.P-Tu-046 Barnacles as Bioindicators of Microplastic Pollution in Mangrove Estuaries

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Microplastic pollution has been an ongoing problem that affects the environment and living beings. Most microplastics reach aquatic ecosystems, such as estuaries, and end up in the ocean. One way to determine microplastics' abundance, distribution and type is using bioindicators. Barnacles have proven to be potential species for the assessment of microplastics in coastal waters, due to their position in the water column and its feeding behavior. For this study, a protocol of microplastic isolation from barnacles was developed. One species of barnacle was recollected from buoys in the Manglares El Salado Fauna Production Reserve. All samples were prepared, through dissection and digestion, to detect the presence and abundance of microplastics. The short-term goal of the study is to establish a monitoring program for microplastic contamination. Our hypothesis was that barnacles attached to the buoys located outside the estuary accumulate more microplastics than the ones located inside the estuary due to the pollution coming from the city of Guayaquil. Preliminary results suggest that a higher concentration of microplastics is found in the barnacles from the buoys placed in the outer estuary of El Salado reserve. It was also found that fibers constitute the predominant type of morphology. Overall, the outcome of this research shows that barnacles can be used as bioindicators for plastic pollution in estuarine ecosystems. The methodology applied was practical in terms of profitability and efficiency, making it a great alternative for monitoring programs.

1.10.P-Tu-047 Endocrine Disruptors (Perchlorate and Thiocyanate) in Drinking Water: A Case Study in Chile

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Perchlorate and thiocyanate exposure are a potential health concern because it interferes with hormone production by thyroid gland. Food and drinking water (DW) are an important source of human exposure. However, little is known about the occurrence of perchlorate and thiocyanate in Chile. The purpose of this study was to determine the occurrence of perchlorate and thiocyanate in DW in Chile and estimated daily intakes. A total of 85 samples of DW were collected during 2019 in II region (Antofagasta: 11, Calama: 19), IV region (Illapel: 14; Ovalle: 15) and RM (Maipú: 12; Independencia: 14). The quantification was performed on an LC-MS/MS. The LOD were 0.05 ngmL-1 for perchlorate and thiocyanate. The estimated daily intake (EDI) values were calculated according to the average concentration (ngmL-1) of each ED in DW and the corresponding consumption rates (L) and divided by the body weight (kg). The highest concentration of perchlorate and thiocyanate were observed in DW from II region (Perchlorate: ND - 25.8 ngmL-1; Thiocyanate: 64.9 – 10200 ngmL-1), while those lowest concentrations were observed in IV region (Perchlorate: ND – 16.8 ngmL-1; Thiocyanate: 2.7 – 8.0 ngmL-1) and RM (Perchlorate: ND – 23.7 ngmL-1; Thiocyanate: 1.3 – 12.9 ngmL-1). Perchlorate concentrations in DW were higher than the guideline value suggested in France (15 ngmL-1). There is currently no regulation for thiocyanate in DW. The EDI for perchlorate were lower than the reference dose (7000 ng kg-1 day-1). For thiocyanate the EDI (2.9x105; II region) exceeds the lowest observed adverse effect level (LOAEL), which is 1.9 × 10 5 ng kg−1 day. The results show that perchlorate and thiocyanate are a common contaminant in DW in Chile. Inhabitants of northern Chile were exposed to higher-dose perchlorate and thiocyanate via intake of DW than inhabitants from other regions of Chile.

1.10.P-Tu-048 Bromate an Emerging Environmental Pollutant in Drinking Water and Vegetables: A Case Study in Chile

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Bromate (BrO3−) is classified as a group 2B, possible human carcinogen by the International Agency for Research on Cancer (IARC). Although some studies have reported the occurrence of bromate in drinking water (DW), very little is known about its presence in Chile. In this study, we quantified bromate in DW and vegetables collected across Chile and estimated daily intakes of bromate via DW and vegetables. A total of 85 samples (DW: 43; Vegetables:42) were collected during 2017 from supermarkets (non-traceable) and by the National Surveillance Program of the Ministry of Health (traceable), Chile. Quantification of bromate was performed on an LC-MS/MS. The LOD were 0.08 ngmL-1 for DW and 0.05 ngg-1 for fruits/vegetables. The estimated daily intake (EDI) values of bromate was calculated according to the average concentration (ngmL-1 and ngg-1) of bromate in DW, fruits and vegetables, and the consumption rates (L and g) and divided by the body weight (kg). Forty-two percent of DW (n = 18) contained bromate. The largest number of positive samples (n = 11) were collected from the northern region and 3 samples contained levels higher than 10 μg L−1. Bromate concentrations in vegetables (traceable and non-traceable) were 0.15 ng g−1 and 2.14 ng g−1, respectively. The highest bromate levels (non-traceable) occurred in leafy vegetables, such as chard, lettuce, and arugula. Leafy vegetables (traceable), such as lettuce, produced in Regions I, II and III contained bromate levels of 23.6, 9.66 and 7.68 ng g−1. The EDI of bromate (DW) ranged between 58.6 and 447 ngkg-1 day for all age groups and regions. The EDI for all sources and age groups were lower than the RfD of 4000 ngkg-1 day. The results show that bromate is a common contaminant in DW and vegetables in Chile. Inhabitants of northern region were exposed to higher doses bromate via DW and vegetables inhabitants from other regions of Chile.

1.10.P-Tu-049 Bioadsorption of Pb and Cd by the Invasive Aquatic Plant Egeria densa in San Luis Potosi, Mexico

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Bioadsorption is an alternative in wastewater treatment for the removal of heavy metals. The use of agroindustrial waste and aquatic plants is an option that has received attention in recent years since hydrophytes such as *Egeria dens*a have the ability to assimilate nutrients, which is why it has been used to remove contaminants from tributaries, although there are few studies on it. It has been reported that it is an invasive species in natural and artificial water bodies and its biocontrol is quite limited. The research project addresses challenges of SDG 6 “Clean water and sanitation” and SDG 12 “Responsible production and consumption”, and aims to study the potential of *Egeria densa* as a Pb and Cd bioadsorbent for wastewater treatment and its management as an invasive species in the Valles River in San Luis Potosi, Mexico. The bioadsorbent was obtained from the biomass of *Egeria densa* coming from the Valles River. It was washed with deionized water and dried at 65° C for 72 hours. It was subsequently ground in an electric mill and sieved to 300 µm and stored in airtight bags. Solutions of 10, 25, 50, 75 and 100 mg/L of Pb (II) and 0.5, 1, 2.5, 5 and 7.5 mg/L of Cd (II) were prepared from certified standards of each element in HNO_3 at 3%. In 50 mL Falcon tubes, 1 g of bioadsorbent was added with 25 ml of each of the solutions prepared above and placed in a shaker at 150 rpm for 120 minutes. All experiments were carried out at room temperature (25°C) at pH 6.0. Every 15 minutes, samples of 1 ml of the solutions were taken and volumetric to 10 mL with deionized water. The samples obtained were centrifuged for 15 minutes at 3000 rpm and preserved at 4°C for the analysis of Pb and Cd by flame atomic absorption spectrophotometry (Thermo Scientific Model iCE 3000 Series). According to the data obtained, the removal of Pb (II) is 97% for 100 mg/L at 60 minutes and for Cd (II) is 91% for 1 mg/L at 30 minutes. With these results, it is concluded that *Egeria densa* biomass has good adsorbent characteristics, such as low equilibrium time, removal of Pb and Cd (≥90%) than other bioadsorbents such as sugarcane bagasse or orange peel, and natural availability in large quantities in various points of the Valles River and others rivers in Mexico to be usable and achieve its control and management.

1.10.P-Tu-050 Occurrence and Contamination Level of Ochratoxin A (OTA) in Spices Marketed in Chile (2016-2020): A Case Study of Merken

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The potential ingestion of food products contaminated with OTA is cause of concern for public health. OTA, is classified by the IARC as a Group 2B substance given its carcinogenic potential. The objectives of our study were to 1) quantify and compare the OTA levels in merkén (mixed spice) with paprika and chili (single spices), and 2) estimate OTA exposure (EDI). In total, 319 samples (256 merkén, 39 paprika, and 26 chili samples) were collected in Chile (2016–2020). Analyses (OTA) was performed using a LC-FLD. The EDI value of OTA was calculated according to the average concentration (ngg-1) and the corresponding consumption rates (g day-1) and divided by the body weight (kg). Merkén: The total of 256 merkén samples analyzed a 66% (n=168) were contaminated with OTA. The number of positive samples (63–90%) remained stable from 2016 to 2019. In 73 samples, the OTA concentration exceeded the national OTA limit of 5 ngg-1. Interestingly, the highest OTA concentrations were present in 2016 (71.7 ngg-1) and 2017 (387.9 ngg-1). These concentrations were 4- and 25-fold higher than the international regulation of 15 ngg-1. Paprika: The highest detection frequency (100%) occurred in 2017 (5.7–297ngg-1). However, only 31% of samples (2017) had OTA concentrations that exceeded the international regulations. Chili: In 2017, the OTA detection frequency was 93% with a maximum OTA concentration (71.6 ngg-1). Interestingly, the OTA concentrations in five samples collected in 2017 exceeded the international limit of 20 ngg-1. The EDI values were considerably higher in 2017 than 2020 (paprika > chili > merkén). OTA is a common contaminant in single a mixed spice consumed in Chile. The EDI values were also lower than the Benchmark dose level (BMDL10; 4730 ngkg-1 day) for non-neoplastic effects.

1.10.P-Tu-051 Personal Exposure to Flame Retardants and Pesticides in Uganda and Costa Rica Using Silicone Wristbands

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Costa Rica and Uganda are both tropical countries, and agriculture in these nations plays a crucial role in their economies, export crops being significant sources of revenue. Uganda, with its customs of small-scale farming, and Costa Rica, which is renowned for its leadership in conservation and intense tropical agriculture, are both recognized internationally for their rich ecosystems in biodiversity. However, the reliance on pesticides used in agriculture raises concerns about potential chemical exposure to humans and biodiversity. In this exploratory study, human exposure to Polybrominated biphenyl ethers (PBDEs), Current Use pesticides (CUPs), and organochlorine pesticides (OCPs) were assessed in two population groups in Uganda and Costa Rica using silicone wristbands for the first time. Participants in Uganda (n=39) and Costa Rica (n=54) wore for five days silicone wristbands, which were then analyzed for 21 PBDEs,11 BFRs, 21 OCPs, and 58 CUPs and their metabolites. The most commonly detected chemicals in both groups were BDE-209, DBDPE, Diazinon, and B-HCH among PBDEs, BFRs, CUPs, and OCPs, respectively. Median concentrations of 21 PBDEs were significantly higher in Uganda than in Costa Rica. In contrast, 11 BFRs, 21 OCPs, and 58 CUPs were significantly higher in Costa Rica compared to Uganda. Most individual chemicals showed higher concentrations in samples from Costa Rica than in those from Uganda. This may be a result of the extensive use of pesticides, rapid urbanization, and industrialization occurring in Costa Rica, implying a potentially greater risk of adverse health effects associated with these

chemicals; conversely, results reflect that Uganda is characterized as an "agriculture-based" country dominated by small scale farms. Standardization of protocols for silicone wristband use is an ongoing need. This study highlights the potential of wristbands as a tool for capturing regional differences in human exposure to a wide range of flame retardants and pesticides, indicating their utility as personal exposure monitors for globally focused studies.

1.10.T Emergent Environmental Issues and Perspectives in Latin America

1.10.T-01 Bioassays with Allium cepa for the Monitoring of Toxicity in the Groundwater of Yucatan, Mexico

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Groundwater, Yucatan's sole drinking water source, is highly susceptible to contamination due to its porous karstic geology. This study employed the Allium cepa (onion) bioassay to evaluate toxicity and the potential toxic effects of groundwater pollutants in the Yucatan aquifer. Seven monitoring wells were studied during September and October 2021. The study also involved quantifying pesticides, polycyclic aromatic hydrocarbons (PAHs), nutrients, and the anthropogenic marker caffeine to correlate with the bioassay results. The physicochemical characteristics of the sampled wells revealed that site P2, located in Chuburna, exhibited statistically significant differences from the rest of the sites, attributed to higher pH and dissolved oxygen concentrations. Nutrient concentrations also showed significant variation between sites, with samples closer to the coast (P3 and P7) presenting higher ammonia and phosphate concentrations. Twenty organochlorine pesticides and Chlorpyrifos were detected in the study zone. The pesticides found at the highest concentration were d-HCH and Chlorpyrifos, with 141.44 and 175.92 ng/L, respectively. Lindane, Heptachlor, Aldrin, and DDT metabolites are regulated in NOM-127-SSA-2021, which includes the permitted values of pollutants in water for human use or consumption. In the present study, none of the sites had values above this threshold. Heptachlor and Aldrin were only present in sites P4oct and P2sept. Interestingly, DDT values were highly correlated with caffeine concentrations. This result may indicate that this pesticide may be linked to domestic discharges. Acenaphthylene, acenaphthene, fluorene, phenanthrene, and the sum of B(k)fluoranthene and B(b)fluoranthene presented the highest prevalence. B(k)fluoranthene and B(b)fluoranthene were the PAHs found at the highest concentration. The results of the *A. cepa* bioassay indicated no nuclear abnormalities. The study also found no statistical differences in the mitotic index, root length, biomarkers of oxidative stress, and inhibition of B-esterases between sites and controls. In summary, the low concentrations of contaminants, which can be used as a proxy of anthropogenic discharges, and the lack of effect in the biomarkers used at organism, cellular, and biochemical levels indicated no toxic impact on *A. cepa* roots in the monitored wells. This study was financed with UNAM-DGAPA-PAPIIT grants IN203321 and IN211724.

1.10.T-02 Neurobehavioral Effects of *Danio rerio* Larvae Exposed to Water from Madin Dam

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Water is essential for life, forming the major part of the composition of most living organisms. Therefore, it is crucial to protect it from any threat that could jeopardize its safety. The Madín Dam, located in the municipalities of Atizapán de Zaragoza, Naucalpan de Juárez and Tlalnepantla de Baz, in the State of Mexico, is a vital source of drinking water for more than 20 million inhabitants in the Valley of Mexico. Metals, pesticides, personal care products, pharmaceuticals, and persistent organic pollutants have been detected in this reservoir. These contaminants threaten the reproduction, growth and survival of aquatic species, as well as their behavior. One of the organisms that have demonstrated high sensitivity in neurobehavioral biomarkers is the zebrafish (Danio rerio), which can be an excellent bioindicator organism in ecotoxicological studies, particularly in early stages of development. The hypothesis proposed is that contaminants in the water of Madin Dam can alter the behavior of Danio rerio larvae. To test this hypothesis, water samples were taken from five sampling points near the main sources of contamination. Subsequently, larvae 7 days post-fertilization were exposed for 24 h in fish tanks of 4 L capacity with constant oxygenation, and lethality, acetylcholinesterase (AChE) activity and the behavior of the organisms were assessed. AChE was measured using the commercial Acetylcholinesterase Activity Assay kit (MAK119-1KT), Sigma-Aldrich ®), and behavior by means of locomotor activity, recording the distance traveled after being stimulated with light and darkness during 25 minutes (cycles of 5 minutes of habituation, 5 minutes stimulated with light and finally 5 minutes of darkness, twice) using Danio Vision (Noldus). The study was performed using 150 larvae for each sampling point in triplicate. The results obtained show an increase in the frequency of mortality, particularly at sampling point 5, in addition to a reduction in the locomotor activity and AChE of the organisms exposed to the water at all sampling points, when compared to the control, suggesting that the pollutants in the reservoir have a negative impact on their neurological function.

1.10.T-03 Microplastic Pollution in Groundwater of Two Rural Communities of Tlaxcala, Mexico

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The present study evaluated the amount and types of microplastics (MP) in ten wells where water is extracted for human consumption and domestic activities of two rural communities of the State of Tlaxcala: San Diego Xocoyucan and Ixtacuixtla, Mariano de Matamoros, México. For groundwater sampling, the possible aerial deposition of microplastics was considered; in each well, two samples were taken, one of surface water (from the first 5 cm of depth) and another of water without contact with air; the last one was obtained with a fountain pump submerged into the deepest part of the wells. In both cases, samples were filtered *in situ* with a number 35 sieve. Ten samplings were carried out from February to November 2023, which includes the dry and the rainy seasons. The average abundance of microplastics was 0.34 MP/L, and there was no difference between the communities. The MPs measured 2,100 to 4,400 µm in length, averaging 2,995 ± 437. 100% of the MP found were fragments. Regarding color abundance, black > white > blue > green > gray coincided in both communities. The polymers identified with FTIR spectroscopy were polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET). Seasonally, there were no differences in the abundance of MP by aerial deposition. However, the groundwater deep in the wells presented a statistical difference with the highest abundance in the dry season (Ixtacuixtla 0.49 MP/L; Xocoyucan 0.47 MP/L), which indicates that rainwater dilutes this pollution (Ixtacuixtla 0.36 MP/L; Xocoyucan 0.34 MP/L). Also, the number of MP through aerial deposition was statistically lower than in deep well water. The communities of San Diego Xocoyucan, Tlaxcala, and Ixtacuixtla de Mariano Matamoros, Tlaxcala, generate domestic, agricultural, and industrial waste. Even more, urban solid waste and wastewater are not efficiently managed. All of the above generates plastic pollution in the region and could be the origin of the MP groundwater pollution observed.

1.10.T-04 Presence of Current-Use Pesticides in Surface Waters of the Agricultural Pampa Region of Argentina and Accumulation in Fishes

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Extensive row crop agriculture is largely predominant in the Pampa region of Argentina, where most of the land is dedicated to pesticide-dependent genetically-modified soybean, corn and wheat crops. Diffuse contamination by pesticides is common in the region, and a variety of pesticide mixtures have been previously reported in surface and ground waters. In the present study, four sites were sampled every month from September 2021 until July 2022 along the Pergamino River (Buenos Aires Province, Argentina) to evaluate the nature and the scale of the pesticide contamination. The presence to pesticides in the body of four species of fish (*Cnesterodon decemmaculatus, Jenynsia multidentata, Bryconamericus iheringii, Corydoras paleatus)* inhabiting this river was also determined in November 2021 and March 2022. Forty-four different pesticide molecules were detected in surface water samples. A complex mixture of pesticides being detected at every sampling date. Frequently detected pesticides included atrazine, glyphosate, clothianidin, triadimefon, tebuconazole, cyproconazole and azoxystrobin which were present in more than 50% of the water samples. Furthermore, all four species of fish sampled contained between 20 to 30 different pesticide molecules in their bodies. Most of the pesticides that were frequently detected in water were also present in fish tissues. The potential impacts of these findings on the health of the fishes are evaluated by examining a series of biomarkers and body indices. Overall, these findings provide strong evidence that surface waters of the agricultural Pampa region are contaminated by a complex mixture of current-used agricultural pesticides, and that these are accumulating in fish and the food chain.

1.10.T-05 Endocrine Disruption in Chilean Rivers: Balancing Science and Regulation

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For the past two decades, industrial and urban wastewaters have been implicated in endocrine disruption within Chilean rivers. Despite this recognition, little action has been taken to establish regulations for both wastewater treatment and the protection of receiving water bodies. The main challenges stem from the unidentified chemical compounds responsible and the dependence on specific chemical thresholds for regulation. Additionally, stakeholders have shown limited awareness of this environmental issue. One potential solution is to explore the intricate relationship between endocrine disruption and the broader climate and biodiversity crises—a topic that carries political sensitivity. When individual chemical identities are poorly characterized or when mixtures of chemicals contribute to observed endocrine disruption effects in fish, an effects-based assessment becomes valuable. Such an approach focuses on the effects rather than specific chemicals. Chilean rivers exhibit varying fish distributions, with higher species richness from the Andes to the sea. Notably, the Biobio River stands out as a hotspot of native fish diversity in the country. Unfortunately, it faces multiple challenges due to urban and industrial wastewaters along its course. Remarkably, the Biobio River serves as a crucial water source for over one million inhabitants near Concepcion, Chile's second-largest city. Our research has gathered evidence of endocrine disruption across different levels, ranging from molecular mechanisms to population impacts. Now, the critical task lies in translating this scientific knowledge into actionable regulatory measures for safeguarding freshwater ecosystems. One promising avenue is the implementation of an Environmental Effects Monitoring Program, modeled after Canada's EEM framework. However, our efforts have encountered obstacles, including insufficient stakeholder engagement and gaps in our understanding of Chile's freshwater environment. In summary, bridging the gap between science and regulation requires a concerted effort—one that considers effects-based assessments, stakeholder involvement, and a deeper understanding of our unique river ecosystems. Supported by ANID/FONDAP/ 1523A0001 and Millenium Scientific Initiative ICN-2019-015.

1.10.T-06 A New Regulatory Paradigm for Pesticide Registration in Brazil: Comments on Recent Legislative Amendments (Law 14.785/2023)

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Environmental risk assessment (ERA) schemes in Latin America and preliminary discussions on the adoption of ERA guidelines in Brazil. Up to December 2023, the assessment of pesticide-related risks to environmental and human health within Brazil was predominantly executed through a hazard classification system—it is important to make clear that the classification did not imply nonregistrability. Despite the absence of local requirements before 2023, in the last 3–4 years, IBAMA has been smoothly moving to an ERA approach, mainly regarding new ingredient actives submission, evaluating several compartments such as aquatic organisms, birds and mammals, soil organisms, and pollinators. Except for the latter case, none have local published ERA guidelines. The approval of new Law 14.785 has the potential of bringing Brazil closer to the regulatory science in place not only in developed countries but also in some of its neighboring countries. New risk assessment schemes must be developed to enable the regulatory agencies and registrants to perform ERAs for several ecological compartments, such as aquatic organisms, birds and mammals, soil organisms, and reptiles and amphibians. In the latter case, Brazil may be among the first countries to require an ERA for this taxon; the European Union is still discussing guidelines for reptiles and amphibians Collectively, these regulatory changes may signify a major challenge since potentially new ecotoxicological studies may be required (e.g., ecotoxicological studies with reptiles and amphibians), modeling tools need to be adopted and adapted to local scenarios, and data gaps need to be addressed to provide the necessary local input parameters (e.g., weather data, soil, etc) to be used in the modeling tools. The newly established paradigm constitutes a significant transformation within Brazil's technical and regulatory landscape. This shift heralds the potential requirement for environmental research involving novel species. As previously delineated, implementing standardized ecotoxicological tests for local and new species is financially and temporally demanding, escalating the complexity of study design, execution, and subsequent interpretation. These factors suggest that ERA could be a powerful tool in bolstering Brazil's sustainability objectives, acknowledging that sustainability is an amalgamation of environmental, societal, and economic aspects.

1.10.V Emergent Environmental Issues and Perspectives in Latin America

1.10.V-01 Drought Management in Mexico: 2020-2024

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Mexico is a country that has suffered from droughts since ancient times, however, in the last three decades, these have intensified, recording significant increases both in their duration and in the affected areas. (CONAGUA, 2001; CENAPRED, 2007). On a global scale, droughts are also more frequent, and the countries that suffer them endure great economic losses that condition migration to other regions of the country and even their abandonment, thereby causing loss of labor force (UNESCO, 1979). Based on the statistics of the drought recorded in Northern Mexico at the end of the 20th century, these affected large areas of cultivation, and substantial losses of livestock, as well as the decrease in the availability of fresh water necessary for industrial processes and domestic use. By the end of May 2023, 70% of the national territory in Mexico registered moderate to severe drought conditions, mainly affecting the Central and Western regions of the country. However, historical data on national droughts refer to the period 2011-2013 as the most relevant in terms of intensity and extent. In relation to the current scenario, the persistence of dry to severe drought conditions over the last 5 years stands out, forecasting a compromising scenario for the availability and recovery of water resources, as well as for agricultural activities in the affected regions. It is important to highlight that the causes of the national drought recorded mainly in the winter and spring months were due to the presence of the climatic phenomenon known as El Niño-Southern Oscillation (ENSO) and its cold phase or "La Niña". The development of the rainy season in the face of this phenomenon is favorable with abundant summer rains, but they did not have a positive impact on the recovery of water bodies in the region, such as Lake Chapala. Similarly, the health impacts due to water scarcity are significant in terms of dehydration, intestinal diseases, and thermal stress, especially for people living in large cities in "urban heat island" environments, where high temperatures have also been linked to mental health problems especially among the low-income population, making them more vulnerable to natural disasters and their consequences. In this paper, the synoptic situation for the study period 2020-2024 is analyzed, the impact on the availability and quality of water resources, records of heat waves and their impact on public health in highly built metropolitan areas.

1.11.P-We Environmental Issues in the Gulf of Mexico

1.11.P-We-008 In Vitro Assessment of Thyroid Hormone Agonists on Coastal Fishes Native to Louisiana

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Louisiana's vast coastal wetlands and estuaries provide vital nursery ground for many different fish species that support commercial and recreational fisheries and represent an important part of the state's economy and culture. Thyroid hormone (TH) signaling in fishes is related to many biological functions such as growth, development, and metamorphosis. TH signaling is a conserved process, regulated by a series of coordinated events involving several proteins. Pesticides, chemical additives, pharmaceuticals, and personal care products can antagonize the thyroid hormone receptors in early life stages of fishes, causing impaired TH signaling and disrupted metamorphosis. Metamorphosis is a vital biological process characterized by the transition from larval to juvenile stage of development and has a range of complexity in fishes, although there is no official classification. Disrupted metamorphosis may lead to increased mortality, decreased recruitment, and population decline of fishes that are important both economically and ecologically. Prior research on chemical TH disruption in fish has focused almost exclusively on the zebrafish (*Danio rerio*), a model species with subtle metamorphosis. Thus, it is unknown whether TH disrupting chemicals in mammals share these activities in fishes or if zebrafish represent the sensitivity of fishes with more complex metamorphosis. Using an *in vitro* thyroid hormone receptor transactivation assay, this study will assess a suite of chemical contaminants detected in U.S. surface waters with known TH disrupting properties in mammalian screening assays on six fishes native to Louisiana's coastal and estuarine habitats with varying metamorphic complexity. Specifically, HEK 293 cells will be transfected with the thyroid hormone receptor from gulf killifish (*Fundulus grandis*), sheepshead minnow (*Cyprinodon variegatus*), spotted gar (*Lepisosteus oculatus*), red drum (*Sciaenops ocellatus*), southern flounder (*Paralichthys lethostigma*), and American eel (*Anguilla rostrata*) and exposed to serial concentrations of selected chemical agonists. Results of this study will determine whether mammalian screening assays are predictive of TH disrupting properties among fishes and if species with more complex metamorphosis are more sensitive to chemical disruption and thus at greater risk.

1.11.P-We-009 In Vitro Assessment of Sensitivity to Thyroid Hormone Receptor Antagonism Among Native Louisiana Fishes

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Louisiana's coastal estuaries and wetlands adjacent to the Gulf of Mexico provide important nursery grounds for fish species that support commercial and recreational fisheries, an important aspect of the state's economy. Early life stages of fish in coastal nursery grounds could be uniquely susceptible to chemical contaminants capable of inhibiting thyroid hormone (TH) signaling. TH signaling is a highly conserved process controlled by a series of coordinated events, involving multiple proteins such as thyroperoxidase, deiodinase type 1 and type 2. Pesticides, chemical additives, pharmaceuticals, and personal care products can inhibit protein

activity in early life stages of fishes, causing impaired TH signaling and disrupted metamorphosis. Metamorphosis is a biological process characterized by the transition from larval to juvenile stage of development and has a range of complexity in fishes, although there is no official classification. Disrupted metamorphosis may lead to increased mortality, decreased recruitment, and population decline of fishes that are important economically and ecologically. Prior research on chemical TH disruption in fish has focused almost exclusively on zebrafish (*Danio rerio*), a model species with subtle metamorphosis. Thus, it is unknown whether TH disrupting chemicals in mammals share these activities in fishes or if zebrafish represent the sensitivity of fishes with more complex metamorphosis. A battery of *in vitro* assays representing multiple proteins involved in TH signaling will be used to assess a suite of chemical contaminants detected in U.S. surface waters with known TH disrupting properties in mammalian screening assays on six fishes native to Louisiana's estuarine habitats with varying metamorphic complexity. The present study will investigate antagonism of the thyroid hormone receptor (THR). Specifically, HEK 293 cells will be transfected with the thyroid hormone receptor from gulf killifish (*Fundulus grandis*), sheepshead minnow (*Cyprinodon variegatus*), spotted gar (*Lepisosteus oculatus*), red drum (*Sciaenops ocellatus*), southern flounder (*Paralichthys lethostigma*), and American eel (*Anguilla rostrata*) and exposed to serial concentrations of selected chemical thyroid hormone inhibitors. Results of this study will determine if mammalian screening assays are predictive of THR antagonism among fishes and if species with more complex metamorphosis are more sensitive to chemical disruption and thus at greater risk.

1.11.P-We-010 Comparing the Impacts of 'Forever Chemicals' on the Development of Sheepshead Minnow and Red Drum

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Per- and polyfluoroalkyl substances, also known as PFAS, or 'forever chemicals', are a class of nearly indestructible chemicals resistant to environmental degradation that are of particular concern in estuaries in the Gulf of Mexico. PFAS are extremely mobile in aquatic systems and are detected in both sediment and surface waters globally. As the profiles of PFAS contamination can vary depending on the discharge source, environmentally relevant concentrations significantly vary by region and current environmental conditions. Data are especially lacking in estuarine and marine systems, which is of particular concern as salinity can significantly impact the fate and transport of PFAS compounds. Therefore, we characterized PFAS in estuaries within Matagorda Bay, Texas and quantified abundance to determine environmentally relevant concentrations for laboratory testing. Our data show the presence of various PFAS, including PFOS, PFNA, and PFDA. To assess the impacts of these PFAS on local species, we assessed the effects of PFAS on both sheepshead minnow (*Cyprinodon variegatus*) and red drum (*Sciaenops ocellatus*) to quantify differences in sensitivity across development. Embryos from both species were exposed to environmentally relevant concentrations of PFOS, PFNA, and PFDA and assessed for a suite of morphological parameters at hatch. These data will support risk management based decision making in Gulf of Mexico habitats, as this study includes site-specific PFAS compounds and concentration ranges, as well as ecologically and recreationally important fishes.

1.11.T Environmental Issues in the Gulf of Mexico

1.11.T-01 Alterations in Eastern Oyster Crassostrea virginica Shell Formation by the Ocean Acidification Through Dysregulation of Ca2+-Related Signaling Pathways

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As a major bivalve species, Eastern oyster Crassostrea virginica plays essential ecological and commercial roles in the Gulf of Mexico (GOM). The calcareous shells of Eastern oysters are crucial for providing structural support and protecting their soft bodies from multiple environmental stress. Due to human activities, increased absorption of atmospheric CO_2 by the ocean is continuously threatening the Gulf's oyster aquaculture productivity. Ocean acidification (OA) disrupts the acid-base balance and alters the saturation state of $CaCO_3$, leading to reduced calcification rates and shell deformities in oysters. However, the genetic response of their shell formation to the OA and the precise signaling pathway still need to be fully understood. Using the C. virginica from southern GOM, an in vitro model is established with the oyster mantle cells, the major cells for the biomineralization process, to identify the effects of OA on the potential molecular pathways related to the bivalve shell formation. The intracellular calcium flux is visualized using the Fluo-4 Ca^{2+} indicator under the CO_2 exposure, and the dynamics of Ca^{2+} signals are correlated to the upregulation of the Ca^{2+}-binding proteins, such as calmodulin and calcineurin, which play important roles in several signaling pathways of bone generation in vertebrates. This study also analyzed different oyster larval developmental stages under a mimic acidification environment. The expression profiles of several biomineralization-related genes in the Wnt, TGF-β, and BMP signaling pathways showed significantly different responses to the acidification condition before and after the metamorphosis process during oyster larval development. It is suggested that more than one molecular pathway may regulate the biomineralization process in oysters, and their responses to ocean acidification may vary by the oyster life cycle. To further investigate the role of Ca^{2+}-related signaling pathways in the biomineralization process and their responses to the OA, mutated oyster strains will be generated, and shell formation behavior will be evaluated under acidification conditions. The findings of this project will provide reliable references and a selective breeding strategy for the aquaculture and restoration of shellfish to mitigate the challenge from OA along the GOM in the future.

1.11.T-02 Photodegraded Pyrene and Anthracene Increase Mortality and Induce Stress Responses in Eastern Oyster (*Crassostrea virginica*) Larvae and Adult Stages

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The presence of polycyclic aromatic hydrocarbons (PAHs) in the Gulf of Mexico originating from the incomplete combustion of petroleum compounds poses a considerable toxicity hazard to both human health and the organisms of the nearshore bays. While PAHs are toxic to many different organisms, differential photodegradation by sunlight evolves quinones and other compounds that are potentially more toxic than the parent PAHs. Exposed to these pollutants in boat harbors and bays receiving runoff is the eastern oyster (*Crassostrea virginica*), a valuable aquaculture resource of both the East Coast and the Gulf of Mexico. Compounded abiotic environmental and toxicological stress could amount to considerable economic loss. Therefore, insight into the toxicological effects of photodegraded PAHs is especially relevant as of late. Pyrene and anthracene at 10 ppm were exposed to artificial sunlight using the Suntest CPS+ for 0, 1, and 2 hours, in which 0 hr represents unexposed PAHs. Meanwhile trochophore larvae were produced from strip spawning brood stock oysters obtained from the field site and treated at 0.1 ppm after 12 hours post fertilization for 24 hrs. In addition, medium-sized adult oysters collected from the field site in Corpus Christi, Texas were treated at 0.1 ppm for 4 days, and harvested for gene expression analysis, histological, and cell viability analysis. Exposure to anthracene in larvae resulted in the upregulation of the stress-related proteins *defensin* (*def*) and *heat shock protein 70* (*hsp70*) and a downregulation of *calmodulin A* (*camA*) trending toward longer photodegradation times. Exposure to pyrene in larvae resulted in significantly reduced survival after the 24-hr treatment period. Adults showed similar levels of upregulation in stress-related proteins to pyrene exposure and the examination of histological samples of gill and gonad tissue revealed reduced viability in treated specimens in comparison to those of the control group. Although the effects of photodegraded products and the parent PAHs did not differ significantly, these findings suggest that even degraded PAHs will continue to impart a toxic component to the nearshore bay ecosystems of the Gulf of Mexico.

1.11.T-03 Emerging (Nano(Micro)Plastics) and Persistent (Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls) Pollutant Body-Burdens in Oysters and Fish from Matagorda Bay

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Matagorda Bay is the second largest estuary in Texas, housing various petrochemical industries, including those producing plastics. In this study, we report the concentrations of 12 nano(micro)plastics (NMPs, ^{3}700 nm), 15 polycyclic aromatic hydrocarbons (PAHs), and 29 polychlorinated biphenyls (PCBs, including 12 dioxin-like PCBs) in the muscle and liver tissues of gafftopsail catfish (*Bagre marinus*), red drum (*Sciaenops ocellatus*), spotted seatrout (*Cynoscion nebulosus*), and in the gill/mantle of the eastern oyster (*Crassostria virginica*). Pyrolysis Gas Chromatography Mass Spectrometry (Py-GCMS) was used to quantify the polymers of NMPs, and GCMS was used for PAHs and PCBs quantification. Analysis of the total contaminant concentrations across all the biota showed that NMPs had much higher concentrations (2,000x - 30,000x) in comparison to PAHs and PCBs. PAHs ranged from 120 - 230 ng/g dry weight; and PCBs from 26 - 290 ng/g dry weight. In contrast, NMPs ranged from 355 - 8900 µg/g dry weight. Oysters were found to have higher contaminant levels in comparison to fish, as they exhibited 2-11x higher body burdens for PAHs and PCBs, and 5-25x higher body burdens for NMPs. Toxic equivalents (TEQ) based risk assessment for dioxin-like PCBs indicated the likelihood for adverse effects in fish. We also performed aquatic toxicity tests with representative single or mixtures pollutants exposing embryo-larval zebrafish (*Danio rerio*). Fish were exposed to specific pollutants and their concentrations as prominently detected in fish body-burdens. Taken together, we provide new information on the exposure of emerging and legacy pollutants in biota from Matagorda Bay and assess the likelihood for toxicity effects.

1.11.T-04 Comparing the Interfacial Interactions and Biological Impacts of Legacy PFAS with Next-Generation Replacements in Saltwater

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Research on per- and polyfluoroalkyl substances (PFAS) has largely focused on two C8 "legacy" compounds, PFOS and PFOA. Recently, work with regards to next-generation compounds (C6 and C4) has expanded. As the usage of shorter chained replacement compounds has increased in industrial and consumer applications, their detection in the environment has also increased, with some replacement compounds even outpacing legacy PFAS in terms of detection. Despite being developed with the intention of having a lower bioaccumulative potential, some next generation PFAS have been found to have longer half-lives and similar toxicity endpoints as the legacy PFAS they were designed to safely replace. Yet, there is a paucity of experimental data on the fate and toxicity of these shorter chained replacement compounds to accurately compare them against legacy PFAS, especially with regards to estuaries. Additionally, PFAS work has primarily focused on freshwater models; there are little data regarding marine systems and how abiotic conditions (i.e., salt content) affect the availability of any length of PFAS in the environment. Results of the present study address gaps in our knowledge regarding the effect of salinity on the partitioning behaviors of two legacy PFAS and two shorter-chained replacement compounds (PFHxS, and GenX), as well as how these compounds impact early life stage red drum (*Sciaenops ocellatus*) as individual compounds and as mixtures.

1.11.T-05 Comparative Toxicity of PFAS in Lab-Reared and Wild-Caught Sheepshead Minnow in Estuaries of the Gulf of Mexico

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Estuaries in the Gulf of Mexico in close proximity to industrial sites, military facilities, and urban centers are particularly vulnerable to contamination via runoff and discharge. A class of contaminants of particular concern are per- and polyfluoroalkyl substances (PFAS), which are widely used in manufacturing. PFAS are extremely mobile and persistent in aquatic environments, and are ubiquitously detected in sediment and surface waters globally – though data on PFAS fate and transport in estuarine environments are limited. Additionally, as profiles of PFAS contamination can vary depending on discharge source, environmentally relevant concentrations can significantly vary by region and depending on environmental conditions. Therefore, to fill this data gap, we collected environmental samples from two highly productive estuaries with significant industrial runoff along the Texas Gulf Coast to determine PFAS mixture profiles and environmentally-relevant concentrations of specific compounds to inform laboratory testing. We then tested the effects of two of the most commonly found PFAS in these estuaries, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), on early life stage sheepshead minnows (*Cyprinodon variegatus*) – an important prey species found within these estuaries – at the previously determined environmentally-relevant concentrations. We also compared these impacts on wild-caught and commercially-reared sheepshead minnows to assess the differences in sensitivity between these populations. We assessed morphological impacts related to fitness including size at hatch, and energetics (i.e., yolk size during embryonic and

larval stages). These impacts can result in changes to population structure within estuaries, as size-selection is the primary driver of mortality in early life stage fish.

1.11.T-06 Comparative Photo-Induced Toxicity of Tire Wear Particle Leachate on Two Fishes in the Gulf of Mexico, Southern Flounder (*Paralichthys lethostigma*) and Red Drum (*Sciaenops ocellatus*)

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Tire wear particles (TWP) that run off into aquatic habitats following storm events have been linked to recurrent fish kills in highly urbanized watersheds of the Pacific Northwest. TWPs leach a complex mixture of chemicals, including a subset of photodynamic compounds, that can become orders of magnitude more toxic in the presence of solar radiation. This is particularly important for transparent aquatic organisms that experience intense solar radiation, including biota within Gulf of Mexico (GoM) estuaries. The GoM provides critical nursery habitats for many recreationally and ecologically valuable fish species, with nearly all species having transparent larval stages. Data on the occurrence of TWP-related contamination within estuaries are currently limited, especially with regard to their potential to impact the survival and development of early life stage fishes via photo-induced toxicity. Here, we exposed both red drum (*Sciaenops ocellatus*) and southern flounder (*Paralichthys lethostigma*) to TWP leachate under ambient indoor lighting or simulated solar radiation (as UV-A). Studies were conducted in a fully factorial manner to quantify both survival and developmental morphology. We found significant differences in sensitivity to TWP leachate between species under ambient and UV-A conditions. We also found significant sub-lethal morphological impacts of TWP leachate in both species, though southern flounder were significantly more sensitive to TWP leachate compared to red drum. Overall, these data fill critical data gaps important for the protection of estuaries, especially in regions with increasing rates of urbanization experiencing high levels of solar radiation – such as the GoM.

1.12.A.T Exposure and Effects of Micro- and Nanoplastics in the Environment

1.12.A.T-01 Hazards, Accumulation, and Depuration of Synthetic and Natural Fibers Towards *Daphnia magna* and *Physa acuta*

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Microfibers from textiles are one of the most abundant anthropogenic particles found in environmental samples, with research suggesting most microplastics ingested *in situ* are microfibers. Microfibers released from clothing can be either natural or synthetic fibers, which may have different ecotoxicological profiles. Research has suggested that microfibers may be more toxic than fragments or beads due to their ability to penetrate gut walls and their slower excretion rates. However, there is limited information about hazards from natural and synthetic microfibers with even less information about uptake and depuration. The present study

investigated acute toxicity, uptake and depuration rates for natural (cotton and silk) and synthetic/semi-synthetic (kevlar, nylon, acrylonitrile) microfibers towards *Daphnia magna*, a water flea, and *Physa acuta*, a pulmonated snail. Both species were selected for their ease of culture, geographic distribution, and high reproductive capacity. *Daphnia magna* and *P. acuta* also have transparent bodies making it easy to identify accumulation. Microfibers were well characterized with fibers maintained in suspension counted to assess if uptake in both daphnids and snails was linked to fiber stability in test media. Species were exposed to the fibers in a dose-response format to identify lethal thresholds and if uptake rates were linked to exposure concentrations. Concentrations where uptake was reliably confirmed were used to determine uptake and depuration rates. A time series (0, 2, 4, 6, 8, 24, and 48 hours) was used to characterize uptake. For depuration, organisms were exposed for 48 hours, and depuration was monitored using comparable time increments. Toxicokinetics were analyzed for all microfibers using both mass and number of fibers present. Data collation is in process, but stability of microfibers was identified as an issue for *D. magna* uptake, and a stirrer was used to promote microfiber stability. *Physica acuta* use a radula for feeding and exposure was not contingent on microfiber suspension stability. Microfibers used in the study are transparent, making it difficult to identify retention in the body. Idye and idyePoly were used to stain the fibers after tissue digestion to facilitate counting. The study's results will provide a better understanding of how natural and synthetic fibers are retained by aquatic organisms and how retention may influence ecotoxicity.

1.12.A.T-02 Comparison of the Environmental Toxicity of Micro, Nano, and Leachate Fractions of Three Rubber Materials to *Danio rerio* and *Daphnia magna*

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Rubber materials enter the aquatic environment via anthropogenic sources including playground mulch, athletic fields, and roadway surfaces and break down into micro (<5 mm) and nano (<1 μm) sized plastics. Rubber particles can release chemicals into the aquatic environment, with a complex and variable composition depending on the type, source, and age of rubber. For this study, playground mulch recycled rubber (RR), turf field crumb rubber (CR), and tire rubber (TP) materials were cryo-milled, filtered, and separated into micro (1 – 20 μm), nano (<1 μm), and leachate (<0.1 μm) fractions. Particle counts were quantified using flow cytometry and Nanoparticle Tracking Analysis for micro- and nano-sizes respectively. To investigate the environmental toxicity of the three rubber materials, zebrafish (*Danio rerio*) embryos and water fleas (*Daphnia magna*) were exposed to nano, micro, and leachate rubber concentrations of 0 – 3.6 × 109 particles/mL, 0 – 1.29 × 106 particles/mL, and 0 – 100%, respectively. Embryos were evaluated for several behavioral, morphological, and developmental endpoints throughout a 120-hour exposure. Daphnids were evaluated for mortality and immobility after 48-hours of exposure. Nano-scale RR, CR, and TP particles elicited a hatch delay in zebrafish embryos with EC50 values of 1.4 x 109, 1.3 x 108, and 9.9 x 108 particles/mL, respectively. Leachate fractions of RR, CR, and TP particles elicited a hatch delay with EC50 values of 76.6, 103.4, and 95.5%, respectively. Micro-scale RR and CR particles elicited a hatch delay with EC50 values of 5.35 x 105, and 5.25 x 105 particles/mL, respectively. However, micro-scale TP did not elicit statistically significant effects in zebrafish embryos (ANOVA, $p < 0.05$). Nano-scale particles of all rubber materials resulted in an increased embryo hatch delay response compared to leachate and micro-scale particles, suggesting an adverse nano-particle effect not explained by chemical leaching. Daphnia RR micro and nano exposures resulted in mortality, with LC50 values of 9.8 x 105 micro-particles/mL, 5.0 x 108 nano-particles/mL. Leachate exposures did not elicit significant Daphnia mortality. Hatching delays and immobility pose an elevated risk of predation in the environment.

1.12.A.T-03 Accumulation, Depuration, and Potential Effects of Environmentally Representative Microplastics Towards *Daphnia magna*

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Microplastics pose significant environmental risks, specifically for aquatic organisms. There is a lack of research on how environmentally realistic plastics at the micro- (<5mm) and nanoscale (<1mm) impact aquatic organisms. The presence of biomagnification introduces an additional risk where microplastics accumulate along the food chain of aquatic ecosystems. Plastics collected in the natural environment present testing complications resulting in a lack of repeatability, so primary plastics, or plastics intentionally manufactured on the micro- and nanoscale, are often used in plastic research. This knowledge gap highlights the need for additional research into the adverse effects of environmentally realistic microplastics on aquatic organisms, given their prevalence in their natural habitat. The purpose of this study was to characterize toxicokinetics and hazard potential for environmentally realistic microplastics on *Daphnia magna*. Plastic cups and forks were micronized to <120mm, and five concentrations ranging from 0.01 mg/L to 100 mg/L were prepared in moderately hard water. Both types of plastic particles were confirmed to be polystyrene and similar in size ratios using Raman spectroscopy. *Daphnia magna* were exposed to these five concentrations for 48 hours, and mortality and MP accumulation were documented. The dose-response profiles were inconsistent with the increase in concentration because the microplastics did not establish a homogenous test suspension. The highest concentration of 100 mg/L was then used to test uptake and depuration presence in the individual organisms, where accumulation was confirmed to increase for up to 48 hours. Individual organisms were found to accumulate an average of 295.9 cup particles/organism and 227.44 fork particles/organism. The organisms depurated 95% of the uptake MPs. Although *Daphnia magna* greatly ingested the microplastics, an acute toxic effect was not observed. This suggests that further research is needed, especially for chronic exposure, to examine how environmentally realistic microplastic characteristics, like chemical additives and sizes, influence their accumulation and hazard potential on aquatic organisms.

1.12.A.T-04 Effects of Microplastics on a Freshwater Plankton Food Web in a Whole-Lake Addition Experiment

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Despite the ubiquity of microplastics in the environment, their fate and effects on aquatic ecosystems are largely unknown. At the International Institute for Sustainable Development - Experimental Lakes Area (IISD-ELA) in northwestern Ontario, Canada, a long-term study is being conducted involving three years of microplastic additions to an entire lake. Data collected post- microplastic additions are being compared to three years of baseline data from the same lake, as well as to a nearby unmanipulated lake using a before-after-control-impact (BACI) design. The planktonic food web, including phytoplankton, zooplankton, and their invertebrate predator, *Chaoborus,* is an important part of the overall ecosystem and affects overall water quality of the lake. These organisms are being monitored for changes in community structure (abundance, biomass, and species composition) resulting from the microplastic additions. In addition, zooplankton are being collected and analyzed for ingestion of microplastics at multiple timepoints to better understand the fate of microplastics in the food web. Based on the first summer of microplastic additions (2023), microplastics are being consumed by multiple zooplankton species, but few changes to the phytoplankton, zooplankton, *Chaoborus* community structures have been observed. As this experiment continues, we will gain a better understanding of the fate of microplastics in the food web and the impacts they have on aquatic organisms, both directly and indirectly. This whole-lake manipulation experiment is the first of its kind, and will inform industry and government level policies to mitigate plastic pollution.

1.12.A.T-05 Microplastic Translocation in Yellow Perch (*Perca flavescens*) and the Influence of Particle Size and Shape

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The fate of microplastics in aquatic ecosystems is a topic of growing concern. Of particular interest is the toxicokinetics of microplastics in organisms. Studies of wild-caught fish have definitively demonstrated the presence of microplastics in tissues beyond the gastrointestinal tract (GIT), suggesting microplastic translocation to other organs is occurring. Still, the mechanisms of translocation and the rate at which it occurs are not yet understood. We conducted a controlled in-lake mesocosm experiment exposing young-of-year yellow perch (*Perca flavescens*) to morphologically heterogeneous microplastic fragments, composed of three polymer types, polyethylene (PE), polystyrene (PS) and polyethylene terephthalate (PET), ranging in size from about 20µm – 1mm. Our objectives were to measure the presence of microplastics within the GITs, muscles, and livers of fish after exposure over a 9-week period

and assess patterns relevant to concentrations and morphologies across tissues. We hypothesized that we would observe particles beyond the GIT, and that these particles would vary from particles in the GIT in terms of size and shape. We predicted that microplastics with shorter lengths and narrower widths would be more likely to translocate, informing the mechanism of translocation. We quantified the number of microplastics in the GITs, as well as any translocated microplastics in the liver and muscle of experimental fish. Microplastics in samples were counted, categorized by polymer type and sub-sampled for particle size and shape measurements. Additionally, we used our data to estimate the rate of ingestion and translocation of microplastics into the muscle and liver of exposed fish. Our results suggest that shape and size likely influence a particle's likelihood of translocation as we observed significantly different microplastic shape and size distributions among tissues. Our findings demonstrate the importance of the use of heterogeneous microplastics in studies of both microplastic fate and effects, as well as elucidate the potential influence of particle morphology on the mechanism of microplastic translocation.

1.12.A.T-06 Assessment of Weathering and Fragmentation Rates of Plastic Debris in the Marine Environment

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The weathering and fragmentation of marine plastics are considered significant sources of micro and nanoplastics. However, the absence of standardized testing methods to simulate marine environmental conditions has hindered quantitative assessment. This study aimed to develop a method for estimating the weathering and fragmentation of plastics in the marine environment through accelerated tests. Furthermore, the study aimed to quantitatively evaluate the impact of stabilizers on the weathering and fragmentation processes of marine plastics. We developed an accelerated test method to assess the weathering and fragmentation rates of marine plastics. Accelerated weathering tests and outdoor exposure tests at ten locations in Japan were conducted. Fragmentation rates of plastic samples following accelerated weathering or outdoor exposure were quantified using fragmentation tests designed to replicate the physical effects of the marine environment. Subsequently, the acceleration factor of fragmentation at accelerated conditions was determined, and the accelerated weathering and fragmentation test was formulated. The test was then applied to four sample types: polypropylene (PP) and polyethylene (PE) film samples (thickness: 0.2 mm) and thick PP and PE samples (thickness: 4 mm). Film samples primarily underwent fragmentation into microplastics with diameters ranging from 300 µm to 5 mm, resulting from sample fracture. On the other hand, thick samples mainly generated those with diameters of 1–300 µm, attributed to surface abrasion. Both sample types produced nanoplastics (< 1µm). We also investigated the effects of chemical additives on the weathering and fragmentation rates of plastics. Weathering and fragmentation rates of PP and PE samples containing hindered amine light stabilizers were measured. The findings revealed that samples containing stabilizers exhibited reduced rates, approximately 0.5 times for PP and 0.1 times for PE, compared to samples without stabilizers. Finally, we extrapolated these results to estimate the generation rates of micro and nanoplastics from marine plastics at a beach in Japan. In this study, we quantitatively demonstrated the importance of the effects of shapes and chemical additive contents on the weathering and fragmentation rate of plastics. These factors should be considered in future studies to estimate the weathering and fragmentation of marine plastics.

1.12.B.T Exposure and Effects of Micro- and Nanoplastics in the Environment

1.12.B.T-01 The Role of Digestion on the Toxicity and Bioavailability of Micro- and Nano-Plastics in Fish: An In Vitro Approach

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Microplastics (MPs, 1µm-5mm) and nano-plastics (NPs, <1µm) (MNP) are a rising concern for environmental and human health. Ingestion is a critical route of uptake in humans and fish, which are significantly affected by MNPs. Moreover, MNP can act as carriers of environmental pollutants. Because ingestion is the main route of MNP uptake, gastrointestinal digestion's role in MNP toxicity and the release of environmental pollutants should be investigated. Due to difficult accessibility, studies on the role of gastrointestinal physiology on the effects of MNPs toxicity and bioavailability are lacking. This research aims to use an in vitro digestion system (IDS) to mimic the digestion process of rainbow trout (*Oncorhynchus mykiss*). We hypothesize that the gastrointestinal processes affect MNP surface chemistry and adsorb cadmium (Cd). To test this hypothesis, we ran the IDS with and without food, with and without MNPs (polyethylene and polyvinyl chloride), and with and without Cd. Cadmium adsorption onto MNPs was evaluated during digestion by measuring the Cd concentration in solution over 48 hours. After digestion, fish intestinal cells, RTgutGC, were exposed to the digesta. Toxicity was evaluated in RTgutGC cells using a multiple-endpoint viability assay measuring metabolic activity, cell membrane integrity, and lysosomal integrity. Co-exposure studies showed that the concentration of Cd dropped by ~30% when food was present and dropped by ~40% when food and MNPs were present, suggesting that the Cd in solution is absorbed to the food surface and MNPs during digestion. RTgutGC cells can tolerate direct exposure only after removing the solids and enzymes through filtration. Although none of the treatments were acutely toxic, our studies show that the presence of food protects the cells against MNPs and Cd toxicity. Currently in progress is the development of a procedure to extract these micro- and nano-plastic particles from the fecal matter produced at the end of the digestion process to ascertain the physical changes made to these particles during digestion.

1.12.B.T-02 Effects of Single or Combined Exposure of Micro/Nanoplastics and Three Anthropogenic Chemicals on Larvae Zebrafish (*Danio rerio*)

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Micro and nanoplastics are found world-wide in all environments. Once in the environment, these synthetic particles are often ingested by organisms which can lead to adverse biological effects. Additionally, the environment is contaminated with man-made chemicals and drugs that can also have negative effects on organisms. Studies have demonstrated that plastic particles can modify the transfer of chemicals to organisms in the environment, which can alter their potential toxicity. We tested the hypothesis that different plastic particles could alter the transfer and

potential toxicity of three common chemicals found in the environment. Using zebrafish larvae, we evaluated the toxicity of each plastic type and each chemical, along with their combinations. The NRC Zebrafish Embryo Toxicity (ZET) test (OECD 236) and the NRC General and Behavioral Toxicity (GBT) test were used to assess three different sizes of polystyrene micro and nanospheres, along with triphenyl phosphate, a plastic additive, Tetrabromobisphenol A, a flame retardant, and fluoxetine, a selective serotonin reuptake inhibitor. Following exposure, morphological abnormalities were recorded, behavior analyses were performed and chemical uptake by the larvae was measured. The plastic particles measuring 0.42, 2.25 and 8.87 μm exhibited no significant toxicity at environmental concentrations (0.05 – 50 μg/mL). At the same time, no significant differences in behavior were observed when zebrafish were exposed to micro or nanoplastics. Phenotypic toxicity was observed for all three tested compounds with EC_{50} values between 0.8-1.8, 1.6-3.2, and 3.3-6.1 μg/μL for triphenyl phosphate, Tetrabromobisphenol A, and fluoxetine, respectively depending on the test used (ZET-GBT). Plastic particles did not modify the phenotypic or behavior toxicological endpoints of fluoxetine when plastics were added at their highest concentration (50 μg/mL). The effects of adding plastic with the chemical uptake by zebrafish larvae is currently being evaluated. This study is relevant as it is increasing the knowledge about the effects of concomitant anthropogenic stressors on freshwater fish, specifically during sensitive life stages and will improve the understanding of plastic particles as potential carriers of chemicals to organisms. Furthermore, this work's use in support of environmental risk assessment makes it relevant to this specific session.

1.12.B.T-03 Nanoplastic-Induced Behavioral, Reproductive, and Transcriptomic Disruption that Persists into Adulthood of Zebrafish Larvae

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Nano-plastic (NP) contamination is extensive throughout environmental waterbodies and drinking water sources. While microplastic (MP) targets have been well studied and include the endocrine system, the toxicity and associated health risks of large-scale human and animal exposure to NPs have not been well characterized. Thus, larval zebrafish were utilized to investigate developmental exposures to 50 and 200 nm polystyrene fluorescent NPs, specifically evaluating accumulation of plastics, behavioral response, whole-body transcriptomic outcomes, and adult and next generation effects. From 6 to 120 hours post-fertilization (hpf), zebrafish were exposed to a range of NPs (10-10,000 parts per billion), resulting in size and dose-dependent increases in NP accumulation in 120 hpf larvae. Exposures did not impact mortality, hatching rate, or deformities; however, exposure to higher concentrations of larger sized NPs (200 nm) altered larval swimming behavior, as evidenced by hyperactivity. Exposed larvae were reared to adulthood to assess reproductive and next-generation outcomes. At the highest concentration (10,000 ppb), adult F0 reproductive capacity was significantly diminished, and F1 offspring of 1,000 ppb-exposed fish displayed hyperactive behaviors consistent with that of their parents.
To uncover the pathways contributing to these persistent behavioral and reproductive developmental and adult transcriptomes, collecting pooled F0 whole-body larvae in addition to F0 adult brains and gonads from both sexes. Crucially, we found that endocrine system disorder,

organismal injury and abnormalities, and neurological disease were three of the top five enriched disease and disorder pathways disrupted in both larval and adult tissues at both 100 and 1,000 ppb exposure levels. Preliminary data analysis uncovered estrogen receptor signaling, endocrine system development, oxidative stress, inflammation, dysregulated metabolism, epigenetic modification, and hereditary disorder pathways across many tissue types and concentrations. Taken together, this data suggests that early life endocrine and neurological disruption by NPs can persist into adulthood and potentially future generations, as evidenced by NP accumulation, altered behavior, physiology, reproduction, and dysregulated transcriptomics. The uniquely broad scale of this study during a critical window of development provides crucial multidimensional characterization of NP impacts on human and animal health.

1.12.B.T-04 Nanoplastics Inhibit Adipose Tissue Development and Eco-Corona Alters Internalization Pathways in Preadipocytes

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The potential threat of nanoplastics to human health has raised concerns about this emerging environmental pollutant. Micro- and nanoplastic contamination has been identified in many food and water sources worldwide. While toxicological studies on nanoplastics have not yet been formally linked to severe health issues, the chronic effects on human tissues remain unclear. Adipose tissue is an important organ system, comprising approximately 1/4th of the total body weight in humans, it also houses adult stem cells that are critical for tissue regeneration and healing. Considering the hydrophobicity of many engineered polymers, adipose tissue, a dynamic organ, may represent a major target organ sort for plastic fragments, including nanoplastics. Previous experiments have suggested that nanoplastics do, in fact, accumulate in the white adipose tissue of mice. However, overall inconsistent effects of accumulated nanoplastics on lipid metabolism and adiposity are reported in the literature. Our study aimed to investigate alterations in adiposity induced by environmental nanoplastic exposure. Surface modifications of environmental nanoplastics alter their interactions with biological interfaces. To more accurately reflect environmental conditions, nanoplastics with diameters of 100 and 500 nm were incubated in natural water samples such that an environmental corona formed on their surfaces. These environmentally conditioned nanoplastics were then incubated with preadipocytes, progenitor cells of mature, differentiated adipocytes. We identified the pathways of nanoplastic internalization by preadipocytes and obtained images to colocalize the plastic particles with different organelles. Ultimately, we assessed how nanoplastics altered the differentiation of preadipocytes. We found that the internalization of nanoplastics by preadipocytes was dependent on the particle size. Notably, the surface corona significantly altered the internalization pathway of 500 nm nanoplastics in preadipocytes. Nanoplastics were primarily accumulated in and around the endoplasmic reticulum and mitochondria, key organelles for adipogenesis. We also found that intracellular nanoplastics significantly inhibited the differentiation of preadipocytes, potentially leading to reduced adiposity in humans. Given the widespread distribution of adipose tissue, compromised adipogenesis may lead to a range of negative health effects in exposed humans and animals.

1.12.B.T-05 Understanding the Impact of Plastic Additives on Microplastic Toxicity to Ammonia-Oxidizing Bacteria

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Nitrification is a key nitrogen cycle pathway in which ammonium is oxidized to nitrite and then nitrate, which is essential for nutrient bioavailability and denitrification in various environments. Recent studies have shown that some microplastics can affect the nitrification activities of sedimentary and wastewater-associated microbial communities. However, it remains unclear what role additive chemical content plays. We previously found that while unweathered polyvinyl chloride (PVC) microplastics inhibited the nitrification activity of the model ammonia-oxidizing bacterium *Nitrosospira* sp. AV, UV-treated PVC microplastics had no effect. To investigate the role of additive chemical content in this differential toxicity, we employed solid-liquid extraction and gas chromatography coupled to quadrupole time-of-flight mass spectrometry (GC-QTOF/MS) to extract and detect several organophosphate ester, phthalate, and other plasticizer additives in the PVC microplastics. We quantified several different plastic additives in the microplastics. We also observed that UV exposure altered the concentrations of some additives. Furthermore, we conducted an exposure experiment with *Nitrosospira* sp. AV to investigate the toxicity of one of the additives that was detected in the toxic unweathered PVC microplastics, but absent in the non-toxic UV-treated microplastics. Nitrification rates of *Nitrosospira* sp. AV cultures subjected to five different additive concentrations were measured to calculate the half-maximal effective concentration (EC_{50}) of the chosen additive. Our results showed that plastic additives may be important toxins to microbes involved in the nitrogen cycle.

1.12.B.T-06 Unveiling the Adaptive Potential of *Bacillus inaquosorum* (EC30O5B-F5) for Nanoplastic Degradation and Sustainable Agriculture

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Nanoplastic pollution has emerged as a global environmental crisis, necessitating innovative and eco-friendly solutions. We present a novel study that tackles nanoplastic pollution while harnessing its potential for sustainable agriculture. Our study explores dual-purpose nanoplastic bioremediators by integrating nanoplastic-degrading microbes into agriculture. We focus on a plant growth-promoting *Bacillus* species, *B. inaquosorum* (strain EC30O5B-F5), investigating its adaptability to nanoplastic-rich environments and plastic degradation capabilities. We previously showed that the native strain EC30O5B-F5 can utilize polystyrene nanoplastics as a carbon source, with a 1.6-fold growth observed in the presence of plastic before evolution. Now, we are aiming to ramp up the strain's polystyrene metabolism. Through experimental evolution, we exposed replicate evolutionary lines of EC30O5B-F5 to Luria broth with polystyrene nanoplastics over five transfers. Using the colony-counting technique, we observed a three-fold increase in population density, indicating successful adaptation and confirming that the increase was not an artifact. These findings suggest the potential of EC30O5B-F5 to adapt to and degrade nanoplastics. Intriguing light-cream colony morphologies emerged, distinct from normal morphotypes of this strain. Our ongoing research aims to isolate these novel morphotypes and assess their degradation abilities, potentially leading to more efficient bioremediation strategies. By unraveling the plastic metabolism in EC30O5B-F5, we aim to open up possibilities for

harnessing EC30O5B-F5 to tackle nanoplastic pollution while promoting sustainable agricultural practices.

1.12.P-We Exposure and Effects of Micro- and Nanoplastics in the Environment

1.12.P-We-011 Can Earthworms Detect and Avoid Plastics in Soils?

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Plastics have been found in every ecosystem in the world and it is estimated that a substantial amount of micro- and nanoplastics will end up in the terrestrial environment through aerial deposition, irrigation, tire wear particles and agricultural practices, including biosolid application, fertilizers and the use of agro-plastics such as plastic mulch. To understand if earthworms, a sentinel soil species, may uptake plastics in the environment, *Eisenia andrei* were exposed to field soil before and after biosolids application. Although microplastics were detected in both the biosolids and the field soil post biosolid application, there was no observed effect on earthworm survival, growth or reproduction. To determine if the earthworms would uptake microplastics, worms were exposed to artificial soil (to limit incidence of background plastic) in which polyethylene (PE) beads (38-45µm) were incorporated either in the soil or as part of the food. After 28d, earthworms were depurated, then frozen. Casts produced during the depuration were also frozen. Earthworm tissues and casts were digested in 10% KOH, then subjected to a density separation to allow any plastics to float and be collected for analysis. Samples were filtered and analyzed by Raman spectroscopy. Control worm samples in artificial soils with no plastics exposure confirmed no plastic contribution from the soil, laboratory, or processing. Earthworms that were exposed to plastics via the soil had no detectable plastic in the tissues, however there was plastic present in worms exposed via the food. To understand if earthworms were avoiding the plastics in the soil, an avoidance study was conducted in which worms were free to move between control (no plastic) and 2% PE contaminated soils. After 48hr 37% of the worms avoided the soil with plastic. Initial testing of field soils revealed a heterogenous distribution of plastics in soils amended with biosolids. We present evidence that earthworms may be able to avoid some plastics in the soil but will uptake plastics when they are part of their food.

1.12.P-We-012 A Meta-Analysis of Sorption Capacity of Microplastics for Organic Pollutants

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Large-scale production and consumption of plastic products over last few decades have resulted in the prolonged and sustained emission of microplastics into terrestrial and aquatic ecosystems. These particles play varying roles in influencing the fate and transport of environmental contaminants, in particular those of organic compounds. The released microplastics differ significantly across chemical structure, size, and aging history, and such heterogeneity represents a major challenge in assessing their quantitative environmental influences. This study examines to what extent the different structure, size, and aging history of microplastics matter with respect to their sorption / binding potentials for representative organic contaminants. Sorption isotherms and measurements were reviewed and obtained from primary experimental literature for more than 10 commonly investigated microplastics for 5 chemicals from close to 100+ independent studies. Microplastic-water partitioning data and the associated experimental conditions were carefully documented and examined. The collected partitioning data were analyzed with respect to both chemistry of the sorbates as well as structure, composition, and characteristics of the microplastics. A fraction the measurements may be flawed as they were obtained under problematic circumstances that included adsorption to vessel surface, insufficient equilibration time, and inconsistent mass balance. Sorption data generally suggested a high degree of chemical consistency across different studies or manufacturing batches of the plastics. While chemical aging or weathering will alter the structural characteristics of the microplastics, the final quantitative impact of such processes on sorption capacity of the plastics appeared rather limited (< 0.5 log unit). The possibilities of applying various simplifying assumptions towards chemical identity of the plastic were also examined and visualized. Overall, the results suggested that a more pragmatic approach may be adopted for assessing the environmental impact of mixed microplastic in various environments without the cost of incurring large degree of errors or uncertainties. The implications of the findings with respect to emerging productions of new plastics are highlighted and discussed.

1.12.P-We-013 Analysis of High Mass Polycyclic Aromatic Hydrocarbons (PAHs) Extracted from Microplastics Spilled in the Marine Environment

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In 2021 the container ship M/V X-Press Pearl caught fire and later sank off the coast of Sri Lanka. Over 1600 tons of polyethylene pellets, aka nurdles, were aboard the ship along with 25 tons of nitric acid which began to leak and is suspected to be the cause of the fire that lead to the sinking. To add to the witches brew of ingredients in this fire were fertilizers and explosives being transported and flame retardants deployed to douse the fire. When combined with abundant, naturally-occurring compounds found in the marine environment, the need arises to characterize the composition of intact, burned, and broken pieces of the spilled plastic later collected from kilometers of Sri Lankan beaches. Samples of relatively intact, burnt and aggregated combustion remnants of microplastics were extracted using a microextraction method involving sonication in DCM and the addition of multiple surrogates. Extracts were analyzed on a Xevo ™ TQ Absolute (Waters Corporation) tandem quadrupole system using N_2 CID gas and fitted with the Atmospheric Pressure Gas Chromatography (APGC ™) source in positive ion mode using charge exchange ionization and dry N_2 reagent gas. The focus of this work was on high mass PAHs (314 - 424Da, 6 - 11 rings). However, an overlapping range of PAHs (228 – 302Da, 4 – 6 rings) was included in order to facilitate comparison across techniques and with other published results. NIST Coal Tar SRM 1597 was used to perform method development as it was anticipated to include representative analytes. The final method using the HT column resulted in all analytes being eluted in <23 min while still providing satisfactory chromatographic resolution for the majority of high mass analytes and only requiring ramping the N_2 carrier gas to 3mL/min. Development of MRM transitions was based on extracting prospective high mass PAH molecular weights from multiple sources and using 1597 to optimize response for the most prominent peaks in each RT range. Subsequent quantification of the overlap region analytes found in 1597 were within 10.2% of the reference values for 8 of 9 analytes with coronene being an outlier (+58.9%) due to coelution. Combustion remnant and burnt samples contained >500x and >250x, respectively, of the levels of high mass PAHs found in white nurdles. Quantification of high mass PAHs show that white samples have <1%, burnt have 18.2% and CR has 40.7% by mass when comparing the cumulative response for all analytes detected in 1597.

1.12.P-We-014 Assessing the Microplastic Content of Biosolids and Agricultural Fields in Southern Ontario

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Microplastics (<5mm) are an emerging ecological concern. Primary microplastics are intentionally created for commercial use (e.g., microbeads for cosmetic products) while secondary microplastics are created via the degradation of larger plastic materials. Due to their persistence in the environment, the unknown effects of microplastics are a potential threat to the health of various ecosystems. To date, the overwhelming majority of microplastic research has focused on their impacts on aquatic ecosystems, creating a need for research to be performed on their effects on terrestrial ecosystems. One potential significant source of microplastics to agricultural soils are biosolids. Biosolids are nutrient-rich field amendments that are derived from the processing of wastewater. Biosolid samples were collected from fourteen wastewater treatment facilities. Additionally, soil samples were collected from thirteen biosolid-amended agricultural fields. Soil and biosolid samples were digested with Fenton's reagent and density separated with NaBr to isolate microplastic content. Microplastics were counted and categorized based on size, colour, and shape. Biosolids were found to contain 3.95×10^5 ($\pm 3.19 \times 10^4$) microplastics/kg (dry weight), while biosolid-amended fields contain 1645 (± 98) microplastics/kg (dry weight). On average, 32.8% of microplastics found within biosolids are identified as microfibres. Furthermore, microfibres comprise 22.8% of microplastics found in biosolid-amended fields. Microplastic characterization of biosolid samples identified polyester and acrylic as the two most abundant types of microfibre. In addition, HDPE, LDPE, and polypropylene were identified as the most abundant microparticles. Quantifying and characterizing microplastic content of biosolids and biosolid-amended fields is crucial for developing an accurate risk assessment of microplastics in terrestrial ecosystems.

1.12.P-We-015 Assessment of Microplastics Abundance and Distribution in the Alabama River System

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Rapid increase in plastic pollution has received increased attention since the past decade. Plastics find their way to enter the aquatic ecosystem and remain there for hundreds of years. Fragmentation of plastics occurs with time that converts them into micro-sized fragments and fibers called microplastics (MPs). Studying the abundance, distribution, and transportation of microplastics in water systems is necessary for evaluating potential risk and impacts of MPs in the natural ecosystem. A significant number of studies have been carried out in different countries to assess the presence of microplastics in river waters and sediments. However, limited studies on the microplastics pollution in river systems of the US have been conducted. This study is designed to evaluate the abundance and temporal and spatial distributions of MPs in the Alabama River. Five sites from the upstream to lower stream of the river were selected for sampling and 4 seasons (summer, fall, winter and spring) were assessed for MPs in the surface, middle column, and bottom column of Alabama River water. In addition to MP abundance, various physical (e.g., size, shape, density) and chemical properties (e.g., type of polymer) of MPs were characterized. Microplastics with different types, sizes, and colors were found in the Alabama River water. Among the five sites, MP abundance was highest in Montgomery site and summer season (454 particles/L) and lowest in Dixie Landing site and summer season (76 particles/L). These MP concentrations are comparable to the MP concentrations that have been reported for some polluted rivers in the world. Except for Mobile site, the highest MPs abundance was found in surface water as compared to water in the middle or bottom water column. Black and white microplastics were found to be the dominant colors and PET, PE, and PP were the dominant polymer types. This study is important and will serve as a benchmark for microplastics' presence in the Alabama River water that can be used for evaluating potential risk and impact of MPs in the ecosystem of the Alabama River.

1.12.P-We-017 Effects of Particle Dimensions on Microplastic Toxicity to Marine Invertebrates

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Microplastics exist in the environment in various particle dimensions (e.g., polymer type, particle size, and shape). How the particle dimensions drive the toxicity of microplastics to ecological species has been identified as a major data gap that needs further research. A meta-analysis is conducted to evaluate the effects of particle size, shape, and polymer type of microplastics on the organism and population-level subchronic/chronic toxicity endpoints to marine crustacean and mollusk species. We compile controlled toxicity experiments on the target species and endpoints from the literature which reported particle dimension parameters of microplastics used in the testing, with a focus on review papers on this topic. Dose-response analyses are performed with the compiled data set for each particle shape (e.g., fiber, fragment, sphere), polymer type (e.g., polyethylene [PE], polyethylene terephthalate [PET], polystyrene [PS]), and particle size to compare the impacts of these parameters on dose-response relationships and derivation of effect concentrations. Finally, depending on the data availability and quality, a quantitative and/or qualitative comparison of ecological impacts between weathered and non-weathered microplastic particles will be discussed.

1.12.P-We-019 Emission and Ecological Risk of Microplastics from Mechanical Recycling of Plastic Waste

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The increasing production of plastic products and generation of plastic waste have had increasingly negative environmental impacts. Although recycling could reduce plastic pollution, microplastics can be generated during the process of crushing plastic products during mechanical recycling. In a previous study, we found that microplastics are unintentionally generated at extremely high concentrations and released to the aquatic environment during the mechanical recycling of plastic wastes at facilities. Other studies of plastic recycling facilities have reached similar conclusions. These studies have suggested that plastic recycling facilities may be major point sources of microplastic pollution globally. However, there has been no scientific approach to a quantitative evaluation and ecological risk assessment of the generation and environmental discharge of microplastics during mechanical recycling of plastic waste globally. We conducted crushing tests with 13 different plastics and documented the size distribution of particles generated. We then estimated the discharge of microplastics associated with recycling and their removal in wastewater treatment plants. We estimated that the global discharge of microplastics would increase from 0.017 Mt in 2000 to 0.749 Mt in 2060. Although mechanical recycling was estimated to account for 3.1% of the total emissions of microplastics for 2017, discharges of microplastics from plastic recycling may increase, even if plastic pollution from well-known sources decreases. Non-OECD (Organization for Economic Cooperation and Development) Asia could be a major discharging region and would play a vital role in reducing discharges of microplastics. Preliminary ecological risk assessment suggested the potential ecological risks of microplastics from and around plastic mechanical-recycling facilities for aquatic organisms. Reduction of the discharge of microplastics will require less use of plastic products and upgrading wastewater treatment in many countries.

1.12.P-We-020 Growth Inhibition in Common Carp by Dietary Administered Virgin or Recycled Polypropylene Microplastics

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This study investigated the effects of virgin and recycled polypropylene (PP) microplastics (MPs) on fish growth, focusing on potential differences in impact and threshold levels based on contaminated chemicals and particle size. *In vitro* bioassays were conducted to measure the cytotoxicity and agonist activity of some receptors in extracts of virgin and recycled PP pellets. The pellets were then cryogenically ground and sieved through 300 µm and 106 µm meshes to obtain MPs with two different size distributions (<106 µm, 106–300 µm). Fish exposure tests were conducted by adding MP into the diet at arbitrary rate and feeding the fish for four weeks. During the experiment, the total weight of fish in each tank was measured weekly to monitor biomass gain and feed efficiency, and the feeding amount was recalculated. In Test 1 and Test 2, 10% of each size of recycled or virgin MP was added to the diet, respectively. In Test 3, <106 µm virgin PP was added at 2.1 or 4.5% to the diet. In each test, the MP-contaminated diets were fed at 3% of fish body weight per day. At the end of the experiment, the body length and weight of each individual were measured, and blood samples were collected. Total blood cell count, hemoglobin concentration, and hematocrit values were measured. Additionally, the intestinal tissue of some individuals was observed. The extracts from recycled PP showed relatively high agonist activity for receptors such as AhR and PXR, although cytotoxicity was low. In contrast, no biological activity was detected in virgin PP. When fish were exposed to these MPs, both virgin and recycled PP at 10% in the diet for the <106 µm size significantly reduced feed efficiency and suppressed biomass gain. For 106–300 µm MP exposure, these effects were weaker for virgin PP and not observed for recycled PP. Further exposure to lower concentrations of <106 µm virgin PP revealed a lowest-observed-effect level and no-observed-effect level for fish growth inhibition of 1.4 and 0.62 mg/g-b.w./day, respectively. No hematological effects were observed in any of the tests. These results suggest that while recycled PP contained several chemicals, the toxicity of MPs was similar between virgin and recycled PP. Additionally, smaller MP particle sizes exhibited stronger toxicity. This is likely due to the increased number of particles for smaller sizes at the same weight-based concentration, which can be largely explained by the total surface area (or total volume).

1.12.P-We-021 Isolation and Characterization of Microplastics from Cosmetics and Its Effects on *Artemia salina*

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Micro and nano plastics are commonly found in a wide range of daily used consumer products, with cosmetics being particularly notable for their prevalence. Microplastic beads used in cosmetics are one of the major sources of microplastic pollution in the environment. The commonly used synthetic polymers in microbeads include polyethylene, polyethylene terephthalate, polypropylene, polymethylmethacrylate and nylon. Normally, microbeads often escape wastewater treatment plants and pass into treated water systems and water bodies. Cosmetics industries have been using microplastics as a scrubber material and these wash through drains and ultimately pollute the aquatic environment which in turn affects the aquatic organisms. Therefore, the objective of this study is to isolate and characterize Polyethylene microplastics derived from cosmetic product, specifically from face wash (FW) and examine its effects on aquatic organisms (*Artemia salina*). Our work mainly aims to identify and examine microplastics (MPs) generated by cosmetics via FTIR and FESEM analysis, and then use biochemical tests like reactive oxygen species (ROS) formation and antioxidant enzyme activities to determine whether these MPs are hazardous to *Artemia salina*. To study the effect of MPs on aquatic organisms, we have chosen *Artemia salina*, commonly known as the brine shrimp which is a filter-feeder organism and protein source for higher trophic species that can adapt to a wide range of salinities. FTIR analysis confirms the presence of Polyethylene microplastics in face wash. FE-SEM images determine the morphology, size and shape of this isolated microplastics. According to the findings from the FE-SEM results, the beads had a size of 660 µm. The concentration of protein in treated *A. salina* nauplii decreased relative to the control group throughout time. At 120 hours, the reduction in protein content was at its peak in FW-MPs (59.85±3.35%). Lipid peroxidation was found to be significantly higher in *A. salina* compared to the control groups as the time increased.

1.12.P-We-022 Leaching Behaviors of Antioxidants from Low-Density Polyethylene Microplastics in Pure Water Under Simulated Solar-UV Irradiation

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Microplastics (MPs) are defined as plastics with sizes between 1 mm and 5 mm. MPs draw particular environmental and societal concerns because their small sizes allow them to be ingested by organisms, bioaccumulated in the food web, and transported over large time and space scales. Therefore, MPs have broad impacts on the global aquatic ecosystem and are among the top ten environmental problems. Plastic additives, such as plasticizers, UV stabilizers, antioxidants, and flame retardants, are added to plastic materials to enhance polymer physical performance and longevity. They are not chemically bound with plastics and can leach out when the plastics enter the aquatic environment. Solar exposure is thought to promote the leaching of plastic additives due partly to photofragmentation, which increases the specific area of the plastic particles. Photodegradation thus not only affects the ecotoxicology of the MPs themselves but also that of the associated additives. However, the leaching behavior of common antioxidants from photodegradable MPs remains poorly understood. Low-density polyethylene MPs were selected as the target for this project because they are commonly used in daily life and are the most detected in the environment. Two antioxidants, Irganox 1076 (2660.77 ng/mg) and Irgafos 168 (19.85 ng/mg) were detected in the virgin MPs made of shredded food storage bags; UV stabilizers and plasticizers were not detected. However, only very low levels of antioxidants (Irganox 1076, < 438.24 ng/mg; Irgafos 168, < 0.55 ng/mg) were detected in both the leachates and the MPs during long-term (1 to 8 months) incubations in pure water under both solar UV-simulated radiation and dark conditions. This suggested the majorities of the antioxidants in the MPs were leached out and further transformed to secondary products within the incubation periods. Currently, we are investigating the kinetic profiles of the antioxidants and their transformation products in both the leachate and the MPs during short-term dark and light incubations (hours to days). We are also characterizing the potential alterations of physicochemical properties of the MPs during the incubations using Fourier-transform infrared spectroscopy, elemental analyses, and scanning electron microscopy. This study will improve the understanding of leaching behaviors of antioxidants from polyethylene MPs with and without solar irradiation.

1.12.P-We-023 Micro- and Nanoplastic Contamination in Tiger Shark (*Galeocerdo cuvier*) Blood from the North Atlantic and South Pacific Oceans

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The tiger shark (*Galeocerdo cuvier*) is an apex predator and ingests an array of food and non-food items including microplastics and other anthropogenic materials. Previously, we examined the stomachs and spiral valve of seven sharks captured in the Western North Atlantic Ocean. All previously examined specimens contained microplastics (>45 μm) and other anthropogenic particles, with polypropylene (32%) being the most common polymer. Concentrations in the stomachs were relatively high, compared to other fishes, indicating the need to further

investigate the presence of plastic particles beyond the stomach and spiral valve in this species. To explore the fate of microplastics and nanoplastics in tiger sharks, blood samples were acquired from six of the same sharks, as well as 30 additional sharks from the North Atlantic Ocean including the Gulf of Mexico, Northwest Atlantic Ocean off the eastern coast of the United States, and The Bahamas. Blood samples were acquired from 24 sharks in the South Pacific Ocean near Norfolk Island. Blood samples were chemically digested in 20% potassium hydroxide and 30% hydrogen peroxide to remove organic material and filtered to 0.7 µm. Microwave-assisted solvent-extraction was performed, and the extracted samples were analyzed using pyrolysis gas chromatography with mass spectrometry (Py-GCMS) to detect and quantify six plastic polymers (polymethyl methacrylate, polypropylene, polystyrene, polyethylene, polycarbonate, and polyvinyl chloride). Overall, plastic polymers were detected in 49 of 54 sharks from all regions. We observed a mean (± standard deviation) concentration of 4.38 (± 5.15) µg/mL for the sum of all six polymers across all 54 sharks sampled. The tiger sharks sampled near The Bahamas were most contaminated (6.56 ± 7.24 µg/mL), with plastic polymers detected in 16 of 17 sharks. The most detected plastic polymer of the six analyzed was polyethylene, at a mean concentration (± standard deviation) of 3.16 (± 5.16) µg/mL and it was detected in 30 of 54 tiger sharks from all regions. The detection of plastic polymers >0.7 µm in the blood of the tiger shark indicates the translocation of micro- and nanoplastics outside of the gastrointestinal tract. The methodology we present offers a suitable non-lethal method for detecting plastic particles within an organism. Our research will help us understand the fate of micro- and nanoplastics in large aquatic animals.

1.12.P-We-024 Microplastic Characterization and Screening by Combining DART and High-Resolution Mass Spectrometry

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Microplastic particles result from commercial product development and the breakdown of plastics and are abundant in our environment from a variety of commercial sources like cosmetics, textiles and other pieces of plastics such as water bottles that breakdown with radiation. Microplastics have been detected at an alarming level in our marine life and drinking water. Direct Analysis in Real Time - High Resolution Mass Spectrometry (DART-HRMS) allows for rapid fingerprinting of environmental microplastics, e.g. for the identification of their origin. Typical samples of interest are polymers found in the environment like virgin pre-production pellets, microbeads from personal care products, microplastics found in the aquatic environment, and synthetic fibers. DART was coupled to an impact II VIP QTOF (both Bruker, Germany). While traditional GC/MS methods require sample preparation (10-20 min) and long analysis times (> 20 min), this new method allows for rapid polymer analysis (< 5 min) without sample preparation. A small sample sliver is cut and placed in a copper pot of a thermal desorption system (IonRocket, Biochromato, Japan) which delivers temperature gradients from ambient to 600 ºC with ramp rates of 150 ºC/min. When the thermal program is started, the QTOF collects MS spectra at 5Hz data acquisition speed for the entire run. The resulting mass spectra display thousands of discrete peaks. Depending on the temperature, different additives, the polymeric basis as well as degradation products are released and detected at different time points during the run. Distinct m/z signals and general signal patterns of a microplastic material found in water were compared with standard plastic samples such as a soda bottle, a trash bag and packaging material. While the soda bottle turned out to be made of PET, the microplastic clearly had PE as a basis. PE as an insoluble polymer is difficult to analyze with other MS-based methods like MALDI. The microplastic material showed most similarity with the trash bag, regarding both certain distinct marker m/z values as well as the general pattern released by the temperature gradient, so it can very likely be traced back to that origin.

1.12.P-We-025 Modeling Uptake and Depuration Kinetics of a Heterogeneous Mixture of Nanoplastics in *Daphnia magna*

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Nanoplastics (NPs) (<1 um) have been shown to translocate across biological membranes, inducing oxidative stress and immune responses, and posing an environmental health risk. Yet many of the toxicological reports of NP toxicity rely on qualitative measurements of commercially available spherical polystyrene NPs, thus diminishing the role of fragmented irregularly shaped polydispersions of NPs on uptake and depuration kinetics. The goal of this study was to quantify the uptake and depuration rate of polymethyl methacrylate (PMMA) NPs in Daphnia magna. We employed a novel technique of doping PMMA with tantalum ethoxide to create a mixture of polydispersed Ta-PMMA where particle number and diameter could be distinguished using spICP-MS. Our hypothesis was that PMMA uptake and depuration rates were size dependent. Initial particle detection results indicated that particles could be detected in individual Daphnia at 1 mg/L exposure and pooling 10-30 daphnia would allow us to detect particles down to 100 ng/L exposure concentrations. For this study, Daphnia were exposed to a 1 mg/L concentration of a heterogeneous size mixture of Ta-PMMA ranging in size from 150 to 1000 nm for 24 hours, followed by a 24-hour depuration. There were two types of exposures: individually exposed *Daphnia (n*= 10 reps) and 10 *Daphnia* pooled (n= 3) to examine variation amongst individuals. Collections occurred at 3, 6, 12, and 24 hours, followed by water replacements and depuration collection at 30, 36, and 48 hours. Our initial modeling results show that the uptake and depuration kinetics are similar across all size ranges of NPs. The NPs demonstrated a first order uptake and depuration model in all size ranges. In each size range, the uptake of NPs was maxed at the 12- hour mark and were depurated out of the gut by the 36-hour mark. Modeling using increased replicates or pooling the *Daphnia* together will allow us to reduce the variation and model the uptake and depuration kinetics with a higher accuracy. Future research will focus on how the introduction of PFAS chemicals absorbed to NPs will influence uptake and depuration kinetics in Daphnia and fathead minnows.

1.12.P-We-027 Reconciling the Origin of Nanoplastics and Their Characteristics

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Nanoplastics (< 1000 nm) detected during daily use of plastic products, especially those that might lead to direct human ingestion, have attracted great attention from academia and the public. The current focus in research is predominantly on the size ("nano" aspect) and size distribution of these nanoplastics, often leading to an overestimation of their presence. This is primarily due to inadequate consideration of the reaction intermediates and additives used in

polymer synthesis. In this perspective, we discussed the definition of nanoplastics, polymers, and the limitations of existing characterization techniques. We proposed effective nanoplastic characterization should include not only the measurement of size, particle quantity and polymer type, but also molecular weight. We emphasize the importance of differentiating between primary nanoplastics (polymer matrix, reaction intermediates, and additives), inherent to the production process, and secondary nanoplastics, formed through various degradation processes. This distinction is crucial as it influences how we may assess release, characteristics, and toxicity of nanoplastics. Therefore, we argue that nanoplastics should not be collectively regulated as a single group, as some polymers or their reaction intermediates are more toxic than others due to their structure and chemical composition.

1.12.P-We-028 Simple Detection of Polystyrene Nanoparticles and Effects in Freshwater Mussels; Method Development and In Situ Application to Urban Pollution

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The ubiquity of plastics in environments worldwide is raising concerns about their toxicity to organisms. The purpose of this study was to investigate simple means to determine the exposure and effects of nanoplastics (NPs) in the freshwater mussels *Elliptio complanata*. NP tissue levels were determined using a plasmonic nanogold sensor probe and effects were determined using the refractive index (RI) and thiol-reaction rates (TRR) in protein-dense tissue extracts. This method was adapted to quantitatively measure the concentration of NPs in tissues using a salting out extraction in the presence of ACN. Concentrated solutions of albumin were first spiked with NPs to evaluate changes in RI and TRR to determine crowding effects. The data revealed that NPs readily decreased the RI and TRR in albumin *in vitro*. These 3 simple assays were then applied on freshwater mussels caged for 3 months at various sites in a largely populated area. Mussels downstream of the city center and found at the street runoff discharge sites were highly contaminated by NPs and the RI and TRR were also reduced. In conclusion, simple and readily accessible assays to assess the NP contamination based on a visual nanogold sensor technology, and the effects of these plastics are proposed for freshwater mussels.

1.12.P-We-029 Sorption Behavior of Cd(II) onto PVC and PET Microplastics in Synthetic Soft Water and Synthetic Sea Water

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We studied the adsorption of Cadmium (Cd (II)) ions onto poly vinyl chloride (PVC) and polyethylene terephthalate (PET) microplastics (MPs) in synthetic soft water and synthetic sea water. MPs have garnered significant interest due to their prevalence in aquatic ecosystems and their ability to carry heavy metals, posing a threat to both aquatic organisms and human health. The adsorption heavy metal ions on MPs, the adsorption behaviors of metal ions with MPs in different environmentally relevant aqueous media (e.g., freshwater and seawater with varied dissolved organic carbon concentrations (DOC)) is largely unknown. In this study we first estimated with Visual MINTEQ the distribution of Cd species as a function of aqueous media composition. Visual MINTEQ calculations reveal that 30.7% of Cd precipitates when synthetic soft water is used as the solvent, while 28.2% of Cd precipitates when synthetic seawater is used as the solvent. The experimental findings mirrored the observed trend: 23.7% of Cd precipitated with synthetic soft water, while 24.1% precipitated with synthetic sea water. As the pH is kept constant during the kinetic experiments ionic strength would affect the adsorption of Cd to the microplastic surface. Precipitation reactions likely play an important role in the quantity and form of Cd adsorbed onto MPs. Our initial experiments show that the harder the water the less soluble Cd is present in the aqueous phase. Current experiments are testing the rate and amount of Cd sorbed onto PVC and PET MPs in deionized water, soft water, and seawater with different levels of DOC . Our study will provide relevant information to understand the affinity of Cd to MPs in relevant environmental conditions.

1.12.P-We-030 Sublethal Effects of Microplastics Exposure on Freshwater Amphipods, *Hyalella azteca*

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Microplastics, defined as plastic particles with a size less than 5 mm, are increasingly posing a significant environmental challenge due to their widespread presence in many aquatic ecosystems. The subtoxic effects of microplastics on freshwater organisms have received less attention, despite evidence of their acute effects. This research investigates the subtoxic effects of microplastic exposure on the freshwater amphipod, *Hyalella azteca*, a key component of the aquatic food chain. Our hypothesis is that long-term exposure to microplastics at concentrations commonly seen in the environment will cause sublethal effects on *H. azteca*, which might have negative impacts on individual fitness and community health. Microplastics to be used for exposure have already been prepared in the laboratory using a high-energy milling machine and liquid nitrogen to break down plastic cutlery made of polystyrene into micro-particles. Although this study is still in its early stages, our proposed methodology entails subjecting *H. azteca* to environmentally appropriate concentrations of microplastics (2µg/L – low concentration, and 2mg/L – High concentration) in controlled laboratory conditions. In this study, we plan to evaluate many endpoints, including as survival, oxidative stress, growth, reproduction, and behavior, in order to better understand the possible sublethal impacts of microplastics on these marine organisms. Our goal is to understand the fundamental processes of stress responses in *H. azteca* caused by microplastics by combining molecular and behavioral analysis with laboratory research. By studying the ecological consequences of microplastic pollution on freshwater ecosystems, this research aims to further our understanding of the problem and provide guidance for developing mitigation methods.

1.12.P-We-031 The Acute Toxicity of Microplastics Co-Exposed with a Nano Copper Pesticide on *Daphnia magna* and *Caenorhabditis elegans*

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The presence of microplastics in aquatic ecosystems, along with the co-occurrence of chemical contaminants such as pesticides, poses significant ecological concerns. This study investigates the acute toxicity of microplastics generated from agricultural films co-exposed with a nano copper pesticide on two widely studied aquatic organisms, *Daphnia magna* and *Caenorhabditis elegans*. Micro and nano plastics (MNPS), ubiquitous in terrestrial and aquatic environments, have been recognized as vectors for various contaminants, including pesticides. In this study, environmentally relevant concentrations of microplastics were selected to mimic real-world exposure scenarios, and dose response was performed for both of the organisms tested. Acute toxicity tests were conducted using standard protocols for *Daphnia magna* and *Caenorhabditis elegans*, assessing endpoints such as mortality, and immobility. The experimental design aimed to elucidate potential synergistic or antagonistic effects resulting from the co-exposure to microplastics and nano copper. Preliminary findings suggest that the co-exposure of microplastics with the copper pesticide significantly alters the toxicity on the *Daphnia magna* and *Caenorhabditis elegans*. There is very little impact of the agricultural plastics on the aquatic species *Daphnia magna*, when exposed alone. In the co-exposure of the pesticide and the plastic significantly shifted the dose response curves, where the slope of the curves steepened and the NOEL was shifted effectively shortening the effects window suggesting the presence of plastic decreases the potency of the pesticide. In *Caenorhabditis elegans* there is a significant difference in toxicity between the biodegradable plastic MNPs and the LDPE MNPs, with the biodegradable plastics being the more toxic set of plastics, both compared to the control. The outcomes of this study contribute valuable insights into the potential risks posed by microplastics and chemical contaminants in aquatic ecosystems. Understanding the acute toxicity of microplastics co-exposed with the nano copper pesticide provides essential knowledge for environmental risk assessment and informs strategies for mitigating the adverse impacts on aquatic biota and ecosystem health. Further research is warranted to elucidate the mechanisms underlying the observed effects and to evaluate the long-term consequences of microplastic pollution in combination with chemical contaminants.

1.12.P-We-032 The Role of Surface Chemistry on Nanoparticles Uptake and Toxicity in Fish Intestinal Cells

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Plastic pollution is an emerging environmental problem that has been projected to increase at least until 2050. Due to mechanical and chemical degradation, plastic can break down into the micro size range (1 mm to 5 mm) and nano size range (< 1 mm). Nanoplastics (NPs) can enter cells and are therefore of particular toxicological importance. Our study aimed to test how different functional groups (amine positive charge, carboxyl negative charge, and uncoated) attached to the surface of fluorescently labelled polystyrene nanoplastics (100 nm) affect cellular uptake and toxicity. Considering that the dietary route is the main route of uptake in fish, we used an intestinal cell line (RTgutGC) derived from rainbow trout *(Oncorhynchus mykiss*). The nanoplastics behavior in the exposure solution was measured by dynamic light scattering (DLS) and zeta potential. Cytotoxicity was evaluated using a multiple endpoint viability assay that measures simultaneously metabolic activity and cell membrane and lysosomal integrity. Nanoplastics intracellular accumulation was measured using two methods, fluorescence and pyrolysis gas chromatography mass spectrophotometry (PY-GC-MS). The DLS analysis revealed that nanoplastics remained polydispersed in the exposure medium with an average size of 122, 132 and 99 nm for carboxyl, amine, and uncoated NPs. The zeta potential analysis showed a change of -18.7, -3.9 and -19.6 for carboxyl, ammine and uncoated NPs, respectively.

The fluorescence quantification showed that negatively charged NPs (carboxyl and uncoated) accumulate in cells more effectively than positively charged (ammine) NPs. Moreover, negatively charged NPs induce an increase in lysosome activity while positively charged did not. This effect was also confirmed by imaging. Quantification by PY-GC-MS will be compared to fluorescence measurements to determine sensitivity of the technologies. This data will be instrumental to further understand the mechanisms and factors involved in NPs cellular uptake.

1.12.P-We-036 Combined Effects of Microplastic Fibers from Disposable Face Mask Leachate and Graphene Oxide Nanoparticles on Microalgae *Scenedesmus Obliquus*: Analysing the Effects of Heavy Metals

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Disposable surgical face masks extensively used during the COVID-19 outbreak would release microplastics into the aquatic environment. The increasing usage of Graphene oxide (GO) in various consumer items has led to its ubiquitous presence in freshwater systems. This study determined the quantity and kind of microplastics discharged from disposable surgical face masks. The mask-leached microplastics were identified to be polypropylene of varying shapes and sizes, spanning from 1 mm to 15 mm. In addition, heavy metals like Cd, Cu, and Zn leached from the face masks were quantified. Four concentrations of GO, 0.1, 0.5, 1, and 2 mg L^{-1}, were mixed with leached solution from the face masks to perform the combined toxicity test on freshwater algae, *Scenedesmus obliquus*. A dose-dependent decrease in algal cell viability was observed upon treatment with various concentrations of GO individually. The mixtures of GO and the leached solution from the face masks exhibited significantly more toxicity in the algal cells than in their pristine forms. GO promoted increased production of oxidative stress and antioxidant enzyme activities resulting in cellular damage and decreased photosynthesis. These impacts were elevated when the algal cells were treated with the binary mixture. Furthermore, the heavy metal ions leached from face masks also contributed to the toxic effects. Our study shows that the leachates from disposable surgical face masks, combined with GO, may pose a severe environmental threat.

1.12.P-We-038 Fate of Microplastics in Conventional Drinking Water Treatment Facilities

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Microplastics are ubiquitously present in the environment, and oral consumption has been demonstrated to exert toxicity to the liver and intestines of model organisms. Furthermore, micro- and nanoplastics have been observed in the blood and tissues of humans, though the exact sources and absorption pathways are not clearly defined. Given the toxic effects of microplastic consumption and pervasiveness in the environment, drinking water treatment plants (DWTPs) serve as a crucial barrier for consumers of municipal drinking water. However, without a rigorous understanding of removal processes and their effects on the physiochemical properties of microplastics, appropriate regulation of these contaminants is not possible. In this study, we aim to determine the sources and fate of microplastics in DWTPs. In order to confirm the presence of microplastics in DWTPs, the lagoons, which serve as the final destination for particulates removed from water, were sampled. Using a soil probe, samples were collected from the top 10cm of soil at the shoreline of the lagoons, and a control sample was collected from an empty field directly adjacent to the lagoons. Six 10g samples of each soil were analyzed for microplastic content. Microplastics were extracted from the samples using elutriation, digestion in 7.5% NaOCl, and centrifugation in 6M $ZnCl_2$ in series. Microplastics were then filtered onto 0.8µm polycarbonate filter membranes. Potential microplastics were imaged using Nilered staining and fluorescence microscopy and quantified using automated particle counting through ImageJ. Both lagoon samples demonstrated a significant ($p<0.05$) increase in particle count over the control (36.5 particles g^{-1} for lagoon 1 and 28.9 particles g^{-1} for lagoon 2, compared to 19.0 particles g^{-1} in the control). This result suggests that a significant amount of microplastics are removed from the influent water stream and are deposited in the lagoon sediments, which has implications for proper sediment disposal and lagoon water discharge and/or recycle. Further research will aim to determine the influent water concentration and removal efficiency during water treatment, as well as develop a mechanistic understanding of removal processes for microplastics. Doing so will allow for the prescription of treatment processes to achieve adequate removal of plastic particles from drinking water.

1.13.P-Th-101 Machine Learning Webtool for Chemical Ecotoxicity Prediction

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Evaluating chemical ecotoxicity (HC50) is a valuable approach to identify potential hazardous effects of chemicals on ecosystems and is of great interest in academic and regulatory communities. In silico models based on artificial intelligence provide an efficient way of screening thousands of chemicals, where chemicals are usually represented by different approaches, such as physicochemical descriptors, fingerprints, and graphs. These chemical representations often form high-dimensional data. We hence developed a toxicity prediction website tool integrating multiple machine learning and deep learning methods such as autoencoders and random forest with various molecular features such as physicochemical descriptors and molecular fingerprints as model inputs. Specifically, the autoencoder model can be used to learn low-dimensional chemical representations from hundreds of chemical features. We used Amazon Web Service (AWS) Cloud Development Kit application to streamline backend machine learning models and frontend user input of high dimensional chemical features. Our website can also integrate PubChem Sketcher to transform chemical representations. Our developed website tool provides a useful method to efficiently and conveniently predict chemical ecotoxicity.

1.13.P-Th-102 Pitfalls of Incorporating Historical Control Data in Assessment of Toxicity to Terrestrial Plants From Pesticides

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Historical control data are compiled of control data from past studies and traditionally used in clinical trials and mammalian carcinogenicity tests to improve statistical power of treatment effects analysis. More recently, the historical control approach has become frequently employed in ecological risk assessment to estimate sensitivity of standardized toxicity tests. Specifically, a threshold based on the overall variation of pooled control data from past studies is used to determine whether statistically significant effects observed in individual toxicity tests should be deemed certain. However, combining data from multiple studies without caution may lead to skewed analysis and erroneous conclusions. To evaluate the potential benefits and pitfalls in utilizing historical control data for terrestrial plant toxicity testing, we reviewed relevant studies and conducted additional statistical analyses using publicly available historical control datasets. These datasets are used to propose thresholds (e.g. 10%) at which any effect greater than can be deemed confident and lesser than confers uncertainty. Our goals were to: 1) evaluate the variation within the dataset and 2) determine if the use of historical control datasets to interpret plant ecotoxicological results is biologically and financially appropriate. We found that variation across individual studies was greater than variation within individual studies. Further investigation revealed that the inter-study variation was significantly influenced by time and location indicating that the overall variation in this historical control dataset likely represents differences in non-random, external environmental factors. Moreover, using tomato production in NY State as an example, we demonstrated that biological effects below the threshold values proposed by historical control data analyses could still lead to substantial financial loss in agricultural crop production. Lastly, there is a lack of literature on how model plants used for ecological risk assessment reflect the native community to evaluate if the utilization of threshold values can confidently predict ecosystem-wide effects. Our analyses show that dataset selection for a historical control approach need to be considered carefully to reduce influence from environmental factors. Future research should clarify the utility of historical control application as related to the potential financial burden and biological significance.

1.13.P-Th-103 Development of a High-Throughput In Vitro Chemotaxis Assay in Jurkat T Cells for Hazard Identification of Immunotoxicants

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Current immunotoxicity testing guidelines rely on low-throughput, costly rodent models. To identify potential immunotoxicants rapidly, high-throughput new approach methods (NAMs) are needed for functional immune endpoints, including chemotaxis — the process by which immune cells are recruited to a specific location *in vivo*. Chemotaxis is necessary for immune system development, response to infection, and wound healing. To model chemotaxis *in vitro*, Jurkat T cells, an immortalized $CD4^+$ T cell line, were cultured, stained, and seeded above a transwell membrane with 3-micron pores. Media containing CXCL12, a T cell chemoattractant, was added below the transwell membrane. After 3 hours, the plate was imaged on a high-content imager to count cells that migrated through the membrane. Initial experiments recapitulated previous studies in demonstrating that Jurkat T cells responded to varying concentrations of CXCL12 in a biphasic manner, with less activity observed at the lowest and highest concentrations. Next, we investigated how exposure to selected chemicals impacted this chemotaxis by administering a 30-minute pre-treatment of Jurkat T cells with D-mannitol (10 µM), LY2510924 (10 µM), cytochalasin D (10 µM), or perfluorooctanoic acid (PFOA; 100 µM). Preliminary data revealed that both LY2510924, an inhibitor of the receptor for CXCL12, and cytochalasin D, an inhibitor of actin polymerization, completely inhibited chemotaxis. PFOA, which has been reported to inhibit neutrophil chemotaxis, also inhibited chemotaxis in our model by ~30%, although this result was not statistically different ($p = 0.0766$) from control levels. As expected, D-mannitol did not affect chemotaxis. Future studies will utilize longer exposures and a full range of concentrations to assess responses to these and other compounds. This assay represents a potential NAM for identifying immunotoxic potential of environmental chemicals. *This abstract does not reflect official U.S. Environmental Protection Agency policy.*

1.13.P-Th-104 2-Ethylhexanol Exposure: Autism-Like Symptoms and Neurodevelopmental Disruptions in Zebrafish

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2-Ethylhexanol is a branched-chain alcohol that is synthesized and is mostly used as an essential ingredient in the production of plasticizers such tris-(2-ethylhexyl)phosphate, bis-(2-ethylhexyl)phthalate, and bis-(2-ethylhexyl)adipate. It's a photoproduct of octyl methoxycinnamate and has been shown to stimulate hepatic peroxisome proliferation in rats. We used a multimodal neurotoxicity testing technique to assess the detrimental effects of 2-ethylhexanol on neurobehavioral and neurodevelopmental processes. 2-Ethylhexanol was exposed to wild-type and transgenic lines *tg(elavl3:eGFP)* and *tg(mbp:mGFP)* from < 3 to 120 hpf. Tail coiling at 24 hpf and touch-evoked responses at 72 hpf showed inversely proportional significant changes; this early disruption of motor activity was consistent with later locomotor results, which showed that distance travelled impacted significantly along with an increase in turn angle, and could therefore account for the lower dopamine and acetylcholinesterase levels. Additionally, we found deficiencies in locomotor behavior, which might be linked to the genetic alterations linked to autism. Apart from the demyelination of Schwann cells and oligodendrocytes, we also observed anomalies in neurogenesis, such as thinner spinal cords and a smaller brain. These findings are consistent with aberrant alterations in gene expression occurring. According to our research, 2-ethylhexanol's harmful effects resulted in varied degrees of transcriptional alteration of several neurodevelopmental (*gap43, manf, sox2*), neurotransmitter (*drd1, mao, htr1bd*), and autism-related genes (*adsl, eif4a1, mbd5, vps13b,* and *tsc1b*). Using an array of neurotoxicity tests, we first show that 2-ethylhexanol negatively affects zebrafish neurodevelopmental and neurotransmission systems and may cause symptoms analogous to autism. This work was supported by Korea Environment Industry & Technology Institute through Technology Development Project for Safety Management of Household Chemical Products Program (or Project), funded by Korea Ministry of Environment (2022002980005,1485018893).

1.13.P-Th-105 Neurotoxic Potential and Possible Mechanism of Dibutyl Phthalate and Its Metabolite in Zebrafish

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Dibutyl phthalate (DBP), commonly used as a plasticizer, raises significant neurotoxic concerns; however, the effects of its metabolite, mono-n-butyl phthalate (MBP), remain unclear. This study comprehensively investigates the developmental and neurotoxic effects of DBP and MBP on zebrafish (*Danio rerio*) larvae. Zebrafish from both wild-type and transgenic lines (tg(*elavl3*) and tg(*mbp*)) were exposed to DBP and MBP from 4 hours post fertilization (hpf) to 120 hpf. We assessed developmental toxicity using various parameters, including survival rates, hatchability, eye size, malformations, tail coiling, and touch-evoked responses. Locomotor activities were measured using the DanioVision tracking system. Neurotoxic and oxidative stress markers, along with related gene transcription, were evaluated through ELISA and qPCR. Fluorescence imaging revealed disruptions in neurogenesis and demyelination. Our findings indicate that both DBP and MBP caused significant changes in touch-evoked responses at 72 hpf. Behavioral analyses showed that DBP exposure impacted various aspects such as distance moved, velocity, and turn angle. Gene transcriptional analysis indicated significant effects on neurodevelopmental genes (*sox2, manf, gfap*) and oxidative stress genes (*gsta1, gr*), providing further insights into the molecular mechanisms of MBP. Notably, MBP exhibited a greater impact on molecular markers for neurodevelopment compared to DBP. These findings enhance our understanding of the neurotoxic potential of DBP and MBP in aquatic species, underscoring the importance of considering metabolites in toxicity and risk assessments. *This work was supported by Korea Environment Industry & Technology Institute through Technology Development Project for Safety Management of Household Chemical Products Program (or Project), funded by Korea Ministry of Environment (2022002980005,1485018893).*

1.13.P-Th-106 Impacts of Human-Use Antifungals on Symbiotic Soil Fungi in the Agro-Environment

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Unsustainable agricultural practices which degrade soil quality and reduce crop production are a threat to increasing food demand globally. Reusing municipal biosolids and promoting arbuscular mycorrhizal associations in crops to reduce synthetic fertilizer use is practiced in regenerative agriculture but exposes soil to man-made chemicals, including partially metabolised pharmaceuticals, that enter wastewater treatment works. Here, we evaluate the impact of pharmaceuticals on arbuscular mycorrhizal (AM) fungal function. Mycorrhizal spring onion and lettuce were grown in agricultural soil spiked with three antifungal azole pharmaceuticals at environmentally relevant concentrations and used a combination of radio and stable isotopes (^{14}C, ^{33}P, ^{15}N) to track carbon for nutrient exchange between the AM fungi and the plants. We used DNA sequencing to define the microbial communities within crop roots and soils in both treatments and chemical analysis to quantify the presence of antifungals in soil and plant organs. When exposed to antifungal pharmaceuticals, no significant impacts on root colonisation by AM fungi were observed. However, bi-directional exchanges of AM fungal-acquired phosphorus and plant-fixed carbon between symbionts were reduced in all crops alongside changes in bacterial and fungal community composition. Specifically in spring onion roots, there was a significant decrease in fungal (ITS) alpha diversity and this trend followed for lettuce, although it was not significant. These changes support the results from our nutrient tracing observations which revealed that phosphorus assimilation by spring onion via AM fungi was significantly reduced in the presence of the antifungals. Comparatively for lettuce, the results show that this crop did not respond so dramatically, although there was a general decrease in phosphorus transfer. Our results indicate that pharmaceutical antifungal agents have a direct impact on AM function, impacting the wider soil microbiome. This is likely to have wider effects on soil health and function in agroecosystems. Our research emphasises the unintended consequences and threats posed by emerging contaminants in soils in terms of impacts on AM fungal function and microbial community composition. This emphasises the pressing need for further research and regulation across potential contaminants of soil systems to determine wider impacts of circular agriculture practices.

1.13.P-Th-107 Wildfire Contaminants in a Coral Reef Ecosystem From Chemical Fingerprinting After Destruction of a Historic Coastal Town, Lāhainā, Maui, Hawai'i

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In the aftermath of destruction by wildfire, fire-mobilized chemicals from the dense urban area were immediate risks for public and environmental health in the coastal town of Lāhainā, Maui. Concerns included the health of the Lāhainā coral reef, a natural and cultural resource. Metals, dioxins, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), per- and polyfluoroalkyl substances (PFAS), and N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-q) were quantified in reef/beach sediment and wildfire debris/soil from the built environment, open spaces in Lāhainā Town, outlying fields, and storm runoff from 1 to 6 months post-fire. For the overall burn area, only metals exceeded Hawaii action levels for in situ direct exposure to humans, likely due to combustion of older buildings containing chromated copper arsenate (CCA)-treated wood. In runoff and coastal sediment, dioxins and PCBs also exceeded Hawaii no-effects sediment quality guidelines. Dioxin homologue patterns, or fingerprints, were distinct among built-environment ash, soil with plant ash, and soil with legacy agricultural chemicals. In coral reef sediment, dioxin fingerprints showed offshore transport of wildfire chemicals after the first rains 3 months post-fire. During subsequent months, wildfire materials were dispersed northward along the forereef. Wildfire debris removal did not begin in Lāhainā until 5 months post-fire and is expected to take a year to complete, during which time wildfire-mobilized chemicals could migrate into subsurface soil, surface water, groundwater, and be taken up by biota. The next steps for understanding wildfire effects on coastal zones and ecosystems include quantification of dissolved-phase wildfire chemicals in semipermeable membrane devices (SPMDs), polar organic compound integrative samplers (POCIS), and diffusive gradient in thin-films (DGTs) deployed 1-month post-fire and during storm runoff, longer term process-based studies about transport and incorporation of wildfire chemicals into coastal ecosystems, and possible toxicity testing with contaminant mixtures extracted from the passive samplers. The frequency and severity of wildfires is increasing, and lessons learned from Lāhainā will inform emergency response and recovery priorities for future urban and coastal wildfires.

1.13.P-Th-108 Perfluorohexanoic Acid (PFHxA) Induces Cytochrome P450 Expression and Enzymatic Activity in Well-Differentiated Primary Human Bronchial Epithelial Cells (HBEC) in Air-Liquid Interface (ALI) Culture

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Perfluorohexanoic acid (PFHxA) is a short-chain fluorinated carboxylic acid perfluoroalkyl substance (PFAS) used in surface coatings of consumer products as a replacement for longer-chained PFAS. Recent studies have detected PFHxA in indoor dust, suggesting its contribution to the burden of PFAS exposure via ingestion or inhalation of indoor air. However, scientific evidence linking PFHxA exposure to lung health is lacking. To fill this knowledge gap, we aimed to identify potential adverse effects of PFHxA on lung health using primary human bronchial epithelial (HBE) cells differentiated from basal stem cells, which were isolated from the airways of four human donors (n = 4). In a three-dimensional (3D) air-liquid interface (ALI) culture, well-differentiated HBE cells maintain a pseudostratified epithelium, which recapitulates the cellular composition and barrier function of the human airway. Using this gold standard *in vitro* model of airway cell biology, we performed both dose- and time-dependent experiments to characterize the impact of PFHxA on airway epithelial cells in humans. HBE cells were differentiated in ALI culture for 21 days, then exposed to PFHxA at increasing concentrations (0.1-1000 nM) apically for 4h and basolaterally for 24h. At 24h after the initiation of the exposure, no cytotoxicity was observed at any conditions, as determined by the lactate dehydrogenase (LDH) assay. In a subsequent experiment, cells were basolaterally exposed to PFHxA at 0.001 and 1µM for 24h, 48h, and 72h. At the end of each time point, we determined the mRNA expression of interleukin-1β (*IL1B*), MUC5AC (*MUC5AC*), and cytochrome P450 (*CYP1A1* and *CYP1B1*) by qPCR analysis. At 24h, 1 µM PFHxA significantly increased the expression of *IL1B* (1.5-fold), *MUC5AC* (1.8-fold), *CYP1A1* (1.8-fold), and *CYP1B1* (1.5-fold) relative to controls. At 48h of exposure, there were similar trends towards the induction of *CYP1A1* and *CYP1B1*. However, at 72h, 1 µM PFHxA significantly increased the mRNA expression of *CYP1B1* (1.3-fold). Furthermore, compared to controls, 1 µM PFHxA significantly induced CYP1A1 enzyme activity by 172% at 24h and 178% at 72h, as determined by the ethoxyresorufin-O-deethylase (EROD) assay. Our data indicate that exposure to PFHxA induced the mRNA expression of genes critical to the homeostasis of airway epithelia and the enzymatic activity of cytochrome P450, indicating potential adverse effects on lung health through altered metabolic processes.

1.13.P-Th-109 Examining the Human Health Impacts of Carbon Capture Through Life Cycle Impact Assessment: A Targeted Literature Review

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Carbon capture utilization and storage (CCUS) is anticipated to play a key role in the global energy transition by reducing greenhouse gas emissions in numerous industries. Life cycle impact assessment (LCIA) is a decision-making tool that has been widely adopted in CCUS research. Although the focus of LCIA has been predominantly on climate change, other impacts including human health are influential. However, published LCIA conclusions on human health impacts are highly uncertain. We performed a targeted review of publications in the peer-reviewed literature (2008-2023) that included human health as an impact factor in LCIA of post-combustion carbon capture (CC) technology using aqueous amines as the capture solvent. Data extracted from publications included: LCIA method, impact categories, emissions inventory, and methods for derivation of human toxicity potential (HTP). The purpose of the review was to explore the range of approaches and findings related to human health impacts of the CC technology and identify foundational data driving those impact scores. Out of 82 publications, only 34 included HTP as an endpoint. For each of the publications, a summary of the selected emissions and characterization factors used to evaluate cancer and non-cancer impacts within the LCIA is presented. The respective data sources for each factor were reviewed to identify whether the underlying assumptions in both the LCIA method and the emissions inventories were accurately assessed and applied. Results indicated that the solvent monoethanolamine (MEA) was commonly chosen to characterize toxicity and assessed through either ethanolamine production or CO_2 production, with its characterization factors showing variability based on the LCIA method used. The primary conclusions reached by the authors of the 34 studies were that while a process equipped with CC technology may reduce carbon emissions, solvents associated with amine capture technology pose potential human health hazards. However, the data underlying most studies were associated with high uncertainty. For example, the most widely cited emissions inventory referenced only a single study unrepresentative of full-scale amine CC facilities. Further, the HTP scores for MEA did not reflect MEA specifically and were derived from limited sources. Therefore, if these CCUS LCIA studies are being relied upon to make decisions regarding implementation of this technology, the results should be interpreted with caution.

1.13.P-Th-110 Sediment Quality Assessment Survey of the Matagorda Bay System

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The Matagorda Bay System, one of the seven major estuaries located along the coast of Texas, plays an important role in the local ecosystem, serving as a habitat for many marine species, act as a natural barrier against coastal hazards, and acts as a filter for pollutants. However, this ecosystem is vulnerable to contamination from non-point source pollution by freshwater inflow. The purpose of this study is to determine if sediment contamination is responsible for ecosystem degradation and/or a decline in benthic organisms. A Sediment Quality Triad (SQT) approach was used to assess the health of the Matagorda Bay System. The SQT consists of chemical contaminant measurements in sediments, sediment toxicity exposures, and benthic community structure and diversity to identify the relationship between which contaminants are found in the sediment and how they influence the benthic species health that live within. The goal is to establish a correlation between sediment contamination levels and decline in benthic diversity, not only to understand the relationship, but to contribute to future point source pollution studies. Mean survival rates for the three toxicity test species (polychaetes, amphipods, and clams) indicate the lowest survival rates were from stations located in the upper portions of Lavaca Bay and Tres Palacios Bay, with mean survival rate lower than 68%. Linear regression models were performed to predict survival rates by the distance to shore (DTS). There were varying impacts on different benthic species. Survival of the hard clam, *Mercenaria mercenaria*, shows a significant inverse relationship with DTS, indicating that as the DTS increases, the survival rate of *Merceneria* decreases. Survival of the amphipod, *Leptocheirus plumulosus*, was also inverse, though it was not statistically significant. The polychaete, *Neanthes arenaceodentata*, showed a different trend, with survival increasing with DTS, but not significantly correlated. Future analysis of sediment grain size, chemical contaminants, and benthic community structure will provide more conclusive insights regarding sediment quality within the study area. The findings from this study can support further implementation of policies that protect estuarine environments from future contamination, leading to healthier marine habitats. Additionally, by regulating non-point source pollution, it will prevent continuing degradation and contribute to ensuring the resilience of these vital ecosystems.

1.13.P-Th-111 Adverse Effects of Microplastics to Function of Earthworm Coelomocytes

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Reactions of immune cells in earthworms to microplastics exposure have been still unknown even though the number of studies investing the effects of microplastics on soil invertebrates has been increasing. The purpose of the present study is to examine the effects of microplastics to endocytic activity of earthworm coelomocytes. Phagocytosis and pinocytosis of coelomocytes were evaluated via *in vitro* (12-h) and *in vivo* (14-d) assays using polystyrene microplastics and earthworm *Eisenia andrei*. *Escherichia coli* and the neutral red dye were used for the phagocytosis and pinocytosis assays, respectively. In the *in vitro* assay, inhibitions in phagocytosis and pinocytosis were observed while cellular viability were not affected. The *in vivo* assay also revealed that endocytosis declined in the polluted soil with polystyrene microplastics. These results indicate that microplastics exposure could weaken the immune function of earthworm coelomocytes. *This work was supported by the National Research Foundation of Korea grant funded by the Korea government (2021R1C1C2012628).*

1.13.P-Th-112 Transcriptomic Analyses of Hepatotoxicity and Immunotoxicity Induced by Oral Exposure to Contaminated Groundwater Mixture in Male and Female Sprague Dawley Rats

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Complex mixtures of contaminants are found in various environmental matrices, including groundwater reservoirs. These mixtures of contaminants pose risks to human and ecological health. In this study, we investigated the potential underlying molecular perturbations that may lead to hepatic toxicity of groundwater collected from a legacy contaminated pesticide manufacturing and storage site. We used RNASeq to characterize the hepatic transcriptome of male and female Sprague Dawley (SD) rats orally exposed to 10% groundwater mixture (highly impacted well from the industrial site) for 60 days. Apical endpoints (histological and biochemical) analyses in the same rats showed hepatic inflammation and steatosis, along with a reduction of hepatic-related plasma proteins (albumin and alpha2-macroglobulin). Hepatic transcriptomic profiles showed 70 differentially expressed genes (DEGs) in males, while 169 DEGs were observed in females. Using gene set enrichment analyses, gene ontology (GO) analyses in male rats showed perturbation in biological pathways (BPs) involved in T-lymphocyte activation, differentiation and proliferation, as well as amino acid synthesis biosynthesis, and metabolism of glycine, serine and threonine. In female rats, GO analysis showed non-specific immune BPs involved in immune-cell activation, response and regulation, along with the suppression of peptide metabolism and impairment of cholesterol metabolism. The connection between molecular perturbations and immune response provided essential mechanistic linkages between exposure to a complex mixture of contaminants and adverse outcomes. This study revealed links between the potential health impacts and exposure to a complex mixture of contaminants in groundwater sourced from an industrial site.

1.13.P-Th-113 The Disruptive Potential of DEHTP and DINCH on Lipid Metabolism and Oxidative Stress in Zebrafish Larvae: A Comparative Study With DEHP

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Following bans on traditional phthalates such as DEHP, alternative plasticizers such as DEHTP and DINCH have been used increasingly, resulting in higher exposure levels, particularly during early developmental stages. Emerging studies suggest that these newer plasticizers may also pose health risks, potentially threatening metabolic health. These chemicals might affect the regulation of lipid metabolism, including cholesterol homeostasis, which is crucial for maintaining metabolic stability. Despite its importance, given the limited focus on cholesterol homeostasis in the context of these chemicals, this study aimed to investigate the impact of DEHTP and DINCH compared to DEHP. Zebrafish (*Danio rerio*) larvae were exposed to solvent control DMSO 0.01% (v/v) and these 3 chemicals at concentrations of 0.1 and 1 mg/L in dechlorinated water media for 7 days. The main endpoints measured at 7 dpf were TC and HDL-C in lipids and oxidative stress markers (CAT, SOD, MDA, and GPx). Additionally, genes such as *cyp51* , *sqlea,* and *fdft1* have been chosen to represent the cholesterol biosynthesis mechanism. The exposure results were analyzed using a one-way ANOVA test, and a statistically significant decrease in TC and HDL-C levels in DEHTP and DINCH treated groups was observed. On the other hand, DEHP indicated an increasing trend in the TC endpoint. Although all exposure chemicals indicate a potential disruption in cholesterol homeostasis, the trends of the traditional and alternative plasticizers differed in direction. The Dunnett contrasts revealed statistically significant effects, particularly in the comparison between the solvent control and the 1.0 mg/L exposure groups for DEHTP and DINCH. These results indicate that higher concentrations cause greater disruptions, as shown by the more negative estimates. These findings highlight the need for further research into the long-term health effects of DEHTP and DINCH, especially considering their rising exposure levels in vulnerable populations like children and infants.

1.13.P-Th-114 Perfluorooctanoic Acid (PFOA) Accelerates the Rate of *Drosophila melanogaster* Larval Development and Alters Adult Metabolism

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Per- and Polyfluoroalkyl substances (PFAS) are highly stable pollutants that pose a global risk to public health. One of the most prevalent PFAS molecules is perfluorooctanoic acid (PFOA), which contaminates the environment of all seven continents and pollutes the water supplies of over 100 million U.S. residents. PFOA is readily absorbed by our digestive system, sequestered within internal organs, and is resistant to both metabolic degradation and excretion. Despite the serious health concerns surrounding PFOA and related molecules, the mechanisms by which these pollutants influence animal development and metabolism are not well understood. To address this shortcoming, we used the fruit fly *Drosophila melanogaster*, which has historically been used as a powerful model to study metabolism and disease, to understand how PFOA alters development and metabolic homeostasis. Our studies revealed that levels of PFOA that are relevant to human exposure induce premature activation of genes involved in developmental timing and maturation. Consistent with this finding, PFOA induced a dose-dependent acceleration of larval (juvenile) development, which at the maximum dose caused animals to enter metamorphosis nearly a day earlier than controls. Intriguingly, this accelerated

development had no negative effect on body size, with exposed-female flies exhibiting a significant increase in body size. Additionally, larval PFOA exposure induced lasting metabolic effects on adults, causing a dramatic increase in acylcarnitine levels. Overall, our studies indicate that low doses of PFOA disrupts *Drosophila* growth and development in a manner that alters the timing of metamorphosis, thus raising the possibility that environmental PFOA contamination could have significant effects on wild insect populations.

1.13.P-Th-115 Identification of Functional Genetic Components Modulating Cellular Toxicity Response to PFAS Using Genome-Wide CRISPR Screens

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Per- and polyfluoroalkyl substances (PFAS) pose significant health risks to both human populations and the environment due to their high persistence and widespread presence. These risks arise from their strong chemical bonds, which contribute to their extensive use in a variety of products. PFAS exposure has been linked to numerous adverse health effects, including cancer, liver damage, reproductive and developmental disorders, and immunological impairments. Despite these known effects, the molecular mechanisms underlying PFAS toxicity remain poorly understood. While several '-omics' and physiological studies have provided molecular response profiles to various PFAS exposures, there is a lack of functional approaches to explore the causal links between human genotypes and PFAS-induced phenotypes. To address this gap, we performed genome-wide CRISPR-mediated genetic screens using the HepG2/C3A human liver cell line to identify genes and pathways that modulate cellular toxicity during PFAS exposure. We selected PFOA and PFOS for this study because they are among the most extensively studied legacy PFAS, with substantial evidence of hazardous effects from prior animal and epidemiological studies; however, their molecular toxicity mechanisms remain unclear. We employed a custom minimal genome sgRNA "knockout" library, consisting of 37,837 sgRNAs with two sgRNAs per gene, covering the entire genome, for the CRISPR screens. We carried out the screens in HepG2/C3A to identify candidate genes which modulated the cytotoxicity of each PFAS. Results showed that the PFOA screen identified 299 candidates, while the PFOS screen identified 285 candidates, with 38 targeted genes common to both screens. Pathway enrichment analyses of these targeted genes indicated that PFOA-related gene hits are enriched for the 'TIMM8A-TIMM13' and 'γ H2AX-Ku70-DNA' complexes, suggesting that loss of genes involved in normal neurological development and DNA damage response, respectively, influences the toxicity of PFOA exposure. Conversely, PFOS-related gene hits are enriched for the 'AXIN1-APC-β-catenin' complex, implicating the Wnt signaling pathway as a potential cellular mechanism responding to PFOS toxicity. The common genes identified in the screens for both PFOA and PFOS are associated with epigenetic regulation. We will validate these findings through single-knockout experiments targeting three top-ranked gene hits that are representative of the enriched pathways of interest.

1.13.P-Th-116 Sensitivity and Reproducibility of Transcriptomic Points of Departure for Diverse Chemicals From High Throughput Assays for *Daphnia magna*

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With limited resources available to conduct traditional toxicity assays on the ever-growing list of environmental contaminants, higher throughput tools that can provide a screening level assessment and identify compounds warranting higher tiers of testing are needed. The current study aims to compare transcriptomics-based points of departure (tPODs) derived for *Daphnia magna* exposed to 22 chemicals of varying modes of action to apical toxicity metrics such as LC50s and EC50s. This study also aims to examine the repeatability of tPODs in these high throughput experiments. For the high throughput exposures, 72h old *D. magna* were placed, one individual per well, in a 96 well plate and exposed to 8 concentrations of each chemical for 24 h. Whole body homogenates from three replicate plates were pooled for RNA sequencing (n=5 biological replicates of n=3 pooled organisms per treatment). Samples were analyzed with targeted RNA sequencing using the Biospyder platform, producing count data for 2378 gene targets. BMD Express 3.0 was used to fit concentration response curves for every gene, and a benchmark concentration at which expression differed from that of the controls was determined, where relevant. The tPOD was calculated as the 10th percentile of the benchmark concentrations for all genes that exhibited a concentration-dependent response (concentration responsive genes; CRGs). The resulting tPODs ranged across 7 orders of magnitude. The minimum tPOD across the 22 chemicals was 0.0048 µg/L for lovastatin, while the maximum tPOD, 4937 µg/L, was for lactofen. Methomyl and 3-trifluoro-methyl-4-nitrophenol (TFM) did not show any CRGs. Of the 22 chemicals tested, crustacean toxicity data was available in the ECOTOX knowledgebase for 13. Three of the chemicals' tPODs were higher than the apical PODs in EcoTox, suggesting these tPOD values were protective about 77% of the time. Three chemicals had duplicate exposures. Methomyl showed 0 CRGs in both exposures, aldicarb replicate tPODs were comparable by a factor of 4, and imazalil replicate tPODS were comparable by a factor of 2. We continue to explore the sensitivity and repeatability of the assay. *The contents of this abstract neither constitute nor necessarily reflect U.S. Environmental Protection Agency policy.*

1.13.P-Th-117 Fish Can Be Scared of the Dark: Lessons Learned From Developing a Light/Dark Preference Test To Assess Anxiety in Larval Zebrafish

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Exposure to chemicals during neurodevelopment has been linked to adverse impacts on mental health, including anxiety, but there does not appear to be an accepted, standard method to screen for anxiety in larval zebrafish (*Danio rerio)* after chemical exposure during development. The larval zebrafish light/dark preference test is, however, a promising option to fill this gap. Previous work has shown that larval zebrafish prefer illuminated environments and have an anxiety-related dark aversion, but there is no recommended protocol for the light/dark preference test to assess chemical impact. To adapt this assay, zebrafish embryos were reared in 24-well plates at 26°C. At 6 days post-fertilization, behavior was recorded using an infrared (IR) camera in a Viewpoint Zebrabox. Two different plate types were assessed: dark-sided, 24-well plate or a clear 24-well plate. In both cases, the microtiter plates were placed on a plexiglass base to create dark and light zones. Each base had stripes of black plastic covering half of each well that blocked visible light, creating darkness for the larvae, but transmitted IR light to permit video tracking. Larvae were given a 40-minute acclimation period in the dark, and then exposed to illumination (350 lux) for 10 minutes, followed by 10 minutes in the unilluminated condition (12 lux). Larvae tested in the dark-sided plates demonstrated a markedly stronger preference for the light than larvae tested in the clear-sided plates: in the dark-sided plates the larvae spent 75-85% of the time in the light side compared to the dark side, whereas in the clear-sided plates the larvae only spent 55-65% of the time on the light side. As expected, these differences were not observed in the unilluminated condition, with no visible light to create the zones. Pharmacological treatment using the dark-sided plates indicated that the light/dark preference was anxiety-related: acute treatment with an anxiolytic chemical (diazepam; 0.3 μM) decreased the amount of time the larvae spent in the light zone under the illuminated conditions. On the other hand, acute treatment with an anxiogenic chemical (caffeine; 125 μM) increased the amount of time the larvae spent in the light zone under the illuminated conditions. In conclusion, these findings suggest that the light/dark preference test is a replicable measure of anxiety, but there are experimental variables that affect the outcome. *This work does not reflect the official policy of the U.S. Environmental Protection Agency.*

1.13.P-Th-118 Microplastic Fiber Toxicity in Inland Silversides (*Menidia beryllina*) Exposed Alone or in the Presence of the Pacific Oyster (*Crassostrea gigas*)

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Microplastic fibers (MPF) are the predominate microplastic (MP) morphology detected in environmental samples. Yet, most toxicity data has been collected on MP particles, spheres, and fragments. To address these gaps the current research aimed to assess the impact of MPF exposure on a marine species that display different feeding strategies. Here, juvenile Inland Silversides (*Menidia beryllina*) and Pacific Oyster spat (*Crassostrea gigas*) were exposed to variable MPF concentrations for 21 days. After exposure, accumulation, tissue damage, changes in gene expression and alterations in growth and behavior were analyzed to assess toxicity. The Inland Silverside exposed to low (100), medium (1000) and high (10,000) MPF/L concentrations did not show differences in growth (length or weight). Similarly, there were no statistically significant differences in the number of MPFs accumulated between treatment groups. However, it was determined that there was a statistically significant difference in the feeding rate between the medium and high treatment groups, but there was no difference in the other groups. Oyster data thus far follows a similar trend with no observed impact in growth (weight, shell height or length) or in MPF accumulation. Currently, samples from both species are being prepared for qPCR analysis to evaluate changes in genes related to nutrition/ metabolism, growth and development, inflammation and oxidative stress. Additionally, oyster feeding assay video recordings are currently undergoing evaluation to determine filtration rates across treatment groups. Data from this project will provide key insights regarding concentration specific signs of toxicity, adding further information to the small pool of existing MPF data and that exposures may not lead to toxic effects. Furthermore, this information will help guide future risk assessments regarding MPFs in marine environments.

1.13.P-Th-119 Identifying Protein Expression Changes of the Fathead Minnow to the Cyanobacterial Toxin, Cylindrospermopsin

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Cylindrospermopsin (CYN), a water soluble tricyclic guanidine alkaloid, is an algal toxin produced by cyanobacteria in both freshwater and marine environments in response to eutrophication, climate change, forcing factors, and anthropogenic activities. Algal blooms are increasing in frequency, duration, and magnitude, but despite their widespread occurrence, mechanistic understanding of how CYN impacts organisms remains limited and high quality aquatic toxicology data is lacking. We leveraged prior research from our laboratory that reported behavioral, morphological, and gene transcriptional changes to a range of environmentally relevant concentrations of CYN (0.127- 1660 µg/L) using standardized EPA (EPA WET, 2002) guidelines for larval fathead minnows. As mRNA transcripts often have poor correlation with resulting protein expression- the direct mediator of phenotype- there is evidence that proteomics data may ultimately provide more robust information for assessments. Because proteome characterization is limited in fathead minnows (10 peer reviewed fathead minnow proteomic toxicology studies from 2010-2021), protein expression differences were explored using high resolution shotgun data independent acquisition (DIA) proteomics. Open-source bioinformatic analysis resulted in the identification of > 3000 total fathead minnow proteins which were compared to our previous apical morphology observations, photolocomotor behavioral data, and transcriptional RT-QPCR data highlighting molecular consequences of CYN exposure on proteins implicated in oxidative stress, hepatotoxicity, neurotoxicity, and DNA damage.
As protein interactions can be conserved across species, ongoing work will focus on comparing and contrasting proteomic responses between zebrafish and fathead minnow orthologs to explore the suitability of larval fish models to examine sensitivity across species.

1.13.P-Th-120 In Vitro and In Silico Prediction of Estrogenic Potency and Developmental Toxicity of Estrogenic Endocrine-Disrupting Chemicals Using Zebrafish

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The present study aimed to assess estrogenic potency of estrogenic endocrine-disrupting chemicals (E-EDCs) by *in vitro* and *in silico* approaches and the predictability of *in vivo* estrogenic potency and developmental toxicity using zebrafish. Diethylstilbestrol (DES), zearalenone (ZEN), and genistein (GEN) were used for an *in vitro* luciferase reporter gene assay in which expression plasmids of zebrafish estrogen receptors (zfERs) were transfected into COS-1 cells. The *in silico* simulations were also conducted to predict interactions between E-EDCs

and ligand-binding domains (LBDs) of zfERs. Developmental toxicity was assessed in zebrafish embryos exposed to DES, ZEN, and GEN at different concentrations. In the *in vitro* luciferase reporter gene assay, DES exhibited the highest transactivation potency to all zfERs among all tested compounds, with more preference for zfERα and zfERβ1 than that of zfERβ2. ZEN demonstrated almost equivalent transactivation potency to both zfERβ1 and zfERβ2, which is higher than zfERα. GEN displayed almost equivalent transactivation potency to all zfERs. The *in silico* predicted binding conformations within zfERs-LBDs showed a pattern where DES and ZEN bound at a position corresponding to Glu321/338/323 of zfERα/β1/β2, respectively. On the other hand, GEN had an interaction with Leu355/372/357 corresponding to zfERα/β1/β2, respectively. Positive correlations were exhibited for EC_{50} from *in vivo* induction of the estrogen-responsive *CYP19A1b* and interaction energy of E-EDCs to each of the zfERs from *in silico* docking simulation. A significant impact on cumulative mortality, hatchability, spinal and tail curvatures, pericardial edema, and reduced blood flow was found in DES-treated embryos, while ZEN or GEN exhibited lower incidence and severity of these effects. A binary exposure to an ER antagonist fulvestrant (FUL) with a fixed concentration of DES or ZEN revealed an ER-dependent regulation on the developmental toxicity elicited by these compounds. Conversely, GEN-mediated developmental toxicities were driven by distinct pathways from ER. Altogether, the *in vitro* zfER transactivation assay and the *in silico* simulations of interactions between ligands and zfERs may help predict the *in vivo* estrogenic potency of untested chemicals. The possibility of the *in vitro* ER transactivation assay and the *in silico* simulations to predict the *in vivo* potency of developmental toxicity warrants further investigation.

1.13.P-Th-121 Applying Systematic Review Data to the Adverse Outcome Pathway Framework: Expanding Evidence for Thyroid Serum Binding Protein Interferences Across Domains of Applicability

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Understanding the impacts of chemical exposures on ecological populations involves interpreting complex information encompassing different levels of biological organization. The Adverse Outcome Pathway (AOP) framework facilitates this process, by linking molecular initiating events (MIEs), through one or more key events (KEs), to adverse biological outcomes at the organism or population level. However, AOP development requires high-quality scientific data. Used increasingly in the field of ecotoxicology, systematic literature review (SR) aims to collect and integrate large bodies of data in a consistent, computable, and comparable way. Thus, there is great potential benefit in the development of workflows that incorporate SR data into AOPs. Transthyretin (TTR) is a thyroid distribution protein that transports thyroid hormone to target tissues and helps maintain hormone homeostasis. Several studies have highlighted the ability of environmental contaminants, including PCBs, PBDEs, and PFAS, to competitively bind to TTR in the bloodstream, displacing thyroid hormones and potentially disrupting normal thyroid signaling. To date, two AOPs have been developed based on this MIE; however, evidence for KE linkages in other species and lifestages is lacking. In this study, we explore both the application of SR data to existing AOPs and the use of SR data to develop new AOPs. First, data from a recent SR focused on collecting thyroid hormone serum binding information across species and lifestages at multiple biological levels were obtained, and a survey of the AOP Wiki was conducted to identify potentially relevant AOPs and MIEs. Three AOPs (AOP 152, 367, and 366) were chosen for having MIEs focused on interference with thyroid serum binding proteins. SR data were then manually processed and mapped to existing KEs. Given that the existing AOPs were developed in amphibians (AOPs 366 and 367) and humans (AOP 152), data supporting KEs and KERs from other species were used as evidence for domain of applicability and tagged for future development of putative AOPs. We standardized and manually mapped the data using corresponding KE information in the AOP Wiki as a source for endpoint terms. Where possible, we proposed standardized terms where they did not already exist. Overall, to make maximum use of data curation efforts, this case study showcases the potential use of SR data in the AOP framework.

1.13.P-Tu General: Environmental Toxicology and Stress Response

1.13.P-Tu-052 Genetics Studies of Xenobiotic Metabolic Enzymes UDP-Glucuronosyltransferase(UGT) and Sulfotransferases (SULT) in Elephants Reveals Unique Feature

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Elephants as the largest terrestrial mammals exhibit distinctive metabolic traits that warrant further investigation, particularly concerning their susceptibility to environmental toxins due to genetic adaptations in metabolic enzymes. Previous studies have indicated that certain isozymes of CytochromeP450, Phase I metabolic enzymes responsible for approximately 80% of human drug metabolism, have undergone genetic dysfunction (pseudogenization) in elephants. This uniqueness makes them potentially highly susceptible to environmental chemicals and toxic substances; however, the full extent of elephants' vulnerability to environmental chemicals remains inadequately explored. In this study, we delve into the Phase II metabolic pathway, focusing on UDP-glucuronosyltransferase (UGT) and Sulfotransferases (SULT), pivotal enzymes involved in the metabolism of various chemicals, both exogenous (such drugs and environmental pollutants) and endogenous (such steroids, fatty acids, and cholesterol). We examined the existing isoforms of UGT1-2 and SULT1-2 in African and Asian elephants by comparing annotated gene data from various species. Our results reveal notable species-specific differences, particularly significant diversification in the UGT2B isoforms, which are crucial for metabolizing plant flavonoids, known as steroid-like chemicals. Interestingly, we observed the absence of UGT1A1 in Asian elephants—a highly conserved enzyme responsible for bilirubin conjugation in most mammals—contrasting with its presence in African elephants. Furthermore, our analysis of SULT genes reveals distinct clustering patterns in Afrotheria species, also suggesting potential enzyme absences.Notably, SULT1B1, typically conserved in mammals, is identified solely in the aardvark and Cape elephant shrew among the Afrotheria group, where it participates in the metabolism of phenols and thyroid hormones. Similarly, SULT1D1 is exclusively found in African elephants among the two elephant species. This enzyme catalyzes the sulfation of dopamine, naphthol, and PNP, suggesting intriguing metabolic variations between elephant species. These findings shed light on the unique characteristics of elephant UGT1-2 and SULT1-2 genes, offering novel insights into elephant metabolism. Further research is necessary to reveal the functional aspects of elephant metabolic mechanisms, including

expression patterns, associations with drug sensitivities, and metabolite detection within physiological pathways.

1.13.P-Tu-053 Does AHR1 Genotype Predict Species Sensitivity to PAHs in Birds?

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Species differences in avian sensitivity to planar molecules, like dioxins, are explained by Aryl Hydrocarbon Receptor 1 (AHR1) genotype. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous chemical stressors that also bind to the AHR1; as such, these chemicals are thought to exert some predictable toxic effects through a similar mechanism of action. Yet, results from our group suggest that the AHR1 genotype does not predict species sensitivity in birds. In this project, we used an *in vitro* transactivationassay to determine the potency of PAH congeners to transactivate AHR1 among different bird species, and we determined whether the transactivation potential is predictive of *in vivo* PAH toxicity. A luciferase reporter gene assay was performed using full-length AHR1s cloned from four bird species (chicken (*Gallus gallus*), ring-necked pheasant (*Phasianus colchicus*), Japanese quail (*Coturnix japonica*) and double-crested cormorant (*Nannopterum auritum*)). Five PAH congeners previously shown to induce metabolic activity in cultured chicken hepatocytes and one methylated PAH were prioritized for use in this study. Our results demonstrate that 1) AHR1 *in vitro* transactivation by different PAH congeners in chicken correlates to *in vivo* measures of sensitivity, 2) AHR1 transactivation potential for benzo(a)pyrene (BaP) does not correlate with species sensitivity when defined by AHR1 genotype, 3) the rank order potency of different PAH congeners is the same regardless of AHR1 genotype, and 4) alkylated PAHs are more potent than parent compounds in transactivating AHR1 in birds. Taken together, our results indicate that this AHR1 transactivation potential does not explain differential species sensitivity to PAHs as predicted by their AHR1 genotype. It remains unclear how toxicokinetic and toxicodynamics influence species sensitivity to PAHs. More work is needed to connect AHR1 transactivation potential and mortality following PAH exposure in birds.

1.13.P-Tu-054 Obesogenic Effects of Bisphenol A by Disrupting Leptin Signaling Pathways in Human Neuronal Cells

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Bisphenol A (BPA), an endocrine disrupting chemical, has been recognized to cause a range of negative health effects, especially during early life exposure. Obesity is influenced by various factors, including behavioral, social, environmental, genetic, and economic variables. The hypothalamus, a component of the central nervous system, regulates energy equilibrium and food consumption to maintain the body's physiological needs. Multiple studies have demonstrated that the hypothalamus serves as the main location for the effects of endocrine regulators, such as leptin, in the regulation of hunger and body weight. Moreover, it is possible that leptin signaling affects the reward value of foods by acting on additional brain regions. This is supported by the existence of leptin receptors in various brain regions, including dopaminergic neurons. Recent studies indicate that leptin may regulate food intake and influence energy balance by stimulating dopaminergic neurons in the midbrain. This work aimed to investigate the connection between

BPA and leptin signaling pathways in dopaminergic neurons using human neuroblastoma SH-SY5Y cells. Cells were treated with varying concentrations of leptin or BPA, either alone or in combination. The assessment included measuring cell viability, levels of the orexigenic agouti-related peptide (AgRP), and the signal transducer and activator of transcription (STAT3) protein expression. Results indicated that the tested low concentrations of BPA (0.01–80 μM) did not have a significant effect on cell viability. Leptin significantly increased STAT3 expression in cells after 15min treatment. A specific STAT3 inhibitor (NSC74859) prevented this phenomenon. Exposure to BPA resulted in a notable increase in STAT3 phosphorylation 15min post-leptin treatment. Co-treatment of neuronal cells with leptin and BPA for 24 h resulted in the induction of STAT3 phosphorylation, which remained for up to 48 h. Leptin (100nM) decreased the levels of the orexigenic neuropeptide AgRP. Conversely, the presence of BPA led to a significant increase in AgRP levels. Simultaneous administration of leptin and BPA to neuronal cells led to a significant increase in AgRP levels that lasted for a duration of 24 h. These effects may contribute to the development of leptin resistance and the dysfunction of leptin signaling pathways, both of which are variables associated with obesity. Therefore, BPA may contribute to the risk of obesity. Supported by Title III.

1.13.P-Tu-055 Identifying Misclassified Mutagens to Enhance the Environmental Protection Agency Regional Screening Levels

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The United States Environmental Protection Agency (EPA) Regional Screening Levels (RSLs) website offers screening levels for residential exposures to chemicals in soil, air, and tap water (drinking water), safeguarding human health against noncarcinogens and carcinogens. Some carcinogenic chemicals operate through a mutagenic mode of action (MOA), potentially inducing irreversible DNA changes and exerting a stronger impact during early-life exposure. To address this, the RSLs provide age-adjustment factors for child age segments (0-2, 2-6, and 6-16 years) as an additional margin of safety for sanctioned mutagens. A former EPA website (last updated in 2015) sanctioned 19 mutagens and offered guidance for identifying additional mutagens; however, only 8 additional chemicals have been labeled as mutagens for the RSLs since the website's removal. This current study aims to: 1) demonstrate that an RSL may be up to two orders of magnitude lower (more conservative) when calculated as a mutagen rather than a regular carcinogen, 2) explore the mutagenicity potential for the 247 carcinogenic chemicals currently listed as non-mutagenic in the RSLs, and 3) advocate for EPA to calculate RSLs for newly identified mutagens using existing mutagenic age-adjustment factors. The first phase involves using the RSL calculator with both standard carcinogenic and mutagenic equations for all carcinogens in the RSL database and comparing the results. For the second phase, toxicity profiles for the existing RSL carcinogenic values will be scrutinized for mutagenicity indications. If MOA information is missing or inconclusive in the source profile or other EPA toxicity sources, the European Chemicals Agency (ECHA) Carcinogens and Mutagens Directive (CMD) and National Institutes of Health (NIH) National Toxicology Program Chemical Effects in Biological Systems (CEBS) and PubChem databases will be consulted. Ultimately, this study seeks to urge EPA to reinstate and updated its list of sanctioned mutagens to ensure RSLs incorporate applicable mutagen equations, thus enhancing EPA risk assessments.

1.13.P-Tu-056 Assessing Trace Metal Bioaccumulation in Several Fish Species Near A Coal Ash Landfill (Dumfries, VA)

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Coal combustion residuals (CCRs) are rich in persistent trace metals which can leach into adjacent waterways from unlined or lining-compromised repositories. Several of these contaminants function as neurotoxins, hepatotoxins, and/or carcinogens, with demonstrated adverse effects on aquatic organisms and human consumers. Possum Point Power Station (Dumfries, VA) is an inactive coal-fired power plant located by Quantico Creek and the Potomac River. Elevated concentrations of trace metals have previously been found in surficial sediments and banded killifish (*Fundulus diaphanus*) adjacent to and downstream from the station which have been shown to biomagnify across trophic levels. As such, this research evaluates the presence and concentration of trace metals in several piscivorous fish species near Possum Point Power Station. Northern snakeheads (*Channa argus*), blue catfish (*Ictalurus furcatus*), and bluegill sunfish (*Lepomis macrochirus*) were sampled from Quantico Creek and upstream reference site Powell's Creek. Epaxial muscle and gonad tissues were dissected from whole fish and stored at -8°C. Tissues were oven-dried, weighed, digested in 65% HNO_3 followed by 30% H_2O_2 and analyzed for 13 CCR-associated trace metals (Al, As, B, Cd, Ca, Cr, Cu, Fe, Mg, Mn, Pb, Se, Zn) using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). While this study is still ongoing, our results will allow us to compare uptake between locations, species, and tissue types and assess potential risks to both ecosystem function and commercial or subsistence anglers.

1.13.P-Tu-057 Comparison of Four Common Oral Bioavailability Methods for Measurement of Arsenic, Antimony, Cadmium, and Lead Bioaccessibility in Soil

Grant Walter *and Rachel Zajac-Fay, Geosyntec Consultants*

Oral bioavailability is defined by the USEPA as the fraction of ingested dose that crosses the gastrointestinal epithelium and is available for distribution to target organs, and bioaccessibility is defined as the fraction available for absorption. Human health risk assessments use a default assumption of 100% oral bioaccessibility/bioavailability, which often results in overestimation of risk. However, USEPA guidance and individual investigations of metals indicate oral bioavailability/bioaccessibility is less than 100%. Multiple invitro methods have been generally accepted to accurately estimate oral bioaccessibility of site-specific relative bioavailability (RBA) of metals in soil. However, detailed review and comparison of validation studies using invitro and invivo methods to estimate oral RBA of metals in soil has not been performed. For this study, four invitro methods were selected for review of oral RBA estimates for arsenic, antimony, cadmium, and lead in soil. The four methods reviewed are the invitro-gastrointestinal method (IVG), the Solubility/Bioaccessibility Research Consortium method (SBRC), the physiologically based extraction test method (PBET), and the Unified Bioaccessibility Research Group of Europe Method (UBM). Twelve validation studies comparing IVG, SBRC, PBET, and/or UBM invitro methods to invivo models were identified and soil RBA estimates (both gastric and intestinal phases) were extracted for comparison. The average estimated gastric RBA percentage (intestinal RBA) for arsenic, cadmium, and lead using IVG were 49(28), 62(38.5), and 24.6(0.6), respectively. The average estimated gastric RBA percentage (intestinal RBA) for arsenic and cadmium using PBET were 36.2(19.1) and 64(38.2), respectively. The average estimated RBA percentage (intestinal RBA) for arsenic and cadmium using SBRC were 44.6(19.8) and 85.7(59), respectively. The average estimated RBA percentage (intestinal RBA) for arsenic, antimony, cadmium, and lead using UBM were 29(21), 7.5, 55.9(28.4), and 49, respectively. Overall, IVG, PBET, SBRC, and UBM methods underestimated oral bioavailable concentrations, but were within two times the invivo RBA estimated concentrations. Site-specific RBAs for metals can be evaluated using invitro methods of estimating oral bioavailability that avoid the complication and expense of animal testing, and should consider the potential underestimation of oral RBA of metals in soil.

1.13.P-Tu-058 Effects of Palladium (Pd) and Platinum (Pt) Contaminated Sediments on Freshwater Invertebrates *Chironomus riparius* and *Hyallella azteca*

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Platinum group metals (PGMs) like platinum (Pt) and palladium (Pd) are strategic elements given their essential role in automotive, electronics, and medical fields. The rising PGM concentrations in aquatic environments is posing threats to benthic organisms due to lacking environmental regulations. Understanding PGM behavior and toxicity in aquatic ecosystems remains limited. To address these gaps, we aimed i) to assess the acute and chronic toxicity of Pd and Pt individually in *Hyalella azteca* and *Chironomus riparius*, ii) to determine the interaction and the resulted effects in *Chironomus riparius* when both metals are combined in mixtures. Both species were exposed to Pd and Pt individually for 14 or 10 days respectively, following standard protocols. Parameters such as survival, growth, and bioaccumulation were measured, and toxicity indicators (EC$_{50}$, LC$_{50}$) were estimated from the dose-response curves. Moreover, *C. riparius* underwent a 28-day exposure (chronic) to evaluate survival, sex ratio, and emergence time. Additional experiments included exposing *C. riparius* to sediments containing mixture of Pd and Pt using isobologram approach. Metal concentrations in sediments and organisms were measured by ICP-QQQ. Our survival results (LC$_{50}$ in µg.g^{1}dw) indicated that Pt (*C. riparius*: 187.8 ± 0.1; *H. azteca*: 520.1 ± 0.1) was found to be more toxic than Pd (*C. riparius*: 283.0 ± 0.3; *H. azteca*: 2626.5 ± 0.4) for both organisms. However, when examining growth rate (EC$_{50}$ in µg.g^{1}dw), Pd (*C. riparius*: 216 ± 13; *H. azteca*: 106.2 ± 0.6) was more effective than Pt (*C. riparius*: 1120 ± 1; *H. azteca*: 927.5 ± 0.2). For chronic exposures in *C. riparius*, Pt demonstrated higher toxicity (LC$_{50}$ in µg.g^{1} dw) compared to Pd (Pt: 75.2 ± 0.1; Pd: 163.8 ± 0.9). In contrast, Pd exhibited greater toxicity than Pt when considering the emergence time (days) of the organisms (Pt: 13164 ± 8; Pd: 40 ± 23). Sex ratio analysis revealed no significant difference. These findings indicate that the toxicological effects of these metals differ depending on the measured endpoint. Ongoing mixture experiments will provide further insights into the interaction of both metals. This study offers innovative toxicological data on Pd and Pt in sediment habitats, illustrating effects from both immediate and prolonged exposures. Subsequent research will concentrate on elucidating the mechanisms underlying the toxic effects of these metals.

1.13.P-Tu-061 Modeling Temperature-Dependent Chronic Toxicity of Thiamethoxam in Chironomids with Realistic Dynamic Exposure Profiles

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Freshwater invertebrates, particularly chironomids, are among the most sensitive taxa to neonicotinoid insecticides, such as Thiamethoxam (TMX). To address the need for a comprehensive assessment of the chronic toxicity of TMX to chironomids, we developed an individual-level mechanistic effect model for *Chironomus riparius*, based on Dynamic Energy Budget (DEB) theory, that integrates time-varying temperature and chemical concentration by the incorporation of a toxicokinetic-toxicodynamic (TKTD) module. Our model was calibrated and validated using laboratory experiments that considered both constant and variable temperatures and TMX exposure profiles. We employed a moving-time-window (MTW) approach to simulate the life cycle of *C. riparius* under various exposure scenarios. This method allowed us to determine critical combinations of life stage sensitivity and chemical exposure, particularly useful when the life cycle of the test organism is shorter than the existing exposure profiles used in risk assessment. The endpoints evaluated included survival, growth, time to emergence, and potential reproduction, with a focus on "survival at pupation" as a combined sublethal and lethal effect endpoint, considering the timing of pupation's dependency on toxicity. Furthermore, we quantified potential risk using exposure multiplication factors (EMFs) to determine a pre-defined effect strength, analogous to summary statistics like EC_X, using worst-case TMX exposure profiles obtained from a geographically-wide monitoring program (AEMP). Our investigation also focused on the influence of different temperature integration approaches (constant vs. dynamic temperatures) on predicting EMFs as risk indicators, contributing to a better understanding of the effects of exposure in environmentally dynamic conditions.
In conclusion, our study provides a rigorous investigation of the combination of toxic stress and temperature-dependency using tailor-made extensive experiments, ultimately reducing uncertainty in predicting the risk of TMX exposure to chironomids.

1.13.P-Tu-062 Effects of Glyphosate on Mitochondrial Function and Cell Apoptosis in Human Kidney Cells

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Glyphosate is the most frequently used herbicide in the world. Glyphosate residues found in the urine and blood of pregnant women, children, and workers in both agricultural and non-agricultural fields suggest a likelihood of prolonged environmental exposure. The unique anatomy and physiology of the kidneys make them vulnerable to toxic xenobiotics. The kidneys are also susceptible to oxidant damage because they heavily depend on the mitochondria and energy to support the transport function of the nephron. The proximal tubules possess a greater number of mitochondria compared to other cell types in the kidney, which aids in their ability to perform active transport and secretion. This study aimed to examine the cytotoxic effects of glyphosate on human renal proximal tubule epithelial cells (RPTEC/TERT1). The cells were exposed to glyphosate at concentrations ranging from 0.00001 to 10 mM for up to 24 hours. Cell viability, lactate dehydrogenase (LDH) levels, apoptosis, reactive oxygen species (ROS) production, cytochrome c release, and ATP levels were assessed. Glyphosate exposure resulted in a concentration-dependent decrease in cell viability. Glyphosate exposure resulted in elevated LDH release, suggesting potential harm to the plasma membranes of human RPTEC cells. After 6 hours, even at low concentrations of glyphosate, ROS levels in RPTEC cells were greater compared to 3 hours. Exposure to the antioxidant N-acetyl-L-cysteine (NAC) significantly decreased the ROS levels induced by glyphosate in RPTEC cells, indicating that this effect is due to glyphosate. Exposure to glyphosate increased caspase-3 activity in a concentration-dependent manner. Treatment of RPTEC cells with NAC or caspase-3 inhibitor, AcDEVD-CHO, significantly reduced glyphosate-induced apoptosis. This suggests that Glyphosate induces apoptosis through the caspase-3 pathway. Elevated cytochrome c levels were observed in glyphosate-treated cells. The cellular level of ATP was reduced after exposure to glyphosate. These results imply that loss of mitochondrial function and induction of oxidative stress play a contributing role in nephrotoxicity of glyphosate, and that glyphosate negatively impacts kidney function. Glyphosate may thus be a potential hazard to human health.

1.13.P-Tu-063 Metabolomics Unveiled: Exploring PFAS Impacts on Honeybees Through Citizen Science

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Two key challenges facing regulatory bodies are 1) detecting and monitoring the presence of per- and polyfluoroalkyl substances (PFAS) within the environment and, 2) effectively assessing their risk to organisms at field-realistic concentrations. Therefore, it is critical for regulators to examine the presence, movement and effects of PFAS within an Australian context. While levels in water and soil can provide baseline information on the scale of PFAS contamination, biomonitors can provide an evaluation of exposure. European honeybees (*Apis mellifera*) have unique characteristics that make them an ideal candidate as a biomonitoring species for terrestrial ecosystems. They are widely distributed, globally important pollinators that effectively sample the environment surrounding a colony. The accumulation of PFAS within these species could have sublethal effects, which leads to changes in biomolecular profiles that can be assessed via metabolomics (the study of small biological metabolites such as fats, sugars and amino acids). Non-traditional end-points such as metabolomics are often overlooked in risk assessments. In that context, this study aimed to assess the types and concentrations of PFAS found in honeybees from 21 Citizen Scientist beekeepers around Melbourne, and trial the use of metabolomics to understand if the concentrations of PFAS found are impacting metabolomic pathways. The results will improve our understanding of the presence, fate and chemical transport of PFAS within the environment, which will enhance environmental risk assessment and regulatory guidelines.

1.13.P-Tu-064 Does Exposure to PFAS-Free Aqueous Film-Forming Foams Impact Growth and Feeding in the Juvenile Hard Clam, *Mercenaria mercenaria*?

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One major source of environmental per- and polyfluoroalkyl substances (PFAS) is through the use of traditional aqueous film-forming foams (AFFFs), fire suppression products used in various industries. PFAS are a class of ubiquitous environmental contaminants that have demonstrated chronic and sub-lethal effects on biota. As a result of these effects, policy changes have mandated reductions in PFAS-containing AFFF use. These directives highlight the general trend away from PFAS-containing products and processes. For example, the US Department of Defense began migration to PFAS-free products in October 2023. While studies have documented acute and chronic effects of these products, there is little known about the long-term sub-lethal effects of these new PFAS-free AFFFs. The objective of this study is to quantify the effects of seven AFFFs on growth and algal uptake in the juvenile hard clam, *Mercenaria mercenaria.* Five of the PFAS-free AFFFs negatively impacted growth over a 21-day chronic exposure period while one PFAS-free AFFF and a reference PFAS-containing AFFF had no observable effect. Median effective concentrations (EC50) for shell growth ranged from 5.81 mg/L to > 100 mg/L. Dry and wet weights also significantly decreased with increasing exposure concentration ($p < 0.05$). These toxicity trends are expected to also be observed through inhibition of algal uptake over a 7-day exposure. The results presented here identify potential chronic effects of exposure to these replacement AFFFs in this economically and ecologically important bivalve species. These results can be used to inform decisions regarding PFAS replacement AFFF products.

1.13.P-Tu-066 Towards Comprehensive Understanding and Analysis of Micro- and Nanoplastics Utilizing Libraries Reflecting Environmental Complexity: Cytotoxicity Study of Surface Oxidated Micro- and Nanoplastics

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The presence of microplastics (MPs; particles less than 5 mm) and even smaller nanoplastics (NPs; particles less than 1 μm) in the environment raises concerns about negative biological impacts. Detection of MPs and NPs in various environments, including air, oceans, and surrounding environments like table salt and drinking water, emphasizes the ubiquitous presence of MPs and NPs. In addition, the detection of MPs and NPs in human tissues, including the lung, placenta, plaque, and feces, underscores inevitable human exposure, emphasizing the critical need for assessing health risks. However, environmental MPs and NPs exhibit a wide range of physicochemical properties, including size, shape, and surface chemical modifications (such as degradation due to UV radiation). In contrast, laboratory studies often utilize uniform MPs and NPs, neglecting their complex environmental physicochemical properties. Considering that the hazard and kinetics of MPs and NPs are influenced by their physicochemical properties, it is imperative to account for real environmental conditions in safety assessment. However, standardized samples of MPs and NPs suitable for such investigations are currently lacking worldwide. This study aims to develop libraries of MPs and NPs that accurately reflect their complex environmental attributes. Polyethylene (PE) and Polyvinyl chloride (PVC) were selected as polymer types due to their significant global production. Fragmented particles

ranging from 20 to 250 µm in diameter were obtained for each polymer, and surface-oxidized MPs and NPs were generated by irradiating them with vacuum UV light at 172 nm under air to mimic environmental conditions. ATR-IR analysis revealed the presence of hydroxy and carbonyl groups in surface. To produce NPs, a previously published precipitation-based method was employed. Scanning electron microscopy confirmed the successful generation of NPs. These libraries were then utilized to conduct cytotoxicity tests. Interestingly, the results revealed that surface-oxidized MPs and NPs exhibited higher cytotoxicity compared to non-surface oxidized counterparts. These libraries will facilitate comprehensive safety evaluations through oral and inhalation exposure tests, as well as various hazard analyses. They will be made available for distribution upon request through future collaborations.

1.13.P-Tu-067 Analyzing Oxidative Stress and Bioactivation in a Humanized *Caenorhabditis elegans* upon Exposure to the Environmental Pollutant Styrene

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Reactive oxygen species (ROS) are crucial to human physiology and are used in many processes, including cell signaling and mitochondrial cellular respiration. However, ROS overproduction can lead to oxidative stress. Continuous oxidative stress has been linked to numerous diseases including diabetes, arthritis, cardiovascular issues, and inflammation. Pollutant bioactivation can contribute to the formation of ROS and oxidative stress. The human enzyme CYP2E1, located on the endoplasmic reticulum (ER) and mitochondria (Mt), facilities the metabolism and occasional bioactivation of small organic pollutants. The aim of this project is to explore whether human ER and/or Mt CYP2E1 expressed within the nematode *Caenorhabditis elegans* (*C. elegans*) leads to increased oxidative stress upon exposure to styrene as a probe pollutant and CYP2E1 substrate. In addition, we aim to establish whether subcellular location (i.e., mitochrondria vs. endoplasmic reticulum) leads to differences in biological response after pollutant exposure. *C. elegans* viability and response to oxidative stress was visually assessed using microscopy in three methods: survival, antioxidant rescue, and fluorescence (CellRox Deep Red) assays. The survival assays showed an overall decrease in viability from 0–99% in 0–16mM styrene-exposed worms. The antioxidant assays demonstrated the mechanism of styrene toxicity was linked to oxidative stress and showed that worm viability depended on the location of CYP2E1. The fluorescence assay proved to be the most sensitive method, allowing for visualization of ROS in styrene exposed worms. ROS levels decreased following antioxidant incubation: up to 4x lower in Mt-CYP2E1 strains and 2x lower in ER-CYP2E1 compared to worms without antioxidants. These assays provide a direct link between styrene exposure and ROS over-production, leading to oxidative stress. Further experiments will include measurements of the styrene CYP2E1 product, styrene oxide (via GC-EI-MS), to confirm the presence of the bioactive metabolite. Results of this study will contribute to strengthening the mechanistic link between styrene exposure and oxidative stress as well as differences in CYP2E1 subcellular location activities using a novel humanized animal model.

1.13.V General: Environmental Toxicology and Stress Response

1.13.P-Tu-068 The Effectiveness of Biopolymer-Based Nanocomposites Against Potential Bacterial Pathogens Isolated from Wastewater Environment

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Traditional wastewater treatment technologies have proved costly and ineffective. This is a concern as pathogenic microorganisms mostly found in wastewater environment have developed resistance against conventional techniques. The use of biopolymer-based nanocomposite materials as alternative antimicrobial agents have drawn substantial attention in research. The present study was aimed to evaluate antimicrobial activity of biopolymer-based nanocomposites against bacterial pathogens isolated from domestic wastewater environment. Developed biopolymer nanocomposite pCh-MWCNTs@Ag-TiO2 and the intermediate product (pCh-MWCNTs) were obtained from Vaal University of Technology. Antimicrobial activity of the two biopolymers against bacterial species (*Escherichia coli* and *Staphylococcus aureus*) was determined using agar well diffusion and Minimum Inhibitory Concentration methods. Different concentrations of biopolymers (7 mg/mL, 3.5 mg/mL, 1.75 mg/mL) were subjected to the test organisms. The zone of inhibition was observed only at developed Ch-MWCNTs@Ag-TiO2 (7mg/mL) against *S. aureus*. As compared to pCh-MWCNTs, lower MIC values were seen for pCh-MWCNTs@Ag-TiO2. Furthermore, lower MIC values were observed against *S. aureus* than *E. coli*. The results in the study showed that the developed Ch-MWCNTs@Ag-TiO2 was more effective than pCh-MWCNTs. The two biopolymers were also more efficient against S. *aureus* than *E. coli.* The present study champions for Sustainable Development Goal 6, which stipulates on the provision of clean drinking water and sanitation for all, by 2030. Currently, nanotechnology is considered the most favourable approach for wastewater decontamination, thus warrants further research.

1.13.V-01 Distribution, Bioaccessibility, and Human Health Implications of Potentially Toxic Elements in Top Soils through Gold Mining in the Obuasi Municipality of Ghana

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Gold mining, particularly artisanal and small-scale mining, has increased rapidly due to a lack of alternative employment opportunities, the potential for high earnings, community development, and a lack of proper regulations. While mining provides economic benefits to individuals and communities, it poses numerous challenges, including environmental degradation, health and safety risks, conflicts over land and resources, and releasing hazardous elements into the environment. Potential toxic elements emanating from extracted ores during gold processing present occupational and unintentional health hazards in communities, the general populace, and the environment. This study investigated the concentrations and potential human health effects of metal content in the top soils of Obuasi municipality, which has been mined for gold over the past century. Topsoil samples were collected with a clean dry plastic shovel, screened, and analyzed for their elemental concentrations using Niton XL3t GOLDD+ field portable X-ray fluorescence (FP – XRF) spectrometer, and the results were validated with an ICP-MS. The spatial distribution pattern of the potentially toxic elements was ascertained with the aid of ArcGIS. The metal enrichments in the study area exceeded those in the control site. However, the Cd, Cu, Ni, Pb, and Zn levels were lower than the baseline values for Canadian soil quality. There was a significant enrichment of Cu, Pb, Ni, and Zn in the eastern part of the municipality, where illegal artisanal mining is prevalent. Additionally, the southern corridor, situated near a gold mine, exhibited significant levels of As and Mn. Results obtained for the total metal concentrations and contamination indices confirmed the elevation of the studied potentially toxic elements in the Obuasi community. A hazard index value of 4.42 and 3.30 among children and adults, respectively, indicates that indigens, especially children, are susceptible to non-cancer health effects.

1.14.P-We Linking Molecular Impacts to Organism Health: Empirical and Theoretical Methods to Scale Contaminant Effects

1.14.P-We-040 A Quantitative Adverse Outcome Pathway for Embryonic Exposure of Fishes to Polycyclic Aromatic Hydrocarbons Leading to Decreased Fecundity at Adulthood

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Quantitative adverse outcome pathways (qAOPs) describe the response-response relationships that link the magnitude and/or duration of chemical interaction with a specific molecular target to the probability and/or severity of the resulting apical-level toxicity of regulatory relevance. The present study developed the first qAOP for latent toxicities where early life exposure adversely affects health at adulthood. Specifically, a qAOP for embryonic activation of the aryl hydrocarbon receptor 2 (AHR2) of fishes by polycyclic aromatic hydrocarbons (PAHs) leading to decreased fecundity of females at adulthood was developed by building upon existing qAOPs for (1) activation of the AHR leading to early life mortality in birds and fishes and (2) inhibition of cytochrome P450 aromatase (CYP19) activity leading to decreased fecundity in fishes. Using zebrafish (*Danio rerio*) as a model species and benzo[a]pyrene as a model PAH, four linked quantitative relationships were developed: (1) early life mortality as a function of sensitivity to activation of the AHR2 in a standardized in vitro AHR transactivation assay, (2) plasma estrogen in adult females as a function of embryonic exposure, (3) plasma vitellogenin in adult females as a function of plasma estrogen, and (4) fecundity of adult females as a function of plasma vitellogenin. Predictions from the resulting qAOP were validated using experimental data from zebrafish exposed as embryos to another PAH, benzo[k]fluoranthene. The qAOP developed and validated in the present study demonstrates the potential of the AOP framework in supporting linkages between cause and effect for latent toxicities and in enabling consideration of these toxicities in quantitative ecological risk assessments and regulatory decision-making.

1.14.P-We-041 Not Another Gene-Network Hairball! Leveraging Chemical-Gene Interaction Data to Better Understand and Identify Genomic Responses to PFAS Exposure

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Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent and globally ubiquitous contaminants. Exposure to PFAS has been associated with adverse outcomes in humans and wildlife, however, the specific adverse outcome pathway/s (AOPs) that cause these effects remain poorly understood. Additionally, PFAS usually occur as complex mixtures of multiple PFAS structures and co-occurring non-PFAS contaminants. Transcriptomic tools are becoming more common in PFAS effects assessments, but holistic interpretation of these large datasets is challenging. Here, we leveraged existing chemical-gene interaction data from the

Comparative Toxicogenomic Database (ctdbase.org) to create a network representation of the known genetic responses to PFAS exposure. This network provides an initial framework for the following objectives. 1) A canonical PFAS exposure gene set will be developed. This gene set will represent genes that are commonly affected by exposure to legacy PFAS such as PFOS and PFOA. This gene set will then be used to analyze transcriptomics data for the genetic signature of PFAS exposure, using gene set enrichment analysis. The ability to screen transcriptomics data set for a signature PFAS response would be a valuable tool for environmental monitoring. 2) The PFAS gene network will be used for gene ontology analysis to identify specific biological processes and molecular pathways that are impacted by PFAS exposure. This will provide a better physiological context for interpreting gene expression data and may help identify key events in the PFAS AOP(s), 3) The network will be used to identify genes that are similarly impacted by multiple different PFAS. These genes are likely to be involved in mixtures affects and identifying these genes will be useful for predicting effects of mixture exposures. Ultimately, a better understanding of the genomic impacts of PFAS exposure may be useful for environmental screening, AOP development, and predicting mixture effects.

1.14.P-We-043 Unraveling PFOS's Subtle Impact in Smallmouth Bass Using Both Traditional Toxicology and Omics-Based Endpoints

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Smallmouth Bass (SMB, *Micropterus dolomieu*) are economically important, freshwater game fish found throughout North America. This species is a focus of research due to recent disease and mortality events observed in some areas of the northeastern United States. PFOS levels measured in the plasma of Smallmouth Bass collected in the Chesapeake Bay Watershed ranged from 20-574 ng/mL. In comparison, PFOS levels measured in muscle tissues from Smallmouth Bass collected nationwide ranged from 0.35 – 110 ng/g. Studies conducted for more than a decade have documented episodic mortality events, high prevalence of skin lesions, endocrine and reproductive disruption in populations of Smallmouth Bass collected in various watersheds. Many of these studies indicated this species is likely subjected to multiple stressors and environmental contaminant exposures, yet no single chemical has been clearly associated with observed impacts. The objectives of this study were to conduct sublethal health assessments in early life stage SMB following exposures to environmentally relevant concentrations of PFOS. Smallmouth Bass embryos (24-hpf) were collected from existing brood stock and cultures at the Columbia Environmental Research Center. Exposures (control, low, high treatments) were conducted for a minimum duration of 30-dph in large flow through water systems with pulsed intermittent diluters. The selection of PFOS concentrations is based on surface water concentrations from recent national surveys and highly impacted sites where SMB are found. In this study, the low exposure concentration is the 95th percentile of PFOS concentrations (24 ng/L) reported in nationwide surface water. The high exposure concentration (96 ng/L) represents PFOS concentrations found in surface water from Ashumet Pond, a highly PFAS-impacted site located in Massachusetts. Traditional toxicology and omics-based endpoints (survival, growth, transcriptomics, microbiome, gene transcript abundance, histopathological changes, behavioral assays) were used to unravel the subtle impacts of chronic PFOS exposures in early life stage Smallmouth Bass.

1.14.P-We-044 Histone Methylation-Mediated Reproductive Toxicity and Multigenerational Effects of Environmental Chemicals in *C. elegans*: Epigenetic Adverse Outcome Pathway (AOP) and Cross-species Extrapolation

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The importance of histone methylation in epigenetic inheritance underscores its relevance to disease and the chronic effects of environmental chemicals. However, our understanding of the causal relationships between chemical-induced histone methylation alterations and organismal level effects remains limited. This study explores the contribution of repressive histone marks to the reproductive toxicity of environmental chemicals in *Caenorhabditis elegans* under the adverse outcome pathway (AOP) framework and their applicability in ecotoxicological assessment through cross-species extrapolation. Triclosan (TCS) and tetrabromobisphenol A (TBBPA) exposures caused reproductive toxicity and altered histone methyltransferase (HMT)/histone demethylase (HDM) activities with increased global methylation of H3K9 and H3K27. Notably, treatment with H3K27-specific HMT inhibitor alleviated reproductive defects and transcriptional response of genes related to vitellogenin, xenobiotic metabolism, and oxidative stress. Comparison of points of departure (PODs) based on calculated benchmark concentrations (BMCs) revealed the sensitivity of histone-modifying enzyme activities to these chemicals. Collectively, 'the disturbance of HMT and HDM' can serve as the molecular initiating event (MIE) leading to reproductive toxicity in the epigenetic AOP for TCS and TBBPA. Finally, the biological applicability of these enzymes was expanded by identifying common model species with analogous sequences and functions of proteins. The combined approach of selective inhibitor assay, BMC analysis, and protein conservation assessment enhances the essentiality, empirical support, and the taxonomic domain of applicability, which are crucial considerations for ecotoxicological AOP. Beyond a single generation, it is necessary to assess if chemical-induced histone methylation alterations persist and influence individual or population-level characteristics across multiple generations.

1.14.P-We-045 Advancing Ecotoxicological Studies: Utilizing New Approach Methodologies to Diminish Avian Testing and Enable Cross-Species Extrapolation

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Traditionally, ecological risk assessments of agrochemicals have relied on in vivo guideline tests involving northern bobwhite and mallard to establish relevant endpoints for avian species. However, these studies are encumbered by limitations such as animal welfare concerns, substantial time and cost requirements, and a lack of mechanistic understanding. The scarcity of work in this domain underscores the necessity to develop case studies that demonstrate the use of new approach methodologies (NAMs) to reduce in vivo avian testing. In a proof-of-concept effort, a case study was formulated based on physiologically based kinetic (PBK) modeling for thiamethoxam in three avian species. The model structure for northern bobwhite and mallard comprised ten compartments, with an additional ovulation model incorporated for domestic egg-laying chicken. Initially, the model was parameterized and calibrated for domestic chicken and northern bobwhite using in vitro kinetic measurements and in vivo toxicokinetic (TK) data. The anticipated time courses of compound concentration in blood, tissues, or eggs exhibited favorable concordance with independent in vivo data from domestic chicken and northern bobwhite. Subsequently, the evaluated domestic chicken model was extrapolated to mallard through allometric scaling. The model was then utilized to characterize the TK profiles across species by simulating internal concentration dose metrics linked to the median lethal dose (LD50) and points of departure in various avian toxicology studies. The findings revealed that mallard and northern bobwhite demonstrated comparable peak blood concentrations (Cmax) and area under the blood concentration curve (AUC) across acute, subacute, or chronic toxicity studies, enabling quantitative interspecies extrapolation of dosimetry. This case study represents an important step in refining the design of ecotoxicological studies and conducting extrapolations across species and routes. Not only does it potentially reduce the necessity for avian testing, it also facilitates in vitro-in silico-based environmental risk assessments of agrochemicals.

1.14.P-We-046 Effects of Carbamazepine to Early Life Stage Zebrafish (*Danio rerio*): Transcriptomics to Behavior

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The commonly consumed anti-epileptic drug, carbamazepine, is among the most frequently detected pharmaceuticals in the world's rivers and surface waters globally. Acute and chronic exposures to carbamazepine have been shown to induce sublethal effects to teleosts, although the effects at environmentally relevant concentrations have not been well characterized. To better understand potentially toxic effects of carbamazepine on early life stage fish, zebrafish (*Danio rerio*) were embryonically exposed to 1, 5, 10, or 50 µg/L of carbamazepine and collected for transcriptomic profiling at 48, 72, and 144 hours post fertilization (hpf). The top enriched gene ontology pathways in 48 hpf larvae were involved in eye development and visual perception, regardless of treatment concentration. In 72 hpf larvae, top enriched pathways were involved in visual perception, lens structure, and ATP synthesis, regardless of treatment. Larvae that were 144 hpf had enriched pathways involved in immune response in embryos exposed to 1-10 µg/L carbamazepine, with the highest dose exhibiting changes involved in skeletal system and organ development. Subsequent samples were assessed for vision-specific behavioral assays to anchor transcriptomic results to higher levels of biological organization. There was a significant decrease in the number of eye saccades during an optokinetic response assay in larvae that were embryonically exposed to carbamazepine. These results suggest that carbamazepine can induce sublethal effects to the visual system, influence immune function responses, and impair skeletal development during the early life stage, reducing visually-mediated behavioral function, which may have implications for fish at concentrations commonly reported in natural systems.

1.14.P-We-047 New Approaches Improve Ecological Risk Assessment by Incorporating Omics into Bioenergetic Models: A Case Study of Daphnia Exposed to a Coal Ash Mixture

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The ecological risk of a chemical is traditionally measured by exposing a single organism to a single chemical in idealized lab conditions through standardized toxicity testing. These tests have many drawbacks that mostly stem from the inability to use this data to predict effects outside of the specific empirical parameters of the test, such as the impacts of other chemicals or mixtures or to extrapolate effects to an ecologically relevant outcome. At the other end of biological organization, novel molecular techniques yield increasing amounts of subcellular data. This information can potentially be used to better understand the individual-level impacts of a chemical by connecting exposure to an altered molecular pathway or mode of action, agnostic of any specific chemical or organism. Here we describe a case study looking at the effect of coal ash, a mixture of numerous metals known to exert toxicological effects, on a model freshwater organism, *Daphnia magna*. We exposed *D. magna* to coal ash through dietary algal exposure and measured impacts on survival, growth, and reproduction for 28 days. We measured the transcriptomic response at multiple time points, enabling us to analyze the suborganismal response of the *Daphnia* to coal ash exposure throughout their life cycle. We then modeled the response of *Daphnia* to coal ash using a Dynamic Energy Budget (DEB) model, testing various potentially impacted physiological modes of action (pMoAs). Instead of identifying the pMoA through traditional DEBtox methods which relies on only individual-level data, we correlated these model simulations with significantly differentially expressed genes to identify the best candidate pMoA based on the molecular coal ash signal. For each state variable in the DEB model, we applied a variable selection approach (using ARACNE) to identify a set of genes predictive of the state variable. We then sorted the genes associated with this variable selection approach and added them to the ARACNE model using machine learning. The model with the best fit to the DEB state variables (as measured by R^2) was selected. This process using transcriptomic data and DEB modeling identified the pMoA of "increased costs of reproduction" as the best description of the impact of coal ash on *Daphnia*. This case study represents an exciting development in using suborganismal data to quantitatively identify the bioenergetic mode of action of complex chemical exposure in a model freshwater organism.

1.14.T Linking Molecular Impacts to Organism Health: Empirical and Theoretical Methods to Scale Contaminant Effects

1.14.T-01 Early Life Exposure to Endocrine Disruptors: Understanding the Neuro-Endocrine Impact from Metabolome Mapping of Rat Models

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Exposure to endocrine-disrupting chemicals (EDCs) in humans during pregnancy and early stages of life can impair normal brain development and reproductive function patterns, leading to severe pathologies later in life. Studies have shown developmental neurotoxicity effects of bisphenols (BPA mostly) and phthalates. However, for many chemicals, such as pyrethroids, per- and polyfluoroalkyl substances (PFAS), organophosphate flame retardants, and plasticizers, hardly any information is available on the relation between endocrine disruption (ED) and potential neurodevelopmental (DNT) effects. In order to better understand the link between ED and DNT outcomes, rats (F0 rat dams) were exposed to six EDCs (BPF, BBzP, DINCH, PFOS, permethrin (PMT), triphenyl phosphate (TPHP)) from pre-mating until lactation. One pup/sex/litter was raised to adulthood for testing of activity, learning and memory, anxiety and social behavior. Targeted (steroids, thyroid hormones, neurotransmitters) metabolomics and untargeted lipidomics of hippocampus (PND6) samples were used to map the metabolic pathways affected by these six EDCs. EDC exposure, compared to control, showed sex- and chemical-specific effects. For instance, in male, PMT, BBzP, TPHP, and DINCH downregulated testosterone, while corticosterone and pregnenolone sulfate were downregulated by almost all chemicals in both females and males. Additionally, PFOS and PMT exposure downregulatedT3 and T4 in males and females, and hundreds of different lipids were affect by all EDCs. Memory function (Morris water maze) was impaired in adult male offspring by BPF and BBzP, and in females by DINCH and TPHP. Steroids, neurotransmitters and lipids levels in hippocampus (PND 6) correlated with sex-dependent behavioral outcomes. In females pregnenolone-sulphate (PREG-S), GABA, and in males PREG-S, acetylcholine, histamine and corticosterone, and in both sexes hundreds of lipids were significantly regulated in the memory function group compared to the control. Previously studies demonstrated a significant correlation between PREG-S and cognitive function, and also a relation between PREG-S and the neurotransmitter acetylcholine. Our findings will be primarily useful to define the mode of action of EDCs on developing neuro-endocrine systems, to elucidate possible relationships with behaviour, and to link rodent data with exposures and effects observed in humans. This project received funding from EU Horizon 2020 (No. 825759, ENDpoiNTs).

1.14.T-03 From Molecular to Whole-Organismal Responses of Salmonids to 6PPD-Quinone

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6PPD-quinone (6PPDQ), a transformation product of the car tire additive 6PPD, is a stormwater pollutant prominent in urban watersheds. In 2021 it was identified as the causal toxicant inducing mass pre-spawn mortality events in adult coho salmon in the Pacific Northwest. Induction of 6PPDQ mortality at environmentally observed concentrations is highly species specific, and lethal concentrations can range greater than 500-fold between closely related species such as coho salmon and Chinook salmon. The mechanisms underlying species specific sensitivity to 6PPDQ are currently unclear, and linking molecular endpoints such as transcriptomics and genetics with responses at higher levels of biological organization are crucial for understanding the mode of action (MOA). Our lab is currently investigating the MOA using a suite of *in vitro* cellular assays, *in vivo* exposures, and molecular endpoints to examine the underlying mechanisms of 6PPDQ-induced mortality in fish. Whole-transcriptome sequencing of recently hatched coho salmon alevin exposed to 6PPDQ has indicated that pathways governing organismal development and endothelial signaling may be substantially altered by 6PPDQ, and a time course of effects on coho salmon cells *in vitro* also supported disruptions in epithelial and endothelial processes. At the whole organism level, visible hemorrhaging in unhatched coho salmon embryo, macromolecule accumulation in the brain of exposed juveniles, and increased hematocrit also indicate alterations in endothelial barriers. Further refinements of mechanism of action are a critical step in understanding highly variable species sensitivity to 6PPDQ. Such information could ultimately be used to generate predictions of other sensitivity species and better evaluate the large-scale environmental impacts on biota.

1.14.T-04 Copper Toxicity on *Chlamydomonas*: Combining Bioenergetic Modeling with Omics Data

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Copper is a micronutrient for algae, but it becomes toxic at high concentrations. The toxicity is due to lipid peroxidation, membrane damage, disruption of protein structure, chlorophyll malformation (substitution of magnesium with copper), and production of reactive oxygen species (ROS). Although these mechanisms are well-studied individually, we know less about their bioenergetic implications and about how lipid and metabolomic signals are connected to the organismal and population level. To address these gaps, we cultured *Chlamydomonas reinhardtii* at varying copper concentrations and measured endpoints at the population level (chlorophyll density, cell count), organismal level (cell size), and sub-organismal level (lipid and metabolite profiles). We developed a new Dynamic Energy Budget (DEB) model for this system. Unlike previous models, this model allows for explicit representation of chlorophyll dynamics. By fitting this DEB model to our experimental data, we identified potential Physiological Modes of Action (pMoAs) of copper toxicity using the DEBtox methodology. The model fits the data well under different assumed pMoAs. Finally, we used the omics data to narrow down the pMoAs and we explored how molecular responses correlate with higher-level effects. Our omic analysis particularly highlights a general toxicity response via glutathione production and a specific impact on photosynthesis through the Warburg Effect pathway. In conclusion, our study elucidates the bioenergetic effects of copper toxicity in algae and demonstrates how molecular responses can be connected to organismal and population-level processes.

1.14.T-05 Multi-Scale Risk Assessment of Thiamethoxam Toxicity to Chironomids in Realistic Environmental Conditions

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Thiamethoxam (TMX), a systemic neonicotinoid insecticide, is widely used to safeguard crops against pests. Recent ecological risk assessments by the US EPA have highlighted potential chronic TMX exposure risks to freshwater aquatic invertebrates, with chironomids identified as particularly sensitive, which could indirectly impact other taxa. To comprehensively quantify the chronic toxicity of TMX in chironomids, we developed a multi-scale, multi-tiered risk assessment approach to characterize risks at both individual and population levels under realistic environmental conditions and TMX exposure. Initially, we constructed a quantitative mechanistic effect model for *Chironomus riparius*, simulating the species' life history under dynamic temperatures and exposure concentrations. This model was designed using a state-of-the-art mechanistic approach, combining Dynamic Energy Budget (DEB) theory with toxicokinetic-toxicodynamic (TKTD) modeling, and calibrated using data from tailored experiments. The calibrated model demonstrated strong agreement with independent validation data specifically collected for this purpose. Subsequently, we applied the DEB-TKTD model to estimate potential risks associated with realistic exposure profiles and variable temperatures

using a moving-time-window (MTW) approach. This method facilitated the identification of the most adverse exposure window, considering critical combinations of life-stage sensitivity and the timing and magnitude of chemical exposure. We estimated exposure multiplication factors (EMFs) associated with different levels of mortality effects (EP10 to EP50) for two vulnerable US watersheds affected by runoff. Finally, we parameterized an Agent-Based Model (ABM) integrating the DEB-TKTD model to estimate risks at the population level. This model was parameterized and validated using literature data and tailored laboratory experiments focusing on density dependence. We estimated EMFs associated with different levels of effects (EPx) at the population level for the same two watersheds. In this study, we present a comprehensive multi-scale risk estimation approach that integrates data generation with state-of-the-art data-driven tools. These tools were developed in accordance with the latest guidelines and recommendations, particularly emphasizing rigorous model testing and transparent documentation.

1.14.T-06 Linking Molecular Impacts to Organism Health: Empirical and Theoretical Methods To Scale Contaminant Effects

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In 2022, the Organization for Economic Cooperation and Development's (OECD) Working Parties on Hazard Assessment (WPHA) and Exposure Assessment (WPEA) initiated a project focused on "Using adverse outcome pathways to address combined exposures to chemicals with relevant effect biomarkers." As part of this effort, project partners developed a template for characterizing effect biomarkers for potential use in environmental assessments as well as guidance on deriving effect-based trigger (EBT) values for environmental water quality monitoring and occupational biomonitoring effect levels (OBELs) for human health. A case study, applying these templates and guidance, was developed for two potential effect biomarkers for estrogenic endocrine disruption, notably estrogen receptor (ER) transactivation (measured *in vitro*) and vitellogenin induction in male fish (measured *in vivo*). Application of the effect biomarker characterization template identified both endpoints as having favorable characteristics for their use as effect biomarkers in environmental water quality monitoring. Subsequently, EBTs corresponding to technical limits of detection (technical-EBT), significant elevation of response above reference or baseline conditions (reference-EBT), potential risk for adverse effects (provisional-EBT), or high probability of adverse effect (refined-EBT) were proposed for both ER-transactivation and plasma vitellogenin induction in male fish. Using this approach, EBTs ranging from 0.18-0.56 ng 17β-estradiol equivalents/L were derived for five separate ER transactivation assays. Likewise, EBTs ranging from 60-3000 µg vitellogenin/ml plasma were derived for six different fish species for which baseline concentrations in control males and sexually mature control females had been reported. Potential use of these proposed EBTs is illustrated via application to multiple case studies including laboratory-based evaluation of a single chemical exposure, *in vitro* and *in situ* exposure to treated wastewater, and in vitro and in situ exposures to ambient surface water. Results contribute to on-going international efforts to support risk assessment of complex mixtures by employing effect biomarkers anchored to adverse outcome pathways. *The contents of this presentation neither constitute, nor necessarily reflect, U.S. Environmental Protection Agency policy.*

1.15.T Microbial Metagenomics: An Emerging Tool for Predictive Ecotoxicology

1.15.T-01 Establishing a Framework for Quantifying the Impacts of Environmental Stress on Marine Microbial Communities

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The vitality of coral reef ecosystems and their capacity to withstand environmental stress are inherently linked to the functionality of their microbiomes. However, existing conservation strategies usually neglect the important role of microbial communities in ecosystem processes. Identifying microbial effect thresholds is urgently needed for the development of national water quality guidelines (WQGs), aimed at preserving ecosystem functionality in the face of human development. Here we developed a quantitative 16S sequencing approach for estimating microbial effect thresholds at community and taxon-specific levels, using coral reef seawater microbiomes as a model community. The workflow involved exposure of seawater samples to a subset of stressors, followed by propodium monazide treatment for cell viability assessment. Microbial loads were integrated with quantitative microbiome profiling by normalizing 16S sequencing data to an even sampling depth, enabling the generation of concentration-response relationships to assess stressor impacts on composition, diversity, and abundance. Toxicity thresholds, estimated for individual amplicon sequence variants, were then modelled into *Prokaryotic Sensitivity Distributions* to derive *microbiome* hazard concentrations, such as the *m*HC5, suitable for protecting 95% of the entire community. Our study revealed varying but significant impacts on microbial communities at environmentally relevant stressor levels. For example, microbiomes in distinct reef environments showed differing sensitivities, with pristine microbiomes exhibiting greater sensitivity to copper than moderately and slightly disturbed environments (*m*HC5 = 1.2, 0.81, 0.30 Cu L^{-1}, respectively), and the derived *m*HC5 values were all lower than the Australian and New Zealand default HC5 guideline value of 1.3 Cu L^{-1}. Additionally, we established thresholds for elevated sea surface temperature, with a *m*icrobiome hazard temperature value of 1.4°C identified, which correlates well with temperatures that trigger coral bleaching. Our findings suggest that marine microbial communities are highly sensitive to environmental disturbances, and coral reef microbiomes may be insufficiently protected by existing national WQGs. The results presented enhance our understanding of community responses to environmental stress, but more importantly the framework developed represents an opportunity to include microbial effect thresholds in future environmental risk assessments.

1.15.T-02 Characterizing the Interplay of Gut Microbes and Mercury Exposures in Two Malagasy Populations

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Methylmercury (MeHg) is a well-known neurotoxicant and is associated with cardiovascular health risks in human populations. However, the influence of the human gut microbiome on MeHg demethylation and elimination is less understood. Most MeHg exposure is from dietary sources (fish and rice). Past work across many human populations shows large variability in internal Hg exposure levels at similar levels of dietary intake. We hypothesized this may reflect

heterogeneity in the human gut microbiome and its influence on MeHg metabolism. To investigate this hypothesis, we analyzed the gut microbiome using stool shotgun metagenomes from 192 individuals from two Malagasy cohorts (coastal n=80; rainforest n=112) aged between 16–81 years old, detailed dietary data, and concurrently measured Hg concentrations in fingernails. Coastal and rainforest cohorts differed significantly in their dietary patterns, and this was reflected by measured Hg concentrations in fingernails (coastal: 991±566 ng/g; rainforest: 165±146 ng/g). Significant differences in the coastal and rainforest populations' gut microbiome likely reflect their predominately seafood vs. rice-based diets. Specific bacterial species (e.g., an unclassified *Roseburia* species in individuals; interaction $p<0.05$) were associated with seafood consumption and fingernail Hg concentrations in the coastal cohort. We calculated the difference between modeled Hg exposures using a one-compartment toxicokinetic model expected from external intake and fingernail Hg concentrations as a measure of MeHg metabolism. Within cohorts, variability in MeHg metabolism was significantly associated with differences in the carriage of protein domains and families by gut commensals (PERMANOVA $p<0.05$). Several protein families, mostly contributed by methanogenic archaeal species, were associated with higher MeHg metabolism (FDR<0.25). This work suggests that the archaeal community may play a role in metabolism of MeHg, and results contribute to better understanding of factors that affect inter-individual variability in vulnerability to dietary MeHg intake.

1.15.T-04 Beyond the Name: Functional Changes Implicate the Microbiome in a Broad Array of Metabolic Changes in Diverse Ecosystems

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While many microbial community studies focus on taxonomic alterations under community perturbation, functional changes under those same perturbations—including toxic exposures—are often more indicative of a community's shifting role in the broader ecosystem. Despite this, there are relatively few methods available for functionally profiling a community directly from shotgun metagenomic sequencing data, with HUMAnN being one of the few widely-adopted methods. Like most microbial community methods, functional profiling is limited by our prior knowledge of microbial genes and genomes, which is biased toward microbes associated with humans. As a step toward addressing this problem, we introduce HUMAnN 4 and apply it to a subset of samples from the Earth Microbiome Project 500 (EMP500). HUMAnN 4 has several advantages over previous iterations of the method, with perhaps the largest being the incorporation of pangenomes that include metagenomically assembled genomes (MAGs) grouped into highly resolved species genome bins (SGBs). The addition of MAGs to our previously isolate-genome-restricted database expands known UniRef50-like gene family clusters by roughly 33%. Given HUMAnN's tiered approach to functional assignment, with the expanded pangenomes we were able to assign genes and protein annotation at a high accuracy during the nucleotide search tier in synthetic communities (99.1% sensitivity and 97.9% precision). Reads that were not mapped during this first tier (nucleotide search) will still be translated and compared against a broader protein database to attempt to classify those contributed by unknown, undercharactertized, or low-abundance microbes. We have additionally implemented important improvements to this translated search tier, including the generation of new, high-confidence enzyme activity predictions for known and novel (MAG-contributed) protein families. On the EMP500 data, we identified an additional 1.4 million UniRef50 clusters from a subset of 10 samples sourced from a river impacted by acidic rock drainage (a 40% increase, HUMAnN v4 vs. v3.7), which resulted in an additional 1,277 enzyme activities identified. This vast increase in identified genes and proteins sheds additional light on the functional potential of these impacted communities and their potential role in environmental remediation.

1.15.T-05 Microbial Risk Assessment: Spatio-Temporal Dynamics of Fecal Indicator Bacteria and Source Tracking with a Novel Rodent Mitochondrial Marker

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The Pensacola Bay System (PBS), a subtropical mixed-use coastal catchment, is increasingly affected by urban discharge, land cover changes, and climatic variability, exacerbating fecal contamination issues in coastal and peri-urban streams. This comprehensive study utilized 600 water samples collected from 50 diverse aquatic ecosystems throughout 2022 to explore the spatio-temporal impacts on fecal indicator bacteria (FIB), specifically Escherichia coli and Enterococci, and nutrient fluxes. Advanced analytical techniques, including IDEXX Colilert-18 and Enterolert-18 assays, ion chromatography, and quantitative Polymerase Chain Reaction (qPCR) for microbial source tracking (MST), were employed to assess FIB levels and identify contamination sources. The use of the HF183 Bacteroides marker highlighted significant human sewage contamination, with novel applications such as a mitochondrial marker for rodent feces revealing contamination in 46 recreational sites. Multivariate statistical analyses, including correlation analysis, multiple linear regression, and general linear modeling, elucidated the complex interactions between FIB concentrations and environmental variables like precipitation, temperature, turbidity, total dissolved solids, and conductivity. These interactions were modulated by both anthropogenic and climatic factors, suggesting that urban discharge significantly contributes to water quality degradation. Machine learning algorithms were instrumental in developing empirical models that correlate land-use patterns with water quality, providing critical insights for data-driven land-use and watershed management policies. This research underscores the need for robust surveillance, integrated fecal indicator bacteria monitoring, and the importance of novel molecular techniques like qPCR for precise contamination source tracing. Our findings not only emphasize the economic and public health implications for coastal communities in the Gulf of Mexico but also recommend immediate implementation of bioremediation and best management practices to mitigate contamination and protect water quality in similar subtropical mixed-use watersheds.

1.15.T-06 Perfluorononanoic Acid (PFNA) Modulates the Gut-Brain Axis in Adult Zebrafish

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Evaluating how neurotoxicants affect bidirectional communication between the brain and gut microbiome has become an intriguing new area of research in the field of toxicology. Here we investigate modulation of the gut-brain axis in adult zebrafish exposed to Perfluorononanoic acid (PFNA). Total RNA from brain and DNA from intestinal tissues was collected from 48 adult zebrafish across an exposure gradient of PFNA. RNAseq and 16S taxonomic profiling data were processed using nf-core rnaseq and ampliseq pipelines built with Nextflow, respectively. Relevant features from metataxonomic and transcriptomic datasets were filtered by applying differential abundance analysis utilizing multivariable associations with linear models (MaAsLin2). Hierarchical All-against-All association (HALLA) was used to find any significant relationships between gene expression and relative abundance of gut microbes. All differentially abundant phyla from MaAsLin2 showed a positive correlation across the PFNA dosing gradient. Analysis with HALLA showed a positive correlation between Desulfobacterota and multiple genes associated with properties in the nervous system, including sphingolipid biosynthesis regulator *serinc1* and ornithine decarboxylase inhibitor *oaz2b*. Additionally, Myxococcota, Chloroflexi, Desulfobacterota, MBNT15, Crenarchaeota, and Patescibacteria where all negatively correlated with *gxp3*, which is associated with protection against oxidative stress. Although further study is required, the data suggest that gut-brain axis modulation plays an important role in the toxicity profile of PFNA and subsequent adverse outcomes in adult zebrafish.

1.16.P-Mo-026 Predicting Nanotechnology Exposure the Missing Tiers: A Regulatory Perspective

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Nanotechnology has been widely available for decades. The environmental exposure and toxicological sciences of nanotechnologies is equally now decades old. However, regulatory defined approaches for exposure predictions that are similar to that of traditional chemicals under such regulations as REACH are still missing. There are several reasons for this, not least the lack of structured and thorough reporting of early research leaving its robustness and reliability in question. Often as well, those data seen as unimportant and thus unreported, are those information most useful for regulators and industry. Another issue is the measurement of nanotechnologies in complex matrices or where natural sources of the same elements make monitoring of these chemicals from anthropogenic nanomaterials difficult. As such, modelling approaches are difficult to validate. Moreover it is also difficult to validate regulatory approaches. Under the REACH (Regulation (EC) 1907/2006) regulation exposure modelling for traditional chemicals is conducted using EUSES. This model includes SimpleBox, which simulates the specific environmental behaviours. Thought SimpleBox4Nano exists, this largely predicts regional exposure, omitting local exposures at point source emission. Further to this specific use cases are attributed with expected releases to air, water and soil. These are so called Environmental Release Categories (ERCs). These ERCs drive some realism in the assessment and stop over estimation in each compartment for exposure predictions and subsequent risk assessment. Beyond this, there can be so called specific ERCs, where these factors are refined by industry bodies to represent further realism based on known common practices (operational conditions/uses etc) within that industry. However, due to limited information and no drivers, such ERCs do not exist for nanotechnologies which can lead to unrealistic assessment. Here, we highlight the importance thorough reporting and simple additions which lead to wider use of produced data, and the usefulness and need for ERCs and how this can drive a more appropriate exposure assessment. We also highlight the need for corroboration on which exposure models are considered as accepted by the regulators. Such tools are imperative to generate common understanding between stakeholders and ensure continued growth of nanotechnology as a key enabling technology, not limiting market access through uncertainties or lack of tools.

1.16.P-Mo-027 New Submicron IR <500nm Combined with fluorescence for MP/NP Detection

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Microplastic or Nanoplastic (MP/NP) contamination has been recognized as a global environmental problem. MP/NP particles are found globally in water, air, and soil. These particles are regularly ingested via contaminated water, beverages, food, and by breathing airborne particles. The MP/NP research community has grown quickly to address questions related to environmental/health risks, but chemical characterization remains to be a problem at the smaller sizes, where you also see the largest number of particles which also may be the most damaging to cells and tissues. FTIR or emerging QCL's laser systems have shown to be limited in some studies to >50 mm particles due to limited IR spatial resolution, IR scatter artifacts and IR saturation. Raman micro spectroscopy offers better spatial resolution but suffers from lower sensitivity than IR, autofluorescence, and can burn samples easily. In some studies Raman spectroscopy has been limited to particles sizes of >20 mm. How can we approach measurements >20 mm with automated chemical measurement and report generation. A new approach to IR micro spectroscopy, called "Optical Photothermal Infrared (O-PTIR)" spectroscopy has demonstrated a unique ability to generate submicron IR spectra without common IR scatter artifacts. O-PTIR uses an infrared pump laser to excite the sample and a visible probe laser to measure the absorbed IR providing a 50X improvement in IR spatial resolution. O-PTIR provides mm to sub-micron size MP/NP characterization, with IR chemical specificity, in a non-contact, reflection geometry, and is not affected by fluorescence. O-PTIR can be used at visible powers of 1/10th of the power needed for Raman, eliminating the potential of burning samples. The IR spectra measured are high SN, transmission quality, that are easily interpreted and searchable. Using co-located fluorescence microscopy, we can highlight particles of interest for further discrimination using O-PTIR in cells and tissues. This offers substantial time savings and possible misinterpretation of spectral data. Combining these methods into automated measurement and report generation can provide valuable feedback to the levels or research.

1.16.P-Mo-028 Impact of Wastewater Borne TiO2 NPs on Metal Uptake by Potato Plants Receiving Synthetic Wastewater Irrigation

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Increasingly considered as a source of water for irrigation, wastewater (WW) can pose risks to crops due to the potential presence of contaminants. WW can be a source of metals and organic contaminants that are known to have harmful implications for human and environmental health. The detection of nanoparticles (NPs) in WW has raised questions about their potential interactions with other co-contaminants and their effects in soil-water systems (e.g., WW-irrigated agriculture). Titanium dioxide nanoparticles (TiO_2 NPs), a type of NP commonly used in industrial and consumer products, and reported to be present in WW, could potentially influence the mobility of contaminants in soils and the uptake of metals by crops. Accordingly, a two-year (2017 and 2018) field lysimeter study was conducted to investigate the impacts of TiO_2 NPs on the mobility of metals (Cd, Cr, Cu, Fe, Pb and Zn) and their uptake by potato plants (Solanum tuberosum L. cv. 'Russet Burbank'). Potatoes were grown in a sandy soil under controlled conditions and irrigated with synthetic WW or WW+TiO_2 NPs. At harvest, potato tubers, plant parts and soil samples were analyzed for their metal concentrations. The presence of 1 mg L^1 TiO_2 NPs in the WW irrigation water reduced significantly ($P \leq 0.05$) the uptake of Cd, Cu and Zn by the potato flesh, skin and roots in both treatment years, but had no significant effect on the uptake of Cr, Pb and Fe into any plant parts. The presence of TiO_2 NPs in the WW appeared to reduce the bioavailability of Cd, Cu and Zn in the soil.

1.17.P-Th Not Just Another NAM: Integrated, Intelligent, and Iterative Approaches to Ecological Risk Assessment

1.17.P-Th-008 Towards One Health: Case Studies to Develop & Test an Integrated Animal-Free Next Generation Human and Environmental Safety Framework for Cosmetics

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There is an increasing momentum and ambition to move towards animal-free chemical safety assessments, particularly for the cosmetic sector with its widespread animal testing bans (e.g. 2013 EU and 2023 Canada). To increase confidence in, and experience with, non-animal new approach methodologies (NAMs) and next-generation risk assessment (NGRA) frameworks across stakeholders, case studies are required to 'stress-test' the existing tools. This allows a thorough assessment of how the tools interplay, their applicability in a range of scenarios, and highlighting areas of larger uncertainties to inform additional research needs. While in the past, human and environmental health safety assessments have operated mostly in silos, NAMs and NGRA frameworks allow to drive synergies, envisaging a future integrated, intelligent, and iterative animal-free one health safety assessment. For this, a case studies project will be carried out by the not-for-profit science organization ICCS (International Collaboration on Cosmetics Safety) to develop and test an integrated animal-free next generation human and environmental safety framework for cosmetic and personal care product ingredients. The case studies will span across human health and environmental endpoints, considering effects and exposure including fate, to foster closer ties between the disciplines through commonalities in biological targets and approaches (e.g., PBK). The project will explore the application of well-established and novel NAMs and NGRA frameworks for safety assessment and regulatory data requirements including registrations (e.g. for US and EU REACH tonnage data requirements + risk characterisation ratio). If possible, the project will compare more "traditional" frameworks vs decision protectiveness. The learnings will inform best guidance practices, further future research efforts, and build support for integrated, one-health, risk assessment approaches, moving forward. This presentation will serve as an overview of the planned multi-phase approach for the project, an update on the current status, and an opportunity for stakeholders to provide feedback or share relevant findings in shaping these case studies.

1.17.P-Th-009 Setting the Bar: Characterizing Variability Across Standard Acute Fish Toxicity Assays

__Kristin Connors__, Brian Morris and Christian Geneus, Procter & Gamble

Regulatory environmental toxicology relies on experimental results from a handful of standardized toxicity tests including the fish acute toxicity assay (e.g., OECD 203, EPA OPPTS 850.1075). Several animal alternative assays have been developed to replace the acute fish toxicity (AFT) assay including the fish embryo toxicity (OECD 236) test, RTgill cell cytotoxicity assay (OECD 249), as well as computational approaches. These new approaches must be easily transferred to new labs, statistically robust, reproducible, reliable, and "accurate". That is, the replacement assay must produce the same result as the AFT (e.g., LC50). Implicit in this comparison is the assumption that the AFT is robust, reliable, and reproducible. The reliability and reproducibility of the AFT are largely unknown, and widely taken for granted during animal alternative assay validation exercises. In this presentation, we summarize the development and curation of an AFT reference database containing LC50 values, 95% confidence intervals, and relevant experimental parameters, as available. The database includes over 700 chemicals, 1862 unique studies, and 6 unique freshwater fish species allowed under standard test guidelines. This database was statistically probed to describe variability and uncertainty in the AFT assay. Intra-laboratory, inter-laboratory, and interspecies variability were explored. The influence on physicochemical properties and MOA on AFT variability were also explored. This information can be used to set expectations on AFT assay performance, and help contextualize results and expectations for alternative assays.

1.17.P-Th-010 Evaluation of the Mode of Action (MoA) of Chemicals Based on Time-to-Death Data Obtained from Fish Embryo Acute Toxicity (FET) Test

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Acute fish toxicity (AFT) data are globally utilized for chemical risk assessment purposes in compliance with various regulatory frameworks. However, there are concerns regarding the unnecessary suffering and numerous fish fatalities resulting from this test, as it involves exposing fish to high concentrations of a test chemical to induce mortality. The fish embryo acute toxicity (FET) test and fish cell line acute toxicity test (RT gill-W1 cell line assay) has emerged as a potential alternative to the AFT test, as these tests are considered as a non-protected life stage under certain animal welfare regulations. However, the adoption of FET test and cell line test as an alternative to fish acute toxicity testing raises challenges, primarily due to the uncertainty surrounding its applicability domain. In this study, we aimed to evaluate mode of action (MoA) of chemicals in more detail, with a view of defining the applicability domain of the FET test and RT gill-W1 cell line assay as an alternative to the AFT. For this, we determined LC50 values of FET test and RT gill-W1 cell line assay (referred as $LC50_{embryo}$ and $LC50_{cell}$, respectively) on selected 30 test chemicals, of which 18 were categorized as narcotic chemicals without known specific biological targets, and the remaining 12 were pharmaceuticals and pesticide with specific target or biological activity. The results were compared with LC50 values ($LC50_{fish}$) for Japanese medaka in the AFT test. Our results suggest that a chemical categorization based on time-to-death data obtained from FET test can reflect MoA of chemicals. Chemicals acting via a specific mechanisms, such as those targeting specific organs, were expected not to cause death at the undifferentiated stage, but rather at eleutheroembryo stage after morphogenesis of primary organs. Conversely, non-targeting chemicals were expected to cause death in the early developmental stage. $LC50_{embryo}$ values of these chemicals were found to be similar to $LC50_{cell}$ and $LC50_{fish}$ values, supporting the effectiveness of time-to-death data obtained from FET testing in distinguishing between chemicals with a specifical target and non-targeting chemicals. These results also suggest that the 24h-$LC50_{embryo}$/96h-$LC50_{embryo}$ ratio could show the contribution rate of target-mediated toxicity: the 24h-$LC50_{embryo}$/96h-$LC50_{embryo}$ ratio of chemicals with a specifical target become greater than 1, while it remains close to 1 in non-targeting chemicals.

1.17.P-Th-011 Increasing Regulatory Recognition for the RTgill-W1 Fish Cell Line Acute Toxicity Assay - A Regional View of the Application of the OECD 249

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The OECD 203 test guideline (TG) remains the key regulatory study for establishing the acute toxicity end-point for fish. Growing concerns over animal welfare have led to the development and validation of alternative approaches to determine short-term toxicity to fish. The fish embryo test (FET) TG was introduced in 2013 (OECD 236) and has struggled to obtain regulatory acceptance, the study output often requiring a significant amount of weight of evidence (WoE) in supplement. More recently, in 2021, the RTgill-W1 cell line assay (OECD 249) was added to the OECD battery of test guidelines. An initial validation study on 38 fragrance molecules of diverse chemistries and physico-chemical parameters and which possess robust OECD 203 studies, was performed. A high correlation ($R^2 = 0.90 – 0.94$) was found between the *In vitro* EC_{50} values determined in the OECD 249 and the *In vivo* LC_{50} values established via the OECD 203. The pertinence and adequacy of the OECD 249 is currently being discussed at an international level by several regulatory agencies in various regions of the globe. Revised technical guidance incorporating the use and stipulating the terms of employment of the fish cell line acute toxicity assay is expected over the mid-term. In the interim, and via a series of case studies, this presentation will illustrate how LC_{50} values predicted using the OECD 249 can be complemented by supporting lines of evidence to prepare robust end-point conclusions for inclusion in regulatory dossier submissions. Additional WoE using aquatic toxicity QSAR models and/or targeted read-across can be employed to substantiate the use of the RTgill-W1 assay to derive valid end-point values until regulatory recognition of this non-animal alternative as a stand-alone method is accepted.

1.17.P-Th-012 Screening for Endocrine Bioactivity Potential of Tobacco Product Chemicals Including Flavor Chemicals

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Adolescence and pregnancy involve elevated levels of hormones (e.g., estrogen, androgen) during which exposure to endocrine disruptors could have long-term developmental and reproductive toxicity effects. Although animal studies show e-cigarettes can affect fertility, there is little understanding of e-cigarette exposure on human reproductive health. The use prevalence and abuse liability of electronic nicotine delivery systems among adolescents, and during pregnancy, raises concerns about possible exposure to endocrine disruptors. In addition, endocrine disruptors have adverse effects on wildlife and environmental health. However, research efforts screening chemicals in tobacco products for endocrine disruption potential are limited. In this study, we screened for chemicals in tobacco products, including flavor chemicals that could be added to tobacco products, for endocrine bioactivity using the Estrogen and Androgen Receptor pathway models, which are based on the full ToxCast/Tox21 battery of bioassays and developed through U.S. Environmental Protection Agency's Endocrine Disruptor Screening Program. For the screened chemicals, we characterized the environmental persistence using predicted half-life periods (BIOWIN program) and the bioaccumulation potential using fish bioaccumulation factor estimates (BCFBAF program). We curated 5179 chemicals in tobacco and tobacco smoke, 2803 flavor chemicals, and 156 e-liquid chemicals from literature or openly available databases. Overall, we identified 137 tobacco chemicals, 34 flavor chemicals, and three e-liquid chemicals (Veratraldehyde, (2E)-3-Phenylprop-2-enal, and 2'-Acetonaphthone) as "active", indicating potential endocrine bioactivity. Further, among the chemicals with endocrine bioactivity potential, 80 were environmentally persistent, 29 bioaccumulative, and 23 both persistent and bioaccumulative. Our findings document many chemicals in tobacco products with potential endocrine bioactivity which raises concerns for both human and environmental health. These results also underscore the importance of understanding non-cancerous toxicological effects, including developmental and reproductive toxicity potential, of tobacco products and flavor chemicals. Our study characterizes the endocrine bioactivity hazard potential of tobacco and flavor chemicals and provides regulators and researchers a readily available list to consider in future ecological and health risk assessments.

1.17.P-Th-014 Environmental Chemical Impacts on Reproduction and Development by Evaluating Both the Embryotoxicity and Reprotoxicity in Zebrafish

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The ICH S5 (R3) guideline on reproductive toxicology that applies to all pharmaceuticals for which reproductive and/or developmental toxicity studies are appropriate, proposes the use of alternative assays as part of an integrated testing strategy to minimize the use of animals. The guide provides a list that contains 29 reference chemicals that have been shown to induce specific malformation or embryo-fetal lethality plus 3 negative chemicals that can be used to support the sensitivity of an alternative assay. Our research focused on the predictivity of both the zebrafish developmental and the reproductive toxicity assays. Zebrafish is a prevalent model in environmental toxicology and provides an ethically acceptable small-scale analysis system with the complexity of a complete organism. This model enables continuous developmental monitoring as well as adult testing and has been widely used for the generation of relevant answers on mammalian developmental and reproductive risk assessment and hazard. Our goal is to further validate this model for its use by testing the compounds indicated in the ICH S5 guideline for their teratogenic potential but complementing them with other reproductive endpoints. To determine the teratogenic risk of these chemicals, the presence of morphological alterations in zebrafish embryos is analyzed at two different stages and the Teratogenic Indexes (TI) are established as the ratio between LC50/EC50 for each stage. As this assay is performed in externally fertilized embryos, embryotoxicity is directly analyzed, and reprotoxicity could be studied further evaluating the effect of some of the listed compounds in adult zebrafish males by their *in vivo* sperm quality assessment. In this assay, the development and maturation of gametes is evaluated by assessing the sperm quality (progressive motility, curvilinear velocity, and linearity) by the CASA analysis, even expression of specific marker genes (*ziwi*, *sycp3l*, and *shippo1*) by qPCR. This assay complements the developmental toxicity assay studying the effect on the male reproductive system, having a more accurate evaluation of embryotoxicity and reprotoxicity in the same model, helping to increase the Accuracy and Sensitivity of the teratogenicity assay by more than 80% and 88% respectively, compared to mammals. Therefore, this is a well-integrated strategy to minimize the use of mammals to predict developmental and reproductive toxicity effects, both in the zebrafish model.

1.17.T Not Just Another NAM: Integrated, Intelligent, and Iterative Approaches to Ecological Risk Assessment

1.17.T-01 Data-Driven Decision Making Using Advanced High-Throughput Environmental Risk Assessment of Fragrance Materials

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For the last 20 years, the Research Institute for Fragrance Materials (RIFM) has conducted environmental risk screening and assessments to evaluate the environmental safety of more than 3,000 fragrance materials manufactured and used by its members. Though the RIFM Environmental Framework (Salvito et al. 2002. Environ. Toxicol. Chem., 21: 1301-1308) is an effective tool, in light of rapid growth in global use of fragrance materials, RIFM has updated the framework to expand its geographic scope and to incorporate recent advances in environmental exposure science and ecological hazard characterization. In this presentation we describe the updated Framework, detailing its expanded geographies, real-time access to the most current population and hydrological data, estimated environmental exposure using waste water treatment plant simulation models and biodegradation, material categorization based on mode of action (MoA), application of an MoA-based ecological threshold of concern, and streamlined execution. The framework methods and outcomes are illustrated through a proof-of-concept exercise. The updated Framework is an enhanced risk assessment tool that enables RIFM and users and suppliers of fragrance materials to perform timely assessments of thousands of fragrance materials to maintain a high degree of environmental protection and support science-based decisions related to product formulations.

1.17.T-02 Detours on the Road to Next Generation Ecological Risk Assessment - Pitstops and Potholes Encountered in Addressing Avian Toxicity Data Gaps

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Next generation risk assessment is an exposure-led, hypothesis-driven risk assessment approach that integrates New Approach Methodologies (NAMs) to assure safety without the use of animal testing. We examined the need for new *in vivo* avian toxicity testing for three common industrial chemicals, 1,2 dichloropropane (1,2-DCP), 1,1,2-trichloroethane (1,1,2-TCA) and triphenyl phosphate (TPP) using a tiered assessment approach that estimated potential avian exposures via fugacity and multimedia fate models for current conditions of use and compared them to hazard information including existing *in vivo* test data for the chemicals and analogs, interspecies correlation estimates, information regarding mode of toxic action (MOA) and results from hundreds of acute avian dietary toxicity studies. In the process, we encountered uncertainties associated with identifying analogues with commonly available tools, the appropriateness of cross-species extrapolation, applying MOAs and potentially conflicting experimental data. This presentation will share some of the lessons learned and ongoing conundrums experienced in applying various NAMs to the subject chemicals data to improve risk-based decision-making regarding the value of new *in vivo* data.

1.17.T-03 Design of a Machine Learning Enabled In Silico Workflow for the Proposal of New Bio-Based Agrochemicals

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There is a growing need to move towards a circular chemical economy which encompasses proposing efficacious chemicals while addressing toxicity, fate, and avoiding animal testing. Utilizing machine learning, we present an *in silico* workflow using iterative virtual synthesis of renewable building blocks to produce a library of potential products for greener insecticides. Bio-based building blocks such as fumaric acid, succinic acid, lysine, and ethanol are reacted with each other to form a first generation set of products. Successive generations, reacted with the same building blocks, allow for increased coverage of chemical space and increased complexity of products formed. Cutoffs based on physical-chemical properties of commercial insecticides that have significant relationships with activity, such as molecular weight, volume, and octanol-water partition coefficient (Log $D_{\mathrm{pH=7.4}}$) allow for screening of the product library. Specific candidates were then scored using docking and free energy perturbation studies. As a last pass, potential metabolites of these candidates also entered the post-virtual-synthesis steps in the workflow. Green chemistry metrics, such as E-Factor and Atom Economy, are evaluated to provide a viable path for synthesis. This entire workflow allows for concerns of toxicity and pesticide functionality to be addressed while narrowing the chemical space for potential targets. The workflow also considers drivers such as environmental fate and toxicity earlier in the design process. The goal is to expand the library to facilitate the creation of future approaches for other commercial chemicals to reach a more sustainable economy while avoiding the negative pitfalls associated with current practices.

1.17.T-04 PrecisionTox: Developing New Approach Methods (NAMs) for Chemical Safety Testing that Use Phylogenetic Relationships to Predict Interspecies Differences in Toxicity Pathways

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Cross-species extrapolation is a critical component of chemical safety testing, yet, predicting the human health effects of chemicals is challenging, in part because toxicology has largely excluded evolutionary knowledge of how genes are functionally bound to one another. To address these needs, PrecisionTox was developed to better protect the health of people and the environment by establishing NAMs for chemical safety testing using a mix of toxicogenomics approaches (e.g., comparative genomics, metabolomics), evolutionary theory, quantitative genetics, data science, toxicology, and law. PrecisionTox employs six model species/cells, i.e., human cell lines, embryos of zebrafish and frog, fruit fly, water flea, worm, which together represent major branches of animal evolution and are recognized biomedical model systems. Our approach leverages the shared genetic legacy of toxicity response with other animals, including invertebrates, to uncover molecular toxicity pathways shared across the animal kingdom. We demonstrate that over 70% of gene families associated with disease/health status are shared among the greatest variety of animal species through evolution. Pathway conservation between invertebrates and humans is based on the degree of conservation within vertebrates and the number of interacting genes within the human network. Human gene sets that already serve as biomarkers are enriched by evolutionarily conserved genes across the animal phylogeny. These discoveries are foundational to interpreting results of biomolecular data generated by chemical screens. PrecisionTox is strengthened through the ASPIS Consortium via integration with the ONTOX and RISK-HUNT3R projects, which are developing state of the toxicokinetic modeling and next generation risk assessment approaches. Presented on behalf of the PrecisionTox project (https://precisiontox.org/).

1.17.T-05 A 24-hour Transcriptomic Assay with Rainbow Trout Alevins: Comparisons with Fish Acute and Chronic Toxicity Data

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There is regulatory, scientific, and ethical interest in the development of alternatives to vertebrate animal toxicity tests. The objective of this study was to establish a high-throughput test method for embryo-larval fish that could yield data comparable to both fish acute (i.e., 96-hr LC50s) and chronic (i.e., long-term, sub-lethal) toxicity tests. Rainbow trout alevins (~1 day post-hatch) were placed in individual wells (24-well plates) and exposed for 24 hr to 12 different concentrations per chemical. Twenty-five chemicals with diverse physical and chemical properties have been studied thus far, including negative controls (fish medium water or 0.5% DMSO). To eliminate the need for a range-finding test, test concentrations were based on a tapered design which included an LC50 push zone (10- and 100-fold higher than the empirical LC50s listed in the U.S. EPA ECOTOX Knowledgebase), transcriptomic point of departure (tPOD) zone (six concentrations below the LC50 range on a half log10 basis), and baseline zone (10- and 100-fold lower concentrations). Using this design, the exposure for ethanol was: 1000, 100, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.001, 0.0001, and 0 mg/L (covering 7 orders of magnitudes). Mortality during transport, rearing, hatching, and experimental phases were all lower than guidance from OECD236. To ensure repeatability of the results, copper sulfate was studied four times and yielded consistent LC50 values. Based on current data, the relationship between LC50 data from our work and that of fish acute studies (from databases) is near 1:1 with a r^2 of about 0.9. A tPOD has been derived for five compounds, and in all cases the tPOD concentration seems to be "protective" (i.e., lower) of values associated with sub-lethal outcomes from chronic bioassays. The findings thus far are promising in terms of establishing a new test method, though work continues to expand our sequencing efforts to yield more tPOD data, test more compounds, and deepen studies into exposure measures (i.e., nominal vs. modeled vs. measured).

1.17.T-06 Quantification of Steroid Hormones in Fish Holding Tank Water as a Method for the Non-Invasive Detection of Endocrine Disruption

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Endocrine disruption, caused by the presence of chemical compounds in the environment, poses a significant threat to aquatic ecosystems and the health of fish populations. Many of such endocrine disrupting compounds (EDCs) target steroidogenic pathways, making the assessment of circulating steroid hormone concentrations a valuable tool in assessing their adverse effects. While blood sampling is often chosen for steroid hormone quantification, such methods present challenges, particularly in the case of small fish species like the fathead minnow commonly used in studying EDCs. In many cases, these fish are too small to provide sufficient blood volume for sample analyses necessitating the pooling of multiple samples thus reducing statistical power. Additionally, ethical concerns arise regarding the sacrifice of large numbers of fish for the purpose of blood collection. As such, the objective of this project was to develop a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of steroid hormones excreted into holding tank water to be used in the screening of EDCs. Preliminary results have indicated that 17β-estradiol, progesterone, testosterone and 11-ketotestosterone are detectable using 500 mL of water collected from a tank containing a single male or female minnow at concentrations ranging from 0.08-6 ng/L. Moving forward, this method will be tested for its ability to detect endocrine disruption in fish following exposure to known EDCs. This step will include a comparison of blood and tank water hormone concentrations as well as transcriptomic analyses to anchor responses to known biological pathways. Once fully validated this method will be highly useful in the non-lethal screening of EDCs, and thus, the assessment of fish health and improving fish welfare.

1.18.P-Th Omics Approaches for Assessing Chemical Hazard and Toxicological Response

1.18.P-Th-015 Comparing the Ecological Effects of Fluorinated and Fluorine-Free Aqueous Film-Forming Foams (AFFF) with Metabolomics

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Fluorine-free foams (F3) are being developed to reduce the adverse environmental consequences of aqueous film-forming foams (AFFF) containing PFAS; however, there is insufficient data on their ecological health effects. In the current study, fathead minnow (FHM; *Pimephales promelas*) larvae were exposed for 24 hrs to dilutions of three commercially available AFFFs, two fluorine-free and one containing multiple PFAS classes. Following exposure, endogenous metabolites were extracted, derivatized, and the metabolome profiled using gas chromatography quadrupole time-of-flight mass spectrometry (GC-qToF/MS). Preliminary results show that one fluorine-free AFFF elicited less differentially altered metabolites than the fluorinated AFFF while the second fluorine-free formulation had an overall comparable response. Metabolite classes up regulated by exposure to the fluorinated AFFF included biogenic amino acids as well as those involved in cellular energetics. Ultimately, linking the impacted metabolites to biological pathways will help inform the toxicological risk of both fluorinated and fluorine-free AFFF exposures in FHMs. Incorporating metabolomic-profiling in these short-term toxicity tests

provides an opportunity to develop novel screening tools to evaluate the ecological risk of emerging, alternative AFFF formulations.

1.18.P-Th-016 Lipidomic and Transcriptomic Analyses of Livers from Rats Treated with Increasing Concentrations of Perfluoro-3-Methoxypropanoic Acid (MOPA)

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Lipid profiles and abundances can be indicative of a particular disease state or phenotype and change rapidly in response to xenobiotic exposure. Per- and poly-fluoroalkyl substances (PFAS) are ubiquitous xenobiotics in the environment and may pose significant health risks. Some PFAS have been shown to alter lipid abundances in various tissues at low doses, potentially foretelling toxicity, or negative health effects. Here, untargeted LC-MS/MS analysis was used to relatively quantify lipid abundance changes in the livers of male and female Sprague-Dawley (SD) rats exposed to 0.01-300 mg/kg/day of MOPA (n=5/sex/dose), a PFAS of concern to the Environmental Protection Agency Office of Water, or vehicle control (n=8/sex) for five days. Lipid abundance changes were compared to transcriptomic changes to better understand the biological basis for lipid alteration. Male rats exhibited more statistically significant lipid and gene changes compared to the females. For the males, a dose-dependent decrease in abundance of saturated glycerophospholipids (GPs) and an increase in monounsaturated GPs was observed. In the females, an increased abundance was observed for saturated and monounsaturated GPs only at the highest MOPA dose. No changes were observed in the neutral lipids for the males; however, triglycerides (TGs) and diglycerides (DGs) were decreased in the female rats at the highest MOPA dose. Additionally, both male and female rats exhibited a dose-dependent decrease in fatty acid (FAs) and lyso-glycerophospholipid abundances. The male transcriptomic data correlated well with the lipid changes observed, suggesting MOPA was initiating metabolic changes starting at the gene level. The males had 17 statistically dysregulated genes that were lipid-associated while the females only had one. For the males, 11 genes associated with FA beta oxidation and FA transport were upregulated. Upregulation of these two gene categories potentially explains the decreased abundance of FAs observed with increasing MOPA dose. Additionally, one gene associated with stearoyl-CoA 9-desaturase activity, responsible for formation of monounsaturated FAs (MUFAs), was upregulated in the males. The upregulation of genes responsible for MUFA formation agrees with the increased abundance of GPs and FAs with MUFA tails. Combined with transcriptomics, lipidomic analyses could aid in predicting adverse health effects of PFAS. *This abstract does not reflect U.S. Environmental Protection Agency policy.*

1.18.P-Th-017 Histological and Transcriptomic Effects of Contaminated Richelieu River Water on Early Life Stages of the Endangered Copper Redhorse (*Moxostoma hubbsi*)

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The copper redhorse (*Moxostoma hubbsi)* is an endangered fish species with a limited range and an estimated population of 2000 individuals. Water quality in the Richelieu River, where the

only known spawning sites of *M. hubbsi* are located, is thought to be marginal and deteriorating. Our previous research suggests that the Richelieu River is contaminated with elevated levels of pesticides during the copper redhorse spawning season, and that copper redhorse exposed to Richelieu River water hatch early with dysregulation of gene expression. The objectives of the current study are to (i) document microscopic morphological outcomes of exposure to contaminated river water using semi-quantitative and quantitative histopathology and (ii) correlate these findings with differences in gene expression. We exposed fertilized eggs to water collected from the Richelieu River, from fertilization (0 days post-fertilization or DPF) to swim-up (14 DPF). The numbers of mortalities and deformities were noted daily. At 14 DPF, for each experimental group, larvae were fixed in 10% buffered neutral formalin and processed for histopathology or frozen at -80°C and preserved for RNASequencing. Histopathology included an initial scan of all sections to identify potentially significant recurring changes. This was followed by (i) semi-quantitative and/or quantitative scoring and (ii) comparisons of the scores between groups. RNA sequencing was performed on frozen larvae and whole-organism gene expression profiles were analyzed using Seq2Fun (http://www.seq2fun.ca) and ExpressAnalyst (www.expressanalyst.ca). Exposure to Richelieu River water did not cause significant changes in obvious organismal level outcomes. However, histopathology suggested mild degrees of physiological disturbances characterized by an increase in the severity of vacuolation in the liver, a decrease in the amount of glycogen in the liver, as well as a decrease in the number of protein droplets in the proximal tubules of the protonephros. Analysis of the transcriptomics data is currently underway. These findings suggest that histopathology can be used to detect minimal physiological disturbances that are missed by obvious organismal level outcomes. However, the physiological implications of these findings are yet unknown, and more work is needed to correlate histopathology with other types of outcomes, especially transcriptomics.

1.18.P-Th-018 Transcriptomic Points of Departure Derived for Diverse Chemicals from High Throughput Assays Using Larval Fathead Minnows

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Risk assessors often need to extrapolate chronic toxicity threshold values from acute tests due to limited resources. Transcriptomic-based points of departure (tPODs) derived from the evaluation of global gene expression after short term tests have potential to provide a more reliable and biologically based alternative for estimating chronic toxicity in aquatic organisms. The present study extends previous work describing a high throughput fathead minnow (*Pimephales promelas*) assay that provides targeted transcriptomic data after exposure to a range of well-studied contaminants for which data from standard toxicity tests exists. One day post-hatch fathead minnows were exposed to eight concentrations of 22 separate chemicals, representing a diversity of structural classes and modes of action (e.g., acetylcholinesterase inhibitors, estrogen receptor agonists, ecdysone receptor agonists, photosystem II inhibitors), for 24 hours. Concentration response modeling was performed on whole body gene expression data from each exposure, using measured chemical concentrations when available and tPODs were generated for

19 of the chemicals. The remaining chemicals impacted fewer than 15 genes in a concentration-responsive manner, therefore a tPOD was not estimated. Generally, larval fathead minnow-based tPODs were lower than reported apical effect concentrations across fish species but were not always lower than concentrations reported to elicit apical toxicity in aquatic invertebrates or algae. Additionally, there was significant overlap of differentially expressed genes within modes of action. Considering the increasing number of chemicals requiring toxicity testing and the limitations of traditional testing strategies, tPODs, based on short-term assays conducted in high-throughput format, may serve as an effective alternative to chronic testing. The results of this study provide a robust data set adding information to a pilot fathead minnow high throughput assay for possible use in ecological hazard screening. *The contents of this presentation neither constitute, nor necessarily reflect, U.S. Environmental Protection Agency policy.*

1.18.P-Th-019 Assessment of "Data Rich" Chemicals Using Larval Midge (*Chironomus dilutus*) High Throughput Transcriptomics Methodology

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With growing numbers of chemicals present in the environment and limited resources to gather comprehensive ecotoxicity data, high throughput screening methods that support chemical risk assessment hold the potential to support this growing demand. One proposed approach involves deriving transcriptomic points of departure (tPODs) for aquatic organisms exposed in a multi-well plate format for 24 h. While concentrations that trigger significant transcriptomic change may not be synonymous with an adverse organismal effect, it has been hypothesized that tPODs should be equal to or lower than concentrations that cause adverse effects, even over longer exposure durations. The goal of the current study was to compare tPODs for midge (*Chironomus dilutus*) exposed to 22 chemicals (primarily pesticides and pharmaceuticals, spanning diverse modes of action) with endpoint data derived from traditional toxicity tests. Twelve-day-old midge (3rd instar) were exposed to eight concentrations (dilution steps of 0.4x) of each test chemical for 24 hours in 96 well plates. Whole body homogenates from three replicate plates were pooled for RNA extraction and the transcriptome was evaluated through full RNA sequencing (n=5 biological replicates per treatment). The tPODs were calculated as the 10th percentile of the benchmark doses of concentration responsive genes (CRGs) and compared to traditional toxicological endpoint data, which were compiled from insects and other taxa from the ECOTOX knowledgebase. The resulting tPODs spanned more than three orders of magnitude, with the lampricide 4-nitro-3-(trifluoromethyl)phenol the most potent of the chemicals tested (tPOD = 0.48µg/L) and the fungicide cyproconazole the least potent (tPOD = 1050 µg/L). All tPOD values were at least two orders of magnitude lower than apical effect concentrations and, therefore, provided a protective effect threshold. With the large quantity of transcriptomic data generated from high throughput methodology, assessing tPOD concentrations in relation to traditional endpoint concentrations may prove to be a sustainable way to gather ecotoxicity data for risk assessment screening and prioritization. *The contents of* *this abstract neither constitute, nor necessarily reflect U.S. Environmental Protection Agency policy.*

1.18.P-Th-020 Transcriptomic Points of Departure in *Pimephales promelas* from Whole Versus Targeted Transcriptome Sequencing

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Short-term toxicity studies identifying transcriptomic points of departure (tPODs) have been proposed as alternatives to long-term, resource-intensive, animal assays for understanding chemical toxicity and risk. The tPOD, which indicates a concentration below which a concerted molecular response to a chemical stressor is not expected to occur, can be used to obtain protective screening values for chemical risk assessment. A common method of obtaining transcriptomic data is by whole transcriptome RNA sequencing (RNA-Seq); however, targeted RNA-Seq is becoming an increasingly attractive alternative method because it focuses sequencing depth on a selected subset of transcripts, often curated to be representative of annotated pathways. Therefore, targeted RNA-Seq is higher throughput than whole transcriptome RNA-Seq and presents a more straightforward bioinformatic analysis, as all the transcript sequences are known and selected *a priori*. However, it is unclear if targeted RNA-Seq will provide equivalent tPOD estimates to that of whole transcriptome RNA-Seq or if the targeted approach will bias the tPOD calculation. To address these questions, we analyzed whole transcriptome data from larval *Pimephales promelas* exposed to multiple concentrations of 18 chemicals for 24 hours in 96-well plate format. A subset of the whole transcriptome data was created *in silico* to mimic the results that would be obtained from a targeted approach, based on the 1,832 gene IDs that comprise the BioSpyder S1500+ gene set. Benchmark concentration-response modeling was applied to both whole and reduced transcriptome data for each chemical to calculate a tPOD, which comprised the 10th percentile benchmark concentration of the concentration responsive genes. Of the 18 chemicals tested, 15 had tPODs derived from the targeted transcriptome set that were within 5-fold of their whole transcriptome tPOD. However, for three chemicals, thiacloprid, atrazine, and imidacloprid, the targeted tPOD differed by 1, 3, and 4 orders of magnitude. The results from most of the chemicals do not suggest a bias in tPODs derived from targeted RNA-Seq. While further analysis into the quantitative differences in tPODs derived from whole and targeted RNA-Seq is needed, the targeted approach could rapidly increase assessment of chemical risk. *The contents of this abstract neither constitute, nor necessarily reflect, official U.S. Environmental Protection Agency policy.*

1.18.P-Th-022 Using the *Pimephales promelas* EcoToxChip to Understand Perturbed Molecular Pathways Across Species Following Exposure to Chlorantraniliprole

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Chlorantraniliprole (CHL), a diamide insecticide, has been increasingly used over the past decade to control a broad range of agricultural pest species. Diamides bind to ryanodine receptors (RyRs), which are evolutionarily conserved calcium release channels and lead to uncontrolled releases of calcium stores. Previous work in our lab focused on using the U.S. EPA's Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool to predict CHL's toxicity across species, which guided focused acute toxicity studies using two *Daphnia* species, and larval (24-hours post-hatch) *Pimephales promelas* and *Danio rerio*. The toxicity studies, along with existing empirical data, showed that the *Daphnia* species were sensitive to CHL, while no mortality was observed in the fish species, and this was likely due to the differences in the binding interactions between CHL and the RyR. The SeqAPASS predictions also indicated that those binding interactions are likely conserved across hundreds of other fish species, suggesting other fish species may not be susceptible. While the RyR itself and pathways modulated by a variety of chemicals that bind to the RyR have been extensively studied, there is little known regarding CHL-specific effects at the molecular level, whether RyR-mediated or other. The EcoToxChip, a qPCR array, was developed to aid in chemical risk assessment by evaluating changes in expression of 370 gene targets selected through evidence-based pathway analysis. Here, the *P. promelas* EcoToxChip was used to help elucidate perturbed molecular pathways following acute exposure to CHL. Pooled whole-body larval *P. promelas* RNA samples from six exposure concentrations were evaluated on the EcoToxChips. Data analyses using the EcoToxXplorer tool showed changes in gene expression in a variety of conserved pathways, particularly signal transduction processes. In addition, five gene-level benchmark doses were generated, two of which were environmentally relevant. Since SeqAPASS predictions demonstrated that the CHL-RyR interaction in *P. promelas* is likely conserved across fish species, the conserved pathways identified in these analyses could potentially be extrapolated to other fish species. Overall, this work lays the foundation to address the uncertainties concerning CHL-mediated pathways. *The views expressed in this work are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.*

1.18.P-Th-023 New Insights into Benzotriazole Stabilizer Mediated Toxicity (UV-327) in Rainbow Trout (*Oncorhynchus mykiss*)

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Benzotriazole UV stabilizers (BUVSs) constitute a diverse set of chemicals produced and consumed in great quantities globally. BUVSs prevents plastics from degrading by UV-light, metal from corroding, and functions as an UV-absorbent in personal care products. BUVSs are considered as an emerging environmental contaminant and has been detected and quantified in multiple species, including humans. Toxicologically, it is established that certain BUVSs can act as endocrine disruptors and activator of the aryl hydrocarbon receptor in fish. However, little is known about how BUVSs affect early life stages of fishes. To investigate this, we exposed newly fertilized rainbow trout embryos (*Oncorhynchus mykiss*) to UV-327 using microinjection as the means of chemical delivery, simulating maternal transfer. Microinjected doses were selected to be environmentally relevant (< LOD to 798 ng g^{-1} egg). Following microinjection, embryos were reared and sampled at hatching, Day 11, 14, and 18 post hatching. No signs or symptoms of apical toxicity (body length, yolk area, cardiotoxicity, and physiological parameters) were significantly impacted, irrespective of the day of sampling or dose. By contrast, the body burden of UV-327 decreased with time and throughout the exposure period. On Day 14 post hatching, we observed altered behaviour among alevins exposed to 273 ng g^{-1} egg compared to control: 1.8 times increased distance moved and 1.5 times photokinetic response. Interestingly, several differently expressed genes related to neurology and behaviour, obtain from the whole-body transcriptome, were identified. These potential biomarkers can serve as a stepping-stone for future investigation on how BUVs can affect behaviour. Additionally, the transcriptome point of departure was established at 34 and 93 ng g^{-1} egg and therefore protective of observed toxicity. In this platform presentations, I will cover the current developments on BUVSs-related research and relate our current findings to those.

1.18.T Omics Approaches for Assessing Chemical Hazard and Toxicological Response

1.18.T-01 Insights from Nontargeted Metabolomics into Chlorpyrifos-Induced Neurodevelopmental Toxicity

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Chlorpyrifos (CPF) is an organophosphate pesticide used worldwide as indoor and agricultural pest control, therefore humans can be exposed to CPF through their food and indoor environment. CPF is metabolized via oxidative desulfuration and hydrolysis to chlorpyrifos-oxon (CPF-O) and 3,5,6-trichloro-2-pyridinol (TCPy). CPF and its metabolites have been well-characterized. As with other organophosphates, CPF acts to inhibit acetylcholinesterase (AChE), a regulatory marker for neurotoxicity. Prenatal exposure to CPF has also been linked to adverse reproductive and developmental effects, with a wide array of potential molecular targets. In this work, we evaluated the metabolism and biochemical impacts of CPF and correlated these results with neurobehavioral and toxicological data. C57BL/6 mouse dams and pups were exposed to high (5 mg/kg/day) and low (0.5 mg/kg/day) doses of CPF during gestation. Dams were evaluated for neurobehavioral changes and plasma AChE changes. While AChE inhibition was only apparent in dams from the high dose group, behavioral alterations were observed at both doses, indicating alternative routes of neurotoxicity. In ongoing studies, CPF, CPF-O and TCPy are being quantified in the same dam and pup brain and liver samples as well as dam plasma samples using a SCIEX 7500 triple quadrupole system following a methanol extraction. In a preliminary dam plasma study, TCPy was the only metabolite detected in samples from the high exposure group. This indicates that CPF is rapidly metabolized, and CPF-O may be excreted or accumulated in tissues not tested in the preliminary study. To evaluate metabolic dysregulation following CPF exposure, a nontargeted metabolomics approach was leveraged, where the same tissue and plasma extracts were analyzed using reversed-phase ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry. Feature finding resulted in thousands of chemical features, which were evaluated globally using unsupervised multivariate statistics as well as individually for significant differences between exposure groups via univariate statistical approaches. Ongoing work will correlate and interpret the metabolomics data with the neurobehavioral and toxicological data to better understand changes related to CPF exposure that may lead to adverse neurological and developmental outcomes.

1.18.T-02 Evaluating Individual Perfluoroalkyl Substances and Mixtures with an Integrated Acute Toxicity, Metabolism, Behavior and Transcriptomics Assay in Zebrafish Embryos

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Perfluoroalkyl substances (PFAS) are a ubiquitous class of environmental contaminants that are of regulatory concern due to their potential impacts on human and environmental health. Two main challenges associated with PFAS safety evaluations are the vast number of different compounds within this class and the fact that environmental exposures typically involve mixtures of multiple PFAS. Traditional testing and assessment approaches will not be sufficient to evaluate the safety of PFAS in a timely manner. Here, we used an animal alternative zebrafish embryo-based assay to rapidly generate toxicological data on nine individual PFAS and three PFAS mixtures. The assay is based on a dose-response experimental design and integrates multiple endpoints including acute toxicity, metabolism, swim behaviour, and transcriptomics. A dose-response analysis was conducted to determine points of departure (PODs) for each endpoint. Acute toxicity, metabolism and behavioural PODs were determined based on the *Curvep* algorithm, and transcriptomic PODs were determined with BMDExpress, using a variety of pathway-based and pathway-free methods. We first evaluated the nine individual PFAS and potency ranked them based on the derived POD values. We then tested three mixtures made from different mixing ratios of the nine individual PFAS: one equimolar mixture, one equipotent mixture (based on previously determined acute toxicity PODs) and a mixture intended to mimic concentrations found in Canadian waters based on a literature review. For the next phase of the project, we will compare PODs across all endpoints between the individual PFAS and mixtures to test for evidence of similar mechanisms (i.e. dose addition), independent action, synergism, or antagonism. Overall, we demonstrate that this integrated zebrafish embryo assay is an economical and ethically viable medium-throughput toxicity test to rapidly generate toxicity data and provide novel insight to support the assessment of PFAS mixtures.

1.18.T-03 Sperm Epigenome as an Indicator of Modified Offspring Brain Development

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DNA methylation has the potential to serve as molecular marker for intergenerational inheritance of modified offspring development extending the developmental onset of health and disease (DOHaD) hypothesis towards the pre-conceptional parental environment. The ubiquitous environmental pollutant Benzo[a]pyrene (BaP) reduces DNA methyltransferase activity in vertebrate models and affects offspring methylation profiles. Sperm harbors a repertoire of methylation markers that are associated with different physiological functions, especially neurobehavioral activity. Sperm-borne differential DNA methylation in genes that regulate the development and function of the central nervous system in offspring has been demonstrated in

fish and mammals. Comparing sperm bisulfite sequencing data from adult male Japanese medaka fish (*Oryzias latipes*) and gene expression, acetylcholinesterase activity and swimming behavior in larval offspring upon parental BaP exposure for 21 days (1µg/L) revealed modified methylation in pathways associated with neuronal development and function (netrin signaling; synaptogenesis pathway). Rho guanine nucleotide exchange factor 7, neuroligin 2 and adenylate cyclase 8 were found hypomethylated in the offspring sperm and exhibited reduced gene expression in the larvae, and thus, are possibly responsible for an increased anxiety behavior identified in larval and adult offspring. The data presented here provide a potential connection between parental BaP exposure, neurodevelopmental impacts, and the risk of behavioral and psychiatric disorders in vertebrates over generations and shed new light on the lasting effects of polycyclic aromatic hydrocarbon exposures.

1.18.T-04 Transcriptomic Assessment of Fathead Minnow Brains Following Nitrate Exposure

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Nitrate contamination in aquatic environments is a recognized global concern for human and environmental health. The effects of nitrate exposure on aquatic vertebrates have been extensively studied, with observed changes to reproduction, development, metabolism, and circulation. However, the direction and degree of observed changes are variable, with nitrate being inductive in some scenarios and inhibitory in others. These conflicting findings suggest that nitrate influences multiple physiological pathways simultaneously. To better understand the dynamics of nitrate's effects, we assessed transcriptomic outcomes in juvenile and adult fathead minnow (FHM) brains. For all experiments, FHMs were exposed to 0, 2, 5, 10, 25, or 100 mg/L NO_3-N as $NaNO_3$; representing the range of nitrate reported in surface waters. For juvenile studies, FHMs were exposed chronically from 1 to 79 days post-fertilization (dpf), or acutely from 75 to 79 dpf. At 79 dpf, FHMs are at the end of their juvenile phase. For adult studies, reproductively active FHM pairs were exposed for one spawning cycle (average of 4.0 days). Whole brain transcriptomes were examined using RNAseq and analyzed with DESeq2 likelihood ratio tests and DRomics R packages. Differentially expressed genes were functionally clustered using DAVID functional annotation tools. In acute and chronic exposures of both juveniles and adults, when compared to FHM in the 0 mg/L NO_3-N treatment, all doses of nitrate altered expression of the RNA polymerase II-related gene cluster, suggesting that nitrate affects basal transcriptional activities. For acutely-exposed juveniles, nitrate (all doses compared with 0 mg/L NO3-N) altered expression of 518 genes enriched for cardiac muscle structure and contraction, neural cell migration, carbon metabolism, DNA replication, cell adhesion, egg coat structure, and proteolysis. In chronically-exposed juveniles, nitrate altered expression of 207 genes, including cytokine signaling and cell adhesion transcripts. In acutely-exposed adult female brains, nitrate altered 119 genes, enriched for vitellogenin production and lipid transport genes. In adult males, nitrate affected 427 genes associated with T-cell signaling, oxidative stress, ion homeostasis, and phosphate signaling. These results suggest that nitrate in aquatic environments broadly influences the FHM transcriptome. Further analysis of dose-response relationships between nitrate and the transcriptome will be discussed.

1.18.T-05 Transcriptomic-Based Points of Departure for Algae (*Raphidocelis subcapitata*) Exposed to 22 PFAS for 24-hours

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Due to their environmental prevalence, persistence, and potential toxicity, per- and polyfluoroalkyl substances (PFAS) are contaminants of concern. However, except for a few well-studied PFAS, hazard data required to evaluate their risk to aquatic ecosystems are limited. The present study used a 24-hour, high throughput assay to screen 22 PFAS with varying chain lengths and functional groups for effects on the transcriptome of a green algal species, *Raphidocelis subcapitata*. In addition to transcriptomic endpoints, biomass, viability, and chlorophyll concentration were also evaluated. The tested PFAS included perfluoroalkyl carboxylic acids, perfluoroalkyl carboxylic acid ethers, alcohols, sulfonic acids, and sulfonamides. Algae were exposed in a 96-well microplate format to 8 concentrations (nominally, 100 to 0.03 µM) of each PFAS, in a ½ log dilution series. Analytical measurements of PFAS concentration were made at the initiation of exposure and after 24 hours. Concentration response curves were fit to the resulting transcriptomic data to calculate gene-specific benchmark concentrations. When possible, effect concentrations were based upon measured free concentrations in the media. The distribution of the gene-specific benchmark concentrations was subsequently used to calculate a transcriptomic point of departure (tPOD) which represents a concentration below which no concerted molecular change is detected in response to PFAS exposure. The resulting tPODs were compared with effect concentrations for traditional endpoints and environmentally detected concentrations of specific PFAS. The calculated tPODs ranged from 0.9 µg/L for PFTrDA (perfluorotridecanoic acid; DTXSID 90868151) to 1041 µg/L for PFNA (perfluorononanoic acid; DTXSID 8031863). Generally, longer straight chain carboxylic acids were the most potent and diols the least potent PFAS tested. When data was available for comparison, tPODs were 2 to 4 orders of magnitude lower than traditional endpoint effect concentrations and 2 to 7 orders of magnitude higher than most environmental concentrations. *The contents of this abstract neither constitute, nor necessarily reflect, official U.S. Environmental Protection Agency policy.*

1.18.T-06 Transcriptomic Point of Departure for Ecological Risk Assessment: Standardized Reanalysis of Publicly Available Toxicogenomic Datasets for Fish

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There are currently no validated new approach methods (NAMs) to estimate the long-term (i.e., chronic) *in vivo* toxicity of chemicals for ecological risk assessment purposes. Long-term *in vivo* toxicity testing is the gold-standard approach to inform ecological risk assessment as it provides insight into the subtle adverse effects that are often not revealed during short-term toxicity testing, such as endocrine disruption or developmental defects. Transcriptomic dose-response modeling (TDRM) is a promising quantitative approach to derive estimates of point of departure and that could contribute to replacing, reducing and/or refining the use of vertebrates in toxicity testing. This approach relies on the assumption that changes in the expression of genes following a short-term exposure to a substance lead to, or are indicative of, long-term adverse effects, if the exposure is sustained. By modeling the dose-response relationship of gene expression values, TDRM estimates the lowest concentration of a chemical to induce a significant change in the expression of each gene, or benchmark dose (BMD). These gene BMDs can then be grouped to derive a chronic exposure concentration expected to not cause harm to the tested species, the transcriptomic point of departure (tPOD). In this project, we conducted a literature review of published articles, as of January 26th 2024, to create an inventory of publicly available datasets for toxicogenomic experiments conducted with various fish species. Datasets for chemicals were included in an inventory if 1) gene expression was measured using a full or reduced transcriptome and 2) results were generated from exposure experiments that included ≥3 test substance concentrations and a control group. All datasets were then re-analyzed using a standardized statistical pipeline that compared various data filtering and TDRM parameters. The aims of this project were to 1) evaluate the variability introduced by various statistical approaches on the resulting tPODs, 2) determine how the tPODs compare to chronic *in vivo* toxicity data, and 3) provide insight on best practices for experimental designs and statistical approaches. Our literature search resulted in 29 articles from which 65 different datasets were available (i.e., 8 microarrays, 57 RNAseq). The results of this project will be discussed as well as the potential manner in which tPODs could be integrated into ecological risk assessment if the method was to be validated.

1.19.P-Tu Omics Beyond Transcriptomics: Leveraging Proteomics and Metabolomics to Improve Mechanistic Understanding of Responses to Environmental Stressors

1.19.P-Tu-071 Accelerate Confidence, Reproducibility, and Transparency in Omics Studies Through Generic Experimental Reporting

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Omics based tools have the power to answer pressing health questions including discovery, decision making and diagnosis that span humans, animals, plants, and their shared environment. Spanning numerous scientific disciplines at various levels of biological organization, our community is making progress in the use of these tools to define, adapt, manage, and innovate to address pressing issues such as global chemical pollution, public health care, emerging pathogens and viruses, in addition to expanding knowledge of fundamental processes. However, to fully leverage these tools and the opportunities they provide, consistency and reliability of data is central. With ever increasing toxicological studies available and specifically focused on the aquatic environment, we highlight opportunities to increase transparency of the research process but also reproducibility of studies, thereby increasing scientific credibility. These include reporting recommendations for ecotoxicity data, behavioral ecotoxicity studies, but also omics-based output spanning proteomics, transcriptomics, RT-PCR/RT-qPCR, metabolomics etc. Through the highlighting of these reporting recommendations, we aim to improve the reporting of experiments in the peer reviewed literature, and thereby increase usefulness in anticipatory actions.

1.19.P-Tu-072 Demonstrating the Reliability of Metabolomics-Based Chemical Grouping: Towards Acceptable Practice

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Metabolomics has reached a critical point in determining its value to regulatory toxicology. Building on 20 years of research, the first metabolomics data to support grouping/read-across was recently submitted to ECHA, metabolomics best-practices for various applications including grouping were published in Nature Communications, and the OECD Omics Reporting Framework has been developed. Given these and other examples of its increasing relevance to chemical safety regulations, an assessment of the reproducibility of metabolomics in the context of chemical grouping is required. The aim of the Cefic-funded MATCHING study (MetAbolomics ring-Trial for CHemical groupING) is to determine whether this technology can demonstrate high reproducibility in grouping, and hence high reliability, or whether refinements in analytical or data analysis practices are needed. Through this fully-blinded evaluation, the second aim is to propose 'acceptable practice' for metabolomics-based grouping. The international consortium comprises seven industrial, government and academic metabolomics ring-trial partners, BASF SE, and the European Chemicals Agency (ECHA) as an independent advisor. First, 8 substances were selected for the trial, and all ring-trial partners were fully-blinded to their identities, modes of action, and the number of categories. Plasma samples for the ring-trial were then derived from 28-day rodent tests (8 substances, each 'low' and 'high' dose, plus vehicle controls), aliquoted, and distributed to partners. Each metabolomics laboratory then applied their preferred LC-MS metabolomics workflows to acquire, process and statistically analyse the plasma samples. This included attempting to group the 8 substances into categories based on their metabolomics signatures, and then reporting their findings to ECHA to ensure the blind conditions of the trial were respected. Six ring-trial partners have reported, and 5 have discovered the identical grouping of the 8 substances into 3 unique categories (remaining partner sees similar trends but had more analytical variability). In conclusion, the findings from the MATCHING study have demonstrated high reliability of metabolomics-based chemical grouping.

1.19.P-Tu-073 Optimization of Non-Lethal Fish Epidermal Mucus Collection Methods for Remote Fieldwork and Community Science

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Fish epidermal mucus is one of the first lines of defence against chemical, physical and biological stressors. Acting as a natural, semi-permeable, chemical and biological barrier, fish epidermal mucus plays a critical role in the animal's immunological and protective function due to its mechanical and biochemical properties. As such, fish epidermal mucus is a repository of numerous innate and acquired components of immunity. Fish epidermal mucus is produced and sloughed off constantly, allowing for its non-invasive collection with relative ease compared to other methods (i.e., blood and tissue sampling). While current fish epidermal mucus collection methods can be conducted with relative ease in a laboratory setting, it remains a time-sensitive process that requires scientific knowledge and experience, and laboratory tools and instruments that are not easily transportable due to their size, fragility, and/or safety regulations. The goal of this pilot study was to optimize current fish epidermal mucus collection methods for remote field settings by testing if we could collect epidermal mucus on paper wipes (i.e., Kimwipes™) and allowing them to air-dry, thereby eliminating the need for immediate laboratory interventions (i.e., reagent addition, centrifugal filtering, and cryopreservation). Simplified methods enable laypersons without a science background to easily collect samples, allowing for longer-term, community-based monitoring programs that prioritize humane, non-lethal, and minimally invasive methods, advancing field-based research scopes and opportunities beyond what was previously possible. In a laboratory setting, we collected epidermal mucus samples from chemically sedated rainbow trout (*Oncorhynchus mykiss*) and stored samples in different conditions (i.e., aluminum foil sheets and plastic bags) and durations (i.e., 10, 20, and 30 days). We then measured proteins in rainbow trout epidermal mucus using liquid chromatography and non-targeted tandem high-resolution mass spectrometry paired with chemoinformatics and bioinformatics software. We present preliminary results from this pilot study and discuss the next steps of our research.

1.19.P-Tu-074 The Effects of Hypoxia on Fathead Minnow Behavior and 'Omics

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Behaviour is a beneficial parameter for observing the health of an array of species in the context of environmental changes. Behavioural monitoring is non-invasive and could be utilized as a refinement for fish studies. Traditional ‘omics methods can be applied to determine changes in fish protein, metabolite, and lipid abundance due to toxic exposures. Dissolved oxygen (DO) fluctuations in water bodies have become more prevalent due to climate change and these fluctuations can lead to increased frequency and intensity of hypoxic conditions. We aimed to combine non-invasive methods with traditional methods to determine the effect of hypoxia on fathead minnow (*Pimephales promelas*) behaviour and ‘omics. We exposed 90 fathead minnows to hypoxic (2.10-2.80 mg/mL DO) and normoxic (5.80-7.50 mg/mL DO) conditions over a 7-day period. Fish were video recorded on days 1, 3, 5, and 7. Three blind observers analyzed the videos for fish tank location activity, foraging behaviour, and novel object behaviour. On day 8, we anaesthetized the fathead minnows with 100 mg/L tricaine methanesulfonate (MS-222) and collected mucus, plasma, gill, and brain samples. In this presentation, we will share the integrated results of fish behaviour, brain, mucus, and plasma ‘omics. We found that hypoxia-exposed fish had altered activity levels over the course of the 7-day treatment, and these fish exhibited behavioural plasticity. Our proteomic analyses found that hypoxic fish brain, mucus, and plasma protein abundances were significantly altered when compared to normoxic fish. Our pathway analysis, which demonstrated the effects of hypoxia on the fathead minnow proteome, will also be presented. Furthermore, the metabolomic data showed that asparagine was elevated in hypoxic fish brains ($p\text{-value} < 0.05$), which may indicate apoptosis suppression and support tumor formation.

1.20.P-Tu Pesticide Risk Assessment and Surrogacy for Pollinators and Non-Target Arthropods

1.20.P-Tu-075 Advancements and Challenges in Non-Target Arthropod Risk Assessment

__Frank B Green__ and Eric Peterson, Texas Tech University

Current methods of non-target arthropod risk assessment in the United States relies on a combination of honey bee (Apis mellifera) laboratory, semi-field, and field studies to comprehensively evaluate the impacts to all arthropod species. These studies determine toxicity benchmarks such as lethal doses (LD) and no-observed-effect levels (NOEC) for tier 1 risk assessments, and colony health, foraging behavior, and brood development for higher tier assessments. While there have been a lot of advancements in this field, resulting in robust honey bee risk assessments, it is unclear how this translates to other non-target arthropods – especially those considered threatened and endangered in the United States. Recent advancements have enhanced our understanding of risk by identifying molecular and biochemical biomarkers that can detect early signs of stress and sublethal effects. Computational models, including the use of probabilistic risk assessment and exposure modeling, offer predictive insights and help identify potential risks under various scenarios. Despite these advancements, challenges remain in assessing the impacts on non-Apis pollinators and other non-target arthropods, such as wild bees and butterflies, and in understanding the cumulative and synergistic effects of multiple stressors in comparison to honey bees. For example, differential sensitivity throughout life-cycles, unknown exposure profiles (e.g., repeated exposures, exposure duration, recovery periods, exposure to multiple stressors), and taxa-specific toxic response data. In this introductory talk, we will discuss new methods, critical data gaps, and potential solutions to the issues facing current non-target arthropod risk assessments.

1.20.P-Tu-076 Toxicokinetics of Pesticide Exposure for *Peponapis pruinosa* with Implications for Exposure Modeling

__Tom Purucker__, U.S. Environmental Protection Agency, Office of Research and Development

Peponapis pruinosa, the hoary squash bee, is an important specialist pollinator of cucurbit crops like pumpkins and squash across North America. As a solitary ground-nesting bee that nests within or near cucurbit fields, *P. pruinosa* provides essential pollination services that can enhance fruit yield and quality for these crops. These fields are commonly treated with systemic insecticides applied directly to the soil or coated onto seeds prior to planting. A toxicokinetic-toxicodynamic (TKTD) model is implemented for this bee with a toxicokinetic component that describes uptake and elimination of the pesticide and a toxicodynamic component that translates the internal concentration to a mortality probability. Exposures are integrated across multiple pathways that include dietary and contact exposures for eggs, larvae, pupae, and adult females to allow for identification of principal routes. Alternative forms of TKTD models are calibrated against available semi-field exposure data as a model selection exercise and to identify maximum likelihood input parameter distributions.

1.20.P-Tu-078 Assessing Surrogacy Options in Lepidoptera via Trait-Based Analyses

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Under the Endangered Species Act (ESA), an Ecological risk assessment (ERA) must be conducted for species designated as listed (threatened and endangered) for the registration of a chemical compound. However, laboratory experiments required to describe organismal response to chemical exposure are not possible with listed species, thereby limiting the development of critical ecotoxicological data. Surrogate species can be used in lieu of listed species in laboratory work, but this raises the important question of how to best choose surrogate species. Our project aims to develop methodologies to identify potential surrogacy options for listed Lepidopteran species based on large, multi-faceted trait datasets. To that end, we collated multiple global, continental, and national published datasets covering physiological, life-history, geographic, ecological, and evolutionary information for thousands of Lepidopteran species and integrated them into a centralized accessible database. With aid from representatives from USFWS, USGS, and academia, we identified the most descriptive and immediately operational trait variables to serve as inputs in multiple unsupervised learning algorithms aimed at clustering species based on our collated trait dataset. Results presented here detail how potential surrogate options are chosen and the unique benefits offered by different clustering algorithms, such as higher interpretability in k-means clustering vs increased flexibility of hierarchical clustering. Overall, our results indicate that comprehensive trait data across species can facilitate the search and development of surrogacy options.

1.20.P-Tu-079 Differential Toxic Effects of Lambda-Cyhalothrin on the Larval, Pupal, and Adult Life Stages of the Painted Lady Butterfly (*Vanessa cardui*)

***Frank B Green**, Texas Tech University*

Agricultural intensification in the United States has threatened pollinator populations, which have seen a decline in recent years. The conversion of tallgrass prairie to monocultured agroecosystems has limited pollinator habitat to fragmented patches along field margins and hedgerows. These areas, often adjacent to row crop agriculture and beef cattle feedlots, are commonly contaminated by pesticides from these practices, inadvertently exposing non-target pollinators. Of particular concern to pollinators are insecticides, which target invertebrate agricultural pests, 70% of which are lepidopterans. Pyrethroids are a class of insecticides used to protect crops from chewing pests by disrupting their nervous systems, leading to paralysis or death. Lambda-cyhalothrin is a pyrethroid that is filially applied to crops to defend against a variety of pests such as aphids, the Colorado beetle, and thrips. The foliar application method contaminates adjacent habitats through direct spraying and more distant habitats through aerial transportation. Most studies on the impact of insecticides on non-target pollinators have focused on the honeybee, *Apis mellifera*. There has been a push to diversify the invertebrate species used in these studies to assess wider ecological impacts and evaluate the suitability of *A. mellifera* as a model species. *Vanessa cardui*, the painted lady butterfly, is a Lepidopteran that has been successfully used in single-chemical toxicity testing. Its distinct life stages (five instars, pupa, and adult) offer an opportunity to assess insecticides effects across various life stages. We examined the toxic effects of lambda-cyhalothrin on five life stages of *Vanessa cardui* through 96-hour acute contact toxicity tests, estimating LD_50 values for instar 1 (1.20114 ± 0.25395 ng/mg), instar 3 (1.85015 ± 0.85015 ng/mg), and instar 5 as well as pupae (0.800255 ± 0.048195 ng/mg), and adult butterflies. Sublethal effects such as changes in mass and wing deformities were also quantified across treatments. Early instars showed higher sensitivity compared to middle instars, while pupae were more sensitive than both early and middle instars. Higher treatment levels often resulted in significant mass loss for individual instars, whereas lower and control treatments typically led to mass gain. Pupae in higher treatments exhibited more wing deformities upon emergence than those in lower and control treatments. Variation of LD50 values across *Vanessa cardui* life stages suggests that the timing of lambda-cyhalothrin application will influence the impact on *V. cardui* populations. Comparing the obtained LD50 values and those found for other pollinator species presents the opportunity to assess the suitability of *V. cardui* as a model species for evaluating the impact of agricultural pesticides on non-target pollinators.

Identification of *Isotomidae* sp. and its skin pattern from Punjab, India by molecular and scanning electron microscopy analysis

***Joydeep Dutta**, Zoology, Lovely Professional University, India*

The amount of agricultural production in India contributes significantly to the global food production and particularly in production of rice and wheat, the nation is ranked second globally. One of the most important aspects of producing food in a sustainable manner is the relationship that exists between agriculture and soil. Plant growth, yield, and the overall health of the ecosystem are all directly impacted by the quality and condition of the soil. A deep understanding of the complex relationships between soil properties, soil structure, agricultural methods, and soil-dwelling organisms is essential for maintaining soil health and productivity. Numerous diverse species of Collembola have been found in agricultural land, indicating that these organisms are able to adapt to a broad range of microhabitats found in a wide variety of soil depths. To identify and categorize the species of *Isotomidae* sp. from agriculture soil using scanning electron microscope (SEM) technology and molecular characterization. Species of collembola species were prepared for morphological identification using JEOL FESEM JSM-7610F-PLUS. Molecular identification involves DNA extraction, amplification by polymerase chain reaction (PCR) with primers, and sequencing. Molecular data is used for species identification and genetic variability assessment, whereas scanning electron microscopy images show morphological features. The taxonomy, phylogeny, and ecological roles of Collembola are revealed by combining DNA data with scanning electron microscopy (SEM). The results indicated that there were several species of Collembola. *Isotomidae* sp. was discovered in Punjab during the survey and subsequently identified genetically. The cuticle's properties of the species using scanning electron microscope (SEM) was used to analyze the surface structure of *Isotomidae* sp. This study identified and described *Isotomidae* sp. by analyzing COXI and skin patterns. Our findings suggest that COXI barcoding could be utilized to establish a precise DNA barcode reference collection for *Isotomidae* sp. and to resolve any disputes regarding species identification.

1.20.V Pesticide Risk Assessment and Surrogacy for Pollinators and Non-Target Arthropods

1.20.V-01 Relative Sensitivity of *Apis mellifera* Versus Other Terrestrial Invertebrates

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The United States Environmental Protection Agency (USEPA) uses available insect toxicity data to assess potential effects of pesticides to federally endangered and threatened (listed) species of insects. These data are also used to assess risks to non-target insect pollinators, which include honey bees (*Apis mellifera*), other species of bees, and many other species of insects. Pollinators are crucial to both listed and nonlisted plants that rely upon insect pollinators to maintain healthy ecosystems and ensure food security. However, it is not feasible to test pesticide risks for every species, so only a few are tested to inform pesticide registration decisions. The honey bee is the most frequently used model/test organism to assess potential risks to insects, but there are concerns that they may not adequately represent the diversity of insect sensitivity to chemicals. We compiled ecotoxicity data from the USEPA's ECOTOX database (www.epa.gov/ecotox) and from unpublished data submitted to the USEPA by pesticide companies to support their pesticide registrations. This comprehensive database provides the ability to assess the relative sensitivity of *A. mellifera* to other tested terrestrial invertebrates in the orders Hymenoptera (other bees, wasps, and ants), Lepidoptera (butterflies and moths), Coleoptera (beetles), and Diptera (flies), all of which are represented by currently listed insect species. We employ a range of methods to structure the data and assess relative sensitivity including pairwise hypotheses tests, generalized linear models, and fitted species sensitivity distributions. Additionally, we consider these comparisons within subsets that condition the analyses on pesticidal modes of action. We evaluate our results with taxonomic hypotheses based on current literature understanding of terrestrial invertebrate sensitivity to pesticides. This includes hypotheses that *A. mellifera* are generally protective (with exceptions) of other hymenopteran pollinators, more sensitive than Lepidoptera and Coleoptera due to a deficit of genes that manufacture detoxification enzymes relative to these orders, and less sensitive than terrestrial Diptera with aquatic larval life stages.

Track 2: Aquatic Toxicology, Ecology and Stress Response

2.01.P-Mo Advances in Ecotoxicology of Scleractinian Corals and Other Coral Reef Organisms

2.01.P-Mo-031 Contamination Assessment of Coral Reef Habitats of the Virgin Islands National Park

Tim Bargar[1], *David A Alvarez*[2] *and Kathy Echols*[3], *(1)U.S. Geological Survey, Columbia, United States, (2)U.S. Geological Survey, (3)US Geological Survey, Columbia Environmental Research Center, Columbia MO*

Declines of live coral on reefs of the U.S. Virgin Islands (USVI) are well documented. The potential role that contaminants play in those declines is largely unknown because of the lack of data on contamination in those habitats. A contamination assessment of coral reefs of the Virgin Islands National Park and Coral Reef National Monument (Park) was conducted to address this data gap. Five matrices — water, detritus, plankton, fish, and coral — were sampled from four reef sites. Passive samplers (semipermeable membrane devices [SPMD] and polar organic chemical integrative samplers [POCIS]) were used to measure contaminants in water. No organic contaminants were detected from SPMDs while several (e.g., bromoform, naphthalene, phenanthrene, cholesterol, diethylhexylphthalate) were detected from POCIS. Flame retardants (PBDEs), legacy organochlorine pesticides (chlordanes and DDXs), and various musk fragrances among other compounds were detected in tissue and detritus samples. Musk fragrances (up to 3,191 ng/g lipid for galaxolide in coral) were the contaminants detected in the highest concentrations. The highest PBDE detection was 114 ng/g lipid (PBDE 47 in plankton).

Generally, tissue dry weight normalized concentrations were highest in coral and lowest in fish, but lipid-normalized concentrations did not differ significantly among fish, coral, and plankton. Detection of organic contaminants in the coral reefs surrounding St. John of the USVI indicate contamination may be a factor in the coral decline. These data may be indicative of the contamination of coral reef habitats surrounding other small, sparsely populated islands in the Caribbean Sea and Gulf of Mexico.

2.01.P-Mo-032 Toxicity of Arsenate and Arsenite to *Acropora cervicornis* and *Orbicella faveolata*

Cailey Elise Dorman[1]*, Dorothy-Ellen Abigail Renegar*[2]*, Ellen Regina Englert Skelton*[3]*, Amy C Hirons*[3] *and Dimitrios G Giarikos*[3]*, (1)Nova Southeastern University, United States, (2)Dept. of Marine and Environmental Science, Nova Southeastern University, United States, (3)Nova Southeastern University*

Port Everglades is a large seaport in Fort Lauderdale, Florida, located adjacent to the coral communities at the northernmost end of the Florida Reef Tract. There are questions regarding how resuspended sediments from the planned dredging and expansion of the port could impact nearby coral reefs. Numerical sediment quality assessment guidelines as well as threshold effect levels (TELs) and probable effects levels (PELs) have been derived for nine metals in Florida coastal waters. A recent study found concentrations of arsenic above TEL (7.24 µg/g) and PEL (41.6 µg/g) values in the port's sediments. Arsenic enters coastal waterways through anthropogenic activities and natural sedimentary processes. In corals, exposure to arsenic-contaminated sediments or solubilized arsenic may disrupt vital biological functions and symbiotic relationships, leading to coral bleaching and mortality. To assess the possible effects of soluble arsenic exposure on adjacent reef systems, the acute toxicity of arsenic species arsenate (As(V)) and arsenite (As(III)) were tested with coral species *Acropora cervicornis* and *Orbicella faveolata* using 96-hour static renewal assays. Each exposure assay included six treatments, consisting of five concentrations [ranging from 0.06-5 mg/L for As(V) and 0.03-2.5 mg/L for As(III)] and a negative control, with six replicate beakers per treatment. Effects were evaluated based on coral mortality (LC50), coral condition (EC50), and the photosynthetic efficiency of the dinoflagellate symbiont (IC50). Arsenic concentrations were analytically verified. During the exposure assays, arsenate and arsenite were found to induce polyp discoloration and polyp retraction. The three highest concentrations of As(V) solutions resulted in 100% mortality after a 24-hour period, while the highest concentration of As(III) led to 100% mortality after 48 hours. Arsenite, despite being tested at lower concentrations and within a narrower range, exhibited greater toxicity than As(V). This observation is consistent with prior research conducted on other marine organisms. The results of these experiments provide new data to support management decisions relating to the testing and disposal of arsenic-contaminated sediments in tropical coastal environments.

2.01.P-Mo-033 A Proposed Standard Laboratory Method for Acute Exposure Toxicity Tests of UV Filters on Scleractinian Coral

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Coral reefs make up 25% of the ocean's diversity and provide crucial environmental services to coastal communities. In addition to global stressors, there is concern on how UV filter ingredients in sunscreen impact corals, especially in near shore reefs that are heavily used by beachgoers. In 2022, the National Academies of Science (NAS) released a comprehensive report reviewing the available literature on the impacts UV filters have on our environment. Studies reporting the effects of UV filters in laboratory exposure show a lack of standardized methods and endpoints within and among marine species, particularly coral. Lack of data standardization make comparisons across species and chemicals challenging, confounding potential differences in species sensitivity with variability of test conditions (i.e., exposure duration, test endpoint) and increasing uncertainty in chemical effects assessments. We developed an acute toxicity test method for the scleractinian coral, *Acropora cervicornis*, with a focus on implementing best practices of the USEPA test guidelines for pesticides and toxic substances to generate data that can support USEPA chemical evaluations. Methods development focused on a test design that is readily transferrable to other laboratories while controlling for factors that could influence concentration response functions. Through various pilot studies, we exposed coral to several UV filters to determine test chamber design, coral density, materials, water quality measurements, and coral sample preservation that will result in a reproducible test consistent with USEPA best practices for acute toxicity endpoints. Exposures following this acute toxicity test method will produce data that can contribute to USEPA assessment of UV filter on corals.

2.01.T Advances in Ecotoxicology of Scleractinian Corals and Other Coral Reef Organisms

2.01.T-01 Developing Standard Toxicity Assays in the Scleractinian Coral *Acropora cervicornis*

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Coral reefs are among the most biologically productive and economically important ecosystems in the world, with corals supporting significant diversity of marine life in tropical regions. Corals are directly or indirectly impacted by a broad range of stressors including a diverse array of chemical contaminants, the impacts of which can be enhanced when combined with co-stressors, such as, UV radiation, ocean warming, and acidification. Clear assessment of the environmental risk of chemical contaminants to scleractinian corals is hampered as there are currently no US EPA or OECD standard test protocols for corals, and it is uncertain how representative other standard test species (i.e. marine invertebrates and algal species) may be, given the complexity of the coral host-symbiont-holobiont system. Toxicity tests with corals have been conducted but the lack of a standard methodology has limited comparisons between studies and highlighted concerns regarding data quality and reliability. Building upon experience conducting coral and other cnidarian toxicity tests with crude oil, polycyclic aromatic hydrocarbons, chemical dispersants, heavy metals, pesticides and UV filters we provide guidelines and discuss considerations for the design and conduct of standard toxicity tests for scleractinian coral species. Factors include the relative sensitivity of individual coral species and/or life-stages to chemical contaminants, their availability and suitability to laboratory culture, specific test designs and replication requirements, inclusion of appropriate parameters for quality control (i.e. water quality, health assessments and appropriate negative and positive controls), timing of exposures, analytical verification of exposures, and finally the choice of appropriate biological endpoints for acute and chronic assessments to provide reliable and statistically appropriate measurements. The flow-through exposure system and methodology which was developed based on these principles was tested with two positive controls (copper and diuron) in acute and chronic exposures with the Atlantic staghorn coral *Acropora cervicornis*. Analytical verification in each replicate confirmed that reliable and reproducible concentrations in expected ranges (based on nominal dosing) were achieved, water quality parameters remained within guidelines, and coral health was maintained throughout the 21-day duration for chronic tests.

2.01.T-02 Acute Toxicity Assays with Adult Coral Fragments: A Method for Standardization

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As coral reefs face global decline from multiple anthropogenic stressors, including chemical substances such as pesticides, pharmaceuticals or personal care products (e.g., sunscreens), the need for an accurate toxicity assessment is essential. Many existing studies on coral toxicity lack consistency and reproducibility. Addressing this gap, we present a standardizable acute toxicity test method for the common reef-building coral *Montipora digitata*. By testing multiple substances (i.e., BP-3, DCMU, Cu^{2+}), our study reveals that the endpoints bleaching, and mortality offer the most robust and reliable results. Aligned with international testing standards, our method promises to enhance toxicity assessments of substances that potentially enter the marine realm, paving the way for evidence-based regulatory decisions.

2.01.T-03 Assessment and Prioritization of Aquatic Contaminants of Concern on Florida's Coral Reef

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Florida’s Coral Reef (FCR) is the third largest barrier reef in the world and faces significant stress from localized, land, water, and air-based sources of pollution. Several chemical contaminants have been found in waters surrounding reefs and within corals, including pesticides, hydrocarbons, personal care products, pharmaceuticals, and trace metals. However, for most current and emergent environmental contaminants, little to no consistent information exists regarding environmental concentrations or toxicological effects on corals. Assessment of the levels of contaminants in coral reef environments is needed to conduct risk assessments to guide management, restoration, and recovery strategies, and the coral reef resource management community has recognized the need for improved, readily available comprehensive information. Geospatial databases for 12 contaminant classes, consisting of over 2.9 million data points for water and sediments, and covering over 32,000 sites in the five counties encompassing FCR (Broward, Miami-Dade, Palm Beach, Martin, and Monroe), were compiled from resources including USGS, STEWARDS, NWIS, NWQMC, MusselWatch, NOAA'S National Status and

Trends, and peer-reviewed literature. Concurrently, toxicological data for scleractinian corals was compiled from the EPA Ecotox database and peer reviewed literature for the same range of contaminant classes. These two datasets were then compared, to establish existing knowledge gaps in both environmental monitoring data and contaminant effects on corals, and to identify any emerging contaminants of concern along the FCR. Where toxicological data for corals was not available, data for related organisms (i.e. another cnidarian) was used to evaluate potential hazard, and where environmental data was not available, then potential sources and likelihood of occurrence for key contaminants was evaluated to estimate exposure risk. The data, analysis, and evidence-based recommendations support informed conservation management decisions and will be widely disseminated via incorporation into Florida's Department of Environmental Protection's (FDEP) coral reef decision support system (CRDSS).

2.01.T-04 Anthozoan Responses to Anthracene: A Comparative Investigation of Tropical and Temperate Sea Anemones

Michael B Morgan[1], *Amari Toussaint*[2] *and Kirt L. Onthank*[3], *(1)Biology, (2)Biology, Berry College, (3)Biology, Walla Walla University*

Anthracene, a polycyclic aromatic hydrocarbon, is more toxic in the presence of ultraviolet radiation. Anthozoans can be exposed to aromatic hydrocarbons from accidents with crude oil spills associated with drilling, shipping, and refining. Previous studies used larval development and transcriptional oxidative stress responses as endpoints for characterizing and quantifying anthozoan responses to anthracene. Additional transcriptional responses to anthracene have not been evaluated. This investigation used tropical and temperate sea anemones to generate transcriptional profiles for anthozoans exposed to anthracene. The tropical anemone *Exaiptasia diaphana* and the temperate anemone *Anthropleura elegantissima* were exposed to nominal 20ppb anthracene in the lab, as well as under full sunlight, for 4hrs. Using qPCR, this investigation sought to develop transcriptional profiles for genes associated with hypoxia, UV damage/repair, chromatin remodeling, and apoptosis. After the 4hr anthracene exposure, subsets of anemones were placed in clean seawater for an additional 7 days. Respirometry was performed on subsets of anemones and treatments to measure rates of oxygen consumption. Results compare tropical vs temperate responses, significant differences in transcriptional responses between presence/absence of direct sunlight, and any significant differences in the transcriptional profiles for the genes of interest after the recovery periods of 24hrs, 96hrs, and 7 days. Respirometry data coupled with transcriptional data provides a mechanism for correlating rates of oxygen consumption with transcriptional hypoxia responses. Significant differences in transcriptional responses from presence versus absence of sunlight provides a quantitative measurement for assessing the phototoxicity of anthracene. Responsive genes presented herein should be considered candidate biomarkers of exposure for future transcriptional ecotoxicology investigations.

2.01.T-05 Acute Toxicity of an Oil Spill Herding Agent to Atlantic Shallow-Water Corals

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Oil spills pose a significant threat to coral reefs and other nearshore environments. Emergency spill response methods can be deployed to contain and minimize the effects of spills, including mechanical containment and recovery, in-situ burning, use of sorbents, bioremediation, and application of dispersants. Chemical herding agents, which can contain and control the spread of oil, have emerged as a promising addition to the spill response toolbox as a potential alternative to other response agents. Herders are designed to be biodegradable and minimize potential harm to aquatic ecosystems. However, the oil spill response community needs data about the toxicity of herders to assess their usefulness in sensitive ecosystems such as coral reefs, where other chemical agents are not typically authorized for use. To address this knowledge gap, the acute toxicity of the herder Thickslick 6535 on two species of shallow water Atlantic scleractinian corals (*Acropora cervicornis* and P*orites divaricata*) was determined in two separate 96-hour static renewal tests. The corals exhibited a range of dose-dependent responses to herder exposure; low concentrations (72.5 mg/L and 145.5 mg/L) resulted in mild to moderate polyp retraction, while higher concentrations (582 mg/L and 1164 mg/L) resulted in moderate to severe polyp retraction, bleaching, and thinning of tissue, leading to mortality. The 96-hour acute exposure thresholds (LC50) were 285.1 mg/L for *Acropora cervicornis* and 329.5 mg/L for *Porites divaricata.* The LC50 falls within the range previously reported for other marine invertebrates such as *Tisbe battagliai* (copepod) and *Mysidopsis bahia* (shrimp). This study provides new data on the effects of herders on shallow-water corals to support spill-response decision-making and inform Net Environmental Benefit Analysis (NEBA)/Spill Impact Mitigation Assessment (SIMA).

2.01.T-06 Developing Standardized Metrics for Analytical and Biological Assessments in Corals

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Corals are impacted by a broad range of physical, biological and chemical stressors often in combination. To assess coral health and conduct environmental risk assessments of chemical contaminants biological and chemical assessments from field-collected corals or from those exposed to stressors in the laboratory are conducted. In addition to a lack of standard test protocols for coral toxicity testing there are also a number of metrics (normalization indices) that have been used when investigating changes in a biological endpoint or the levels of chemical in coral tissues. Typically used metrics in other species, such as, wet and dry weight are challenging in corals given the extraction procedures used. In many studies relating the data to coral surface area has been used with various approaches employed to estimate this. Furthermore, many normalization metrics that have been used, such as, chlorophyll and protein levels are themselves often impacted by the stressor, which is dependent upon the stressor under study. This presentation summarizes lessons learnt from conducting health assessments, toxicity tests and bioaccumulation studies in various coral species exposed in the laboratory to a diverse array of inorganic and organic contaminants, together with corals collected from the field. The goal of this summary and review is to initiate a discussion on best practices and standardized methods moving forward in the assessment of coral health.

2.02.P-Th Advancing Aquatic Toxicity Test Methods: Developments in Testing and Data Analysis of Toxicity Test Methods for Effluents, Sediments, and Receiving Waters

2.02.P-Th-024 Machine Learning-Based Water Quality Prediction for Biological Early Warning System Using In-Situ Daphnia magna Behavior Data

***Tae-yong Jeong**, Environmental Science, Hankuk University of Foreign Studies, Korea, Republic of (South)*

When a harmful contaminant is introduced into the surface water at the BEWS site, *Daphnia* is exposed to the harmful contaminant and shows significantly different behaviors as reactions. *Daphnia magna* BEWS has successfully detected sudden spills of contaminants into surface waters. However, the index calculated from multiple behavioral parameter measurements still causes frequent false alarms in monitoring stations owing to ambient physical, chemical, and biological variations. Therefore, a new approach must be considered to improve *Daphnia magna* BEWS performance. In this study, we obtained eight years of long-term data from a water quality monitoring station and developed ML models to develop the BEWS performance. The objectives of this study include predicting water quality alerts from *Daphnia* behavior and investigating the underlying relationship between behavioral variations and water pollution. Therefore, two independent models, 1) water quality early warning (WQEW) models and 2) *Daphnia* behavior prediction (DBP) models, were constructed separately depending on these specific purposes. The LGBM model demonstrated the most significant improvement in predicting alarms from Daphnia behaviors. Compared with the traditional BEWS alarm index, the ML model enhanced the precision and recall by 29.50% and 43.41%, respectively. The speed distribution index and swimming speed were significant parameters for predicting water quality warnings. These findings suggest that ML models have the potential to advance the predictive capabilities of BEWS.

2.02.P-Th-025 Challenges with Toxicity Identification Evaluation (TIE) Manipulations on Steel Mill Wastewater

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National Pollutant Discharge Elimination System (NPDES) permits can require a Toxicity Reduction Evaluation (TRE) plan for entities not meeting their regulatory permit limits for *Ceriodaphnia dubia* and *Pimephales promelas*. As part of a TRE plan, Toxicity Identification Evaluation (TIE) manipulations can be used to characterize the toxicant(s) in wastewater. In certain situations, TIE manipulations in wastewater can be challenging due to several factors: variability of the toxicant presence, potential for microbial growth, and the necessity to show baseline toxicity. In the current study, wastewater from a steel mill operation had a series of TIE manipulations (Filtration, EDTA, aeration, pH adjustments, C_{18} Solid Phase Extraction) performed on the wastewater. However, these manipulations were inconclusive at identifying the toxicant. It was determined that selected mill processes used an additive coating chemical that was suspected of contributing to toxicity in the wastewater. Product testing was performed on the additive coating chemical, and *C. dubia* and *P. promelas* chronic toxicity tests were run. Expected concentrations of the coating additive were calculated using site wastewater flow measurements. Two proof-of-concept granular activated carbon filtration tests were performed. The first carbon filtration test showed attenuated toxicity (survival, reproduction, and growth) to *C. dubia* and *P. promelas* in the product test. Selected acute and chronic endpoints showed a 3 - 4× reduction in toxicity. A second carbon test was performed with more emphasis on the carbon contact time, carbon mass, and flow rates. The second carbon test results did not yield further reduction in toxicity when compared to the first carbon test. Further testing showed that specific conductivity and pH were increasing in the carbon filtrate. Additional carbon conditioning and rinsing was performed and demonstrated that specific conductivity and pH equilibration was achieved with a ~5 d soaking in moderately hard water with multiple rinses. The TRE process provides a useful vehicle to investigating toxicants to aquatic organisms, and TIE manipulations do have applications in the TRE process. In this study, the product test concentrations filtered through carbon showed that *C. dubia* and *P. promelas* toxicity was being removed. Future carbon filtration tests will test facility wastewater upstream of pretreatment with conditioned granular activated carbon.

2.02.P-Th-026 Method Development for an In Situ, Effects-Based Monitoring Tool Using Lab-Grown Algae Deployed in Dialysis Membrane Devices

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Monitoring chemical contamination in water bodies is crucial for evaluating aquatic ecosystem health and is best achieved using a diversity of techniques. *In situ* bioassays can be an effective compliment to laboratory studies due to their higher environmental relevance and integration of complex, site-related variables (temperature, light availability, osmotic gradients, etc.). To date, the majority of *in situ* studies have utilized caged fish, bivalves, and more recently macroinvertebrates. However, despite their being representative of a critical trophic level (producers) and commonly used in laboratory toxicity testing, algae have rarely been utilized *in situ* due to the technical challenges of deploying small single-celled organisms and reliably determining effects-based endpoints in a dynamic environment. The current study describes initial optimization and characterization of a method using semi-permeable membranes to expose lab-grown algae (*Raphidocelis subcapitata) in situ* and measure effects-based endpoints. Two potential membrane materials; low-density polyethylene tubing, and dialysis tubes were tested. While low-density polyethylene filled with lipid have been widely used as semi-permeable membrane devices, initial dye testing indicated that, in the absence of added lipids, the osmotic gradient was not high enough to cause dye to partition across the membrane. In contrast, a similar dye study with dialysis tubing allowed for rapid exchange of dye across the membrane. To determine viability for use as an *in situ* bioassay device for algae, we conducted experiments to determine baseline variables including the rate of chemical uptake and exchange across the membrane, as well as baseline growth patterns in a controlled laboratory setting. Dialysis tubes were placed in tanks containing 8 mg/L of cyanazine, and samples were taken from the tubes and tank media every hour. Preliminary results demonstrated that equilibrium was reached approximately after four hours, as well as lower growth rates than in flasks. Future studies will optimize algal growth rates and measure inhibition of photosynthesis with a PAM fluorimeter, comparing dialysis exposure techniques to a traditional flask-based assay. *The contents of this abstract neither constitute, nor necessarily reflect U.S. Environmental Protection Agency policy.*

2.02.P-Th-027 Effects of Incubation in River Water Contaminated by Agricultural Runoff on Zebrafish (*Danio rerio*) Embryo Tail Coiling Activity

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Fish worldwide spawn in waters that are contaminated with complex mixtures of environmental chemical. These mixtures often have subtle effects on aquatics organisms that cannot be well detected by traditional toxicity endpoints such as mortality. New and more sensitive methods such as behavioral tests can be used to assess more subtle organismal changes. The level of spontaneous tail coiling activity in fish embryos can be a sensitive and specific marker of developmental neurotoxicity. Spontaneous tail contraction is a simple movement of the tail towards the head, and it is the first behavioral response that can be observed. Here we evaluated a method to assess the neurotoxic effects of exposure to pesticide contaminated river water on zebrafish (*Danio rerio)* embryos by measuring the changes in tail coiling activity. Freshly fertilized (1-hour post-fertilization (hpf)) zebrafish embryos (n=5 per treatment) were raised in either river water collected following a heavy rain event (>30 mm) when pesticide concentrations are the highest, or in control water that matched river water physicochemical parameters. River water was collected in the Richelieu River (Québec, Canada) during the spring/summer spawning season of many native fishes including endangered species. At 11 hpf, embryos were placed inside the Celldiscoverer 7 (Zeiss) in a 96 well plate with 0.2 mL of treatment water until 35 hpf during which time embryo activity was recorded. Video files were broken down in hour long files for each hpf and analyzed using Ethovision (Noldus) to determine the average tail coil and/or activity burst per minute for each hpf. Tail coil per minute ranged from 5.2 (±11.6) to 50.8 (±49.9) and from 0.2 (±0.3) to 55.6 (±57.6) in the river and control water group respectively. Tail coiling initiated at 17 and 18 hpf for control and river water respectively. Thereafter, both groups followed the same trajectory; with increasing activity peaking at 23 hpf, then decreasing until 34 hpf, and completely stopping at 35 hpf. There was no significant difference in tail coiling (average tail coil/min for each hpf) activity between the river and control water groups. Tail coiling activity is a valuable behavioral assessment that has potential, but this system did not allow using high throughput. The results show a promising approach that we will build upon to develop a high throughput assay which will also be used to test the effects of individual chemicals.

2.02.P-Th-028 Standardization of USEPA Short-term Chronic Methods for Evaluating Whole Effluent and Receiving Water Toxicity Using a Freshwater Mussel (Fatmucket, *Lampsilis siliquoidea*)

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Whole Effluent Toxicity (WET) testing is an important component of the U.S. Environmental Protection Agency (USEPA) integrated approach for assessing the water quality of receiving waters (ambient testing) and the potential toxicity of effluents under USEPA's water quality management programs. USEPA's current freshwater toxicity test methods for estimating the chronic toxicity of effluent and receiving water use only three test species (fathead minnow, *Pimephales promelas*; cladoceran *Ceriodaphnia dubia*; and green alga, *Raphidocelis subcapitata*). USEPA is considering adding more standard test species, including a freshwater mussel (fatmucket, *Lampsilis siliquoidea*) which are widely distributed, live burrowed in sediment filtering food from the water column, and exhibit high sensitivity to a variety of contaminants. In the past ten years, U.S. Geological Survey has worked with scientists and managers of federal agencies (including USEPA), universities, and the private sector in the USA and Canada to develop, validate, and apply a short-term 7-day chronic toxicity test method for freshwater mussels. The 7-day mussel test method has been included as part of the 2022 revised ASTM standard E2455-22 for conducting toxicity tests with freshwater mussels. The mussel test duration is consistent with the USEPA's short-term methods for fathead minnow and cladoceran. An independent review indicates that (1) the 7-day mussel method meets the ASTM's internal method testing criteria; (2) the data published in journal articles indicate that fatmucket is as sensitive, or more sensitive, to some toxicants and permitted or mock effluents relative to the two standard species (*P. promelas*, *C. dubia*); and (3) an interlaboratory study demonstrated the ability to achieve reliable test endpoints of survival and growth. However, the procedure of the 7-day mussel method described in ASTM E2455-22 does not meet all the requirements specified for the USEPA's test methods and therefore the mussel method is being revised using an USEPA toxicity test method format. USEPA may then choose to independently review, seek public comment, and potentially approve as an USEPA toxicity test method. This presentation will summarize a newly revised test method for fatmucket and provide updates on its status in the WET testing program.

2.02.T Advancing Aquatic Toxicity Test Methods: Developments in Testing and Data Analysis of Toxicity Test Methods for Effluents, Sediments, and Receiving Waters

2.02.T-01 A New ToxMate Effect-Based Behavioural Test for Micropollutant Toxicity Assessment in Effluents

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As the availability of water resources decreases, the emergence of new contaminants of concern (CEC) continues to rise. Presently, the assessment of effluent toxicity in wastewater monitoring relies on interval-based grab sampling measurements for a select few chemicals, or on punctual effluent toxicity assessment using bioassays for whole effluent testing (WET). Innovative approaches in WET are crucial to capture the impact of the ever-multiplying CECs in aquatic ecosystems and comply with stricter regulations on micropollutant levels. The advantage of effect-based in-vivo WET lies in its ability to reflect the overall toxicity of complex mixtures of micropollutants. One biomarker that has garnered significant research attention in the last decade is behavioral response. The ToxMate biomonitoring system is a laboratory and field-based apparatus designed for locomotor behavioral measurement through videotracking. Utilizing up to three species of bio-indicator invertebrates, the ToxMate has demonstrated effectiveness in non-targeted effect-based screening of micropollutant presence. Locomotor behavior has shown to serve as a biomarker of interest due to its sensitivity and rapid response time to sublethal concentrations of micropollutant exposure. The objective of this communication is to present results from a new WET test developed with the ToxMate for acute short-term toxicity assessment of complex mixtures. This test, named DIAG test for diagnostic, is in the final development stages after extensive laboratory research as well as methodology from proven effluent biomonitoring methods with the ToxMate. Novel bio-model species are used for behavioural analysis: the amphipod *Hyalella Azteca*, the annelid *Erpobdella testacea* and the gastropod *Planorbella duryi*. The toxicity results presented in this communication stem from two distinct studies and sampling campaigns. The first tests were conducted on wastewater samples collected from identified potential hotspot points within the wastewater network upstream of the

WWTP. Meanwhile, the second study compares toxicity levels upstream and downstream of various stormwater outlets in northern France, encompassing both dry and wet weather conditions. The results underscore the utility of the DIAG test for ecologically relevant toxicity assessment of complex mixtures, with potential applications including evaluating the adequacy of extreme weather management systems and identifying hotspots and sources of pollution.

2.02.T-02 Standardization of Acute and Short-term Chronic Methods for Whole Effluent and Receiving Water Toxicity Using the Mayfly, *Neocloeon triangulifer*

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Whole Effluent Toxicity (WET) test methods for measuring the toxicity of effluents and receiving waters are found in 40 CFR Part 136. Short-term chronic freshwater toxicity test methods include the use of three species – a cladoceran (*Ceriodaphnia dubia*), the fathead minnow (*Pimephales promelas*), and a green alga (*Selenastrum capricornutum*, now known as *Raphidocelis subcapitata*). The regulations include no representatives from the sensitive aquatic insect orders used extensively in water quality stream assessments. In 2015, the results of an EPA study were published, establishing 48-hour acute and 14-day chronic test methods for *Centroptilum triangulifer* and comparing its sensitivity to two model invertebrates, *C. dubia* and *D. magna*. Mortality and growth effects were determined using the reference toxicants sodium chloride, potassium chloride, and copper sulfate. Since that time, there have been several papers and presentations about the use of those methods with *Neocloeon triangulifer* (formerly *C. triangulifer*). Currently, EPA is working on standardizing a 48-,72-, or 96-hour acute test method and a 7-day short-term chronic test method to determine mortality and growth effects of three reference toxicants (ammonium chloride (NH_3), zinc sulfate (Zn^{2+}), and diazinon) on *N. triangulifer*. Standardization of *N. triangulifer* culturing conditions and a defined diet of two diatoms, *Mayamaea atomus* and *Nitzschia* cf. *pusilla,* is currently under development. Percent survival and fecundity criteria, using pre-egg laying adult weights and egg hatch rates, are being developed for assessing overall culture fitness. Diatom food quality is being standardized through fatty acid lipid profiles, dry weight, and chlorophyll spectrometry for both culture and toxicological testing. Preliminary acute results for ammonia as N in mg/L averaged LC50 for 3 tests were 4.4 LCL 4.3, UCL 4.5; acute measured zinc in µg/L averaged LC50 using trimmed spearman karber method for 3 tests was 83.1, LCL 71.6, UCL 94.6; and range finder test for diazinon gave an estimated IC25 of 94.4 µg/L. The standardization of the culture and toxicity test methods for *N. triangulifer* will provide a sensitive tool for assessing the toxicity of effluents and in establishing ambient water quality guidelines for aquatic life protection. The progress and results of culture and test method development will be presented.

2.02.T-03 Wastewater Management Strategies: What Happens if Your Water Quality Becomes "Too Good"?

William L. Goodfellow[1] *and Konrad Kulacki*[2]*, (1)Exponent, (2)Ecological and Biological Sciences, Exponent*

Managment of effluent discharges are an important responsibility of every facility. Often discharge permits (e.g., NPDES) require facilities to monitor their effluents for specific chemicals and can also include whole effluent toxicity (WET) testing requirements. However, treatment of wastewater can alter it in ways that may have unexpected impacts on receiving waters and may be counter to the initial priority of maintaining acceptable water quality. For example, following treatment the quality of wastewater in a treatment system may improve to the point of no longer inhibiting organisms in the receiving waters or even within the treatment system. In turn, this can lead to outbreaks of aquatic organisms in treatment lagoons or ponds that can create nuisance issues at the facility or surrounding community. While swarms of emerging insects may be troubling enough, they can also attract other wildlife such as birds and small mammals to the facility or neighboring community. Strategies to mitigate these outcomes may include treatment of wastewater lagoons with biological agents such as *Bacillus* (Bt) products, other inhibitory chemicals, or making use of deterrent applications for larger animals. The treatment of effluent can also alter the composition and concentration of cations and anions contained therein. While releasing wastewater too high inions can lead to freshwater salinization, releasing wastewater too poor in ions can also upset the ion balance in receiving waters, impacting biota. Using case examples, this presentation will provide strategies to be used when situations such as these occur. A discussion will include implications and important considerations for the implementation of various management options when they become necessary.

2.02.T-04 Maternal Age of *Ceriodaphnia dubia* Effects on EPA WET Testing Consistency

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Maternal investment in offspring can vary over the lifetime of the mother. In EPA Whole Effluent Toxicity (WET) testing, although *Ceriodaphnia dubia* neonates are approximately the same age when a test is initiated, the timing of the test initiation often dictates the age of the culture board being used to begin tests. Thus, it is possible that there could be impacts on neonate health and the resulting testing endpoints, meaning tests begun at different times in a mother's lifetime might not produce similar results. Therefore, this experiment set out to determine if the offspring of *C. dubia* mothers as they age produce equivalent sensitivity to toxicants. *C. dubia* offspring from mothers producing their 3rd, 5th, and 7th broods were exposed to the reference toxicant sodium chloride in 48-hour acute and 6–8-day chronic tests. An additional variable considered was if the neonates were from a mother who had reproduced within the past 24 hours. Ten replicates of each brood and timing situation were executed, with a total of 60 acute and 60 chronic tests performed. For acute assays, the resultant LC_{50}s were compared; for chronic assays, the LC_{50}, IC_{25}, and number of test days were compared. The implications of this experiment will address intra- and inter-lab variation concerns, as well as better inform testing laboratories how to achieve consistency with their WET test procedures.

2.02.T-05 *C. dubia* Chronic Toxicity Testing Interlaboratory Study Data Variability and Performance Metrics

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The short-term chronic *Ceriodaphnia dubia* survival and reproduction toxicity test (USEPA test

method 1002.0) is widely used in the United States for compliance monitoring by dischargers of treated wastewater. This is especially true in California where *C. dubia* is often determined to be the most sensitive freshwater species for compliance testing in National Pollutant Discharge Elimination System (NPDES) permits. Although this test is highly important for dischargers and regulators, the regulated community has observed that multiple labs can produce variable results (e.g., ranging from pass to fail) when conducting the chronic *C. dubia* toxicity test on split samples. In response to discharger concerns over the consistency and precision of this test, the California State Water Resources Control Board (State Water Board) conducted interlaboratory testing among 12 labs with blank samples (i.e., prepared culture water) and a reference toxicant, to evaluate the range of test responses and identify potential sources of variability within and among laboratories. An independent analysis of the State Water Board study data for blank samples confirmed that the blank samples could result in significantly lower *C. dubia* reproduction than in the negative control at some laboratories. No single cause of these indications of “toxicity” was identified. Conversely, the study’s Expert Science Panel put forward five draft performance metrics that could be used by laboratories as targets to help improve comparability and consistency in *C. dubia* tests. The metrics were based on survival, reproduction, coefficient of variation for reproduction, percent minimum significant difference, and point estimates for inhibition concentrations. When applied to laboratories that participated in the inter-laboratory study, laboratories that consistently exceeded expectations across the performance metrics also exhibited the most consistent and comparable test results; and, importantly, did not find any indication that blank samples were associated with significant adverse effects. This poster presents the independent data analyses and recommendations describing how toxicity testing laboratories, dischargers, and regulators can use these performance metrics to evaluate and improve chronic *C. dubia* toxicity data quality.

2.02.T-06 Uncertainties in Estimating Low Effect Concentrations in Aquatic Toxicity Tests

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The past 40 years have seen a strong shift toward expressing results of aquatic toxicity tests with regression-based estimates of effect concentrations (ECp) rather than highest no-observed effect concentrations (HNOEC) and/or lowest observed effect concentrations (LOEC). ECp estimates have certain clear advantages for better defining risk; in particular, they address a fixed level of effect (p), whereas HNOECs/LOECs have a variable level of effect dependent on the selected test concentrations and the uncertainty/quality of the test. However, for low values for “p”, an ECp can be highly uncertain and even unrealistic due to various issues regarding estimation methodology, model selection, and data sufficiency. To define and illustrate these issues, analyses were conducted using a rich dataset on the chronic toxicity of NaCl to *Ceriodaphnia dubia* and hypothetical datasets based on other aquatic toxicity tests. Important implications of this for defining risks will be discussed. *This abstract does not necessarily reflect U.S. Environmental Protection Agency policy.*

2.03.P-We Aquatic Mixtures: Characterizing Chemical Composition and Estimating Hazard

2.03.P-We-048 Development of Mathematical New Approach Methods to Assess Chemical Mixtures

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The U.S. EPA's Toxicity Forecaster (ToxCast) program contains targeted bioactivity screening data for thousands of single chemicals to inform prioritization and hazard prediction. However, environmental contaminants in practice are often encountered as coexposures; thus, it is of high importance to also study the bioactivity of chemical mixtures. High-throughput screening data are not available for defined chemical mixtures in part because of the immense number of possible combinations and concentration ratios. The aim of this work is to leverage the readily available single chemical screening assay data in ToxCast to predict the bioactivity of binary chemical mixtures. Mathematical relationships between mixtures and their single chemical constituents are applied with two different models, concentration addition and independent action, resulting in predictions of mixture potencies. To evaluate the performance of these mathematical mixture models, an empirical data set was collected for 21 chemical mixtures and their single chemical constituents screened in concentration-response using a multidimensional *in vitro* platform for transcription factor activity. The chemical mixtures selected were inspired by common household coexposure chemicals from a survey of purchasing data and based on availability in the ToxCast chemical library. Point of departure estimates were compared among the predicted mixture models and the best-fit curves observed in ToxCast. Out of 1701 binary mixture-assay endpoint responses, 237 demonstrated a positive mixture response with at least one active single constituent; in these cases, about 80% of the mixture activity concentrations at the cutoff level predicted from the single chemical constituents fell within 0.5 $\log_{10}$-µM of the observed cutoff concentrations. Furthermore, bootstrap resampling was performed to obtain 95% confidence intervals on the concentration-response models. It was found that 90% of the intervals from the predicted mixture curves overlapped with the observed mixture curve intervals at more than 50% of the tested concentrations. As it is resource-prohibitive to screen all combinations of chemicals, the determination of conservative mixture predictions and related uncertainties is critical for efforts to operationalize existing ToxCast data for the forecasting of simulated mixtures from real-life coexposures. *This abstract does not necessarily reflect U.S. Environmental Protection Agency policy.*

2.03.P-We-049 Role of Alkylated Polycyclic Aromatic Hydrocarbons in Mixture Toxicity from a Legacy Creosote Site

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Derived from distillation of coal tar, creosote is a complex mixture containing mostly polycyclic aromatic hydrocarbons (PAHs) and their derivatives. In particular, alkylated PAHs are abundant constituents of weathered creosote and many petroleum products. While less is known about the

toxicity of alkylated PAHs relative to their parent compounds, they have been demonstrated as important drivers of toxicity in PAH mixtures. This study evaluated the contribution of alkylated PAHs to the toxicity of complex, weathered mixtures from a legacy creosote site. Low density polyethylene passive samplers were used to accumulate freely dissolved organics in the surface water at a former wood treatment site. Samplers were analyzed by gas chromatography-tandem mass spectrometry for unsubstituted and alkylated PAHs. Embryonic zebrafish were exposed to field-collected mixtures and mortality and morphological effects are reported. Three years of sampling at the site demonstrates temporal variability in chemical abundance and toxicity with sum PAH concentrations in adjacent months varying by greater than a factor of two and LC50 values varying by a similar magnitude. Alkylated PAHs constituted the majority of measured PAHs in all samples (83-89%). Fractionation by gel permeation chromatography revealed that two fractions together recapitulated the toxicity of the whole mixture. The fraction containing alkylated PAHs, particularly alkylated phenanthrenes, caused the majority of toxicity while the fraction containing many unsubstituted PAHs caused less than 10% mortality. A third unidentified fraction caused mortality and high incidence of notochord malformation at low concentrations. Target and non-target screening approaches failed to confirm the identity of drivers of toxicity in fraction 3. While toxicant confirmation of alkyl isomers in fraction 4 is unfeasible, their abundance and reported toxicity suggest that alkylated phenanthrenes drive toxicity to zebrafish rather than routinely monitored parent PAHs. Understanding the role of alkylated PAHs informs remediation efforts and improves our ability to protect human health and water quality.

2.03.P-We-050 Toxicity Assessment of Three Rare Earth Elements (La, Gd, Y) in Single and Binary Mixture Exposures to Two Benthic Organisms (*Chironomus riparius*, *Hyalella azteca*)

***Marie Lefranc**[1], Valentin Dupraz[2], Marc Amyot[3], Marie-Soleil Boulay[1], Maikel Rosabal[1] and Noémie Wrzesinski[1], (1)Biological sciences department, University of Quebec in Montreal, Canada, (2)Department of Research, Innovation and Ecological Transition, Bordeaux City Water Authority, France, (3)Biological sciences department, University of Montréal, Canada*

Rare Earth Elements (REEs) are used in multiple applications, including green technologies. Their growing demand has led to the development of numerous mining projects in Canada. In addition to mining effluents, there are other sources of contamination in REE mixtures, such as agricultural or municipal effluents. In aquatic environments, REEs are largely found in sediments when they can cause deleterious effects. However their toxicity in benthic organisms is little known. To fill this knowledge gap, the objective of this study is to provide toxicity data on three REEs (La, Gd, Y) in *Chironomus riparius* and *Hyalella azteca*, then to evaluate whether there are interactions in binary mixtures in the most sensitive species. To do that, concentration-response curves were produced with sediments contaminated by La, Gd, and Y with both species using sub-chronic toxicity tests. Growth inhibition and mortality were assessed and NOEC/LOEC as well as ECx (e.g., EC_{20}, EC_{50}) were calculated. The results showed that *C. riparius* was more tolerant than *H. azteca,* and that there are slight differences in toxicity between the three REEs where $EC_{20}Y > EC_{20}Gd > EC_{20}La$. In addition, toxicity tests with mixtures of La/Gd, Gd/Y and La/Y with *H. azteca* were also carried out and analyzed with the isobologram methodology to determine if there are interactions. According to our results, synergistic interaction was found for the binary combinations studied with the exception of La/Gd (antagonist). Analyzes of REE concentrations in sediments and in *H. azteca* are underway to get more insights about these interactions and the resulted effects. The next steps in this research will focus on understanding the metal-handling strategy of studied REEs using subcellular metal partitioning approach as well as determining their target biomolecules using metallomic coupling techniques.

2.03.P-We-052 PFAS-Free Alternatives: Tools to Avoid Regrettable Substitutions

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Transitioning to PFAS-free alternatives necessitates a systematic evaluation of tradeoffs, considering uncertainties, to identify the most effective short- and long-term strategies. However, standard hazard assessment protocols often overlook critical aspects of PFAS due to their emphasis on bioaccumulation and acute impacts, neglecting the profound implications of chronic low-dose exposure and extraordinary persistence. Additionally, the pervasive presence of PFAS in the environment raises concerns about potential synergistic effects resulting from their ubiquitous mixture, an area that remains largely unexplored. This talk aims to provide an overview of our ongoing activities focused on assessing the environmental impacts of Fluorine-free Foams (F3) as a PFAS-free alternative.

2.03.P-We-053 Evaluating Interactive Effects of PFAS Mixtures on Aquatic Organisms

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Understanding the ecological risks of per- and poly-fluoroalkyl substances (PFASs) is complicated by the fact that they are present in aquatic environments as complex mixtures. It is often assumed that combined effects of chemicals sharing a common mechanism of toxicity can be predicted by dose additivity, and this approach has been applied to chemical groups like PAHs and PCBs. However, PFASs are a much more structurally diverse group, and evidence from our prior work suggests that different toxic mechanisms may be operative for different structural classes of PFASs and/or different test species. In this presentation, we share results from experiments designed to evaluate the interactive toxicity of PFASs suspected to have both similar and dissimilar mechanisms of action. Effects of binary mixtures of two PFASs are evaluated in three freshwater invertebrate species using 7-d exposures measuring sublethal responses (growth or reproduction). From the resulting isobolograms, initial findings are consistent with additivity between PFASs with both similar and dissimilar mechanisms of sublethal toxicity, although the ability to distinguish additivity from independence is limited in some cases by shallowness of the concentration-response curves. There was no evidence of synergy or other extreme interactions. If these results hold broadly true across species and PFAS structural types, it suggests that assuming additivity may be reasonable when estimating ecological risk of complex PFAS mixtures. *This abstract does not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.*

2.03.P-We-054 Use of Transcriptomic Points of Departure to Assess the Toxicity of Environmental Chemical Mixtures in Early Life Stage Copper Redhorse

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In aquatic ecosystems, fish hatch and develop in waters contaminated with hundreds if not thousands of environmental chemicals. Exposure to these complex environmental mixtures may have important consequences on early life stage (ELS) fish, which are known to be sensitive to environmental contaminants. Transcriptomics dose-response analysis (TDRA) is a promising approach for predicting the organismal level toxicity of environmental contaminants from transcriptomics data. It has potential applications in the field of environmental risk assessment but has seldom been used in the context of environmental mixtures. Here, we used a 24-hour microplate-based larval bioassay to assess the toxicity of copper and two pesticides (s-metolachlor and chlorantraniliprole) within the context of environmentally relevant contaminant mixtures. 24 hours post hatch Copper Redhorse larvae (M*oxostoma hubbsi*), an endangered fish species, were exposed to 12 concentrations (including a control) of copper sulphate, s-metolachlor, or chlorantraniliprole. The exposures were performed in 24-well plates with one larva per well. The concentration range for each chemical was based on the estimated LC50, extending two orders of magnitude above this value (to capture acute mortality) and five orders of magnitude below this value (to capture the full transcriptomic response). Each chemical was spiked into one of three water samples to assess the contribution of environmental background contamination on overall mixture toxicity: reconstituted hard water (control), low-contamination river water, or high-contamination river water. RNA sequencing of pooled whole larvae was used to generate transcriptomic data, after which the data were analyzed using ExpressAnalyst to identify significantly deregulated genes. Ongoing analysis for CuSO4, s-metolachlor and chlorantraniliprole will include TDRA to identify differentially expressed genes (DEGs) and transcriptomic points of departure (tPODs), which will be compared for each chemical/water combination. The ongoing analysis will evaluate TDRA's potential for assessing the toxicity of environmental mixtures. It will also provide transcriptomic data on an endangered species and insights into sensitivities to agricultural pesticides found in its native waterways, which can contribute to our understanding of its limited recruitment success.

2.04.P-Tu Assessing Contaminant Effects in Ecosystems with Multiple Stressors

2.04.P-Tu-080 Field Testing of the In-Situ Toxicity Identification Evaluation (iTIE) System as a Novel Approach to Stream Restoration Planning

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The complexity of degraded streams coupled with the high expense of current restoration techniques calls for advancement in the field to reduce cost and better understand which chemicals are causing the most harm. The in-situ Toxicity Identification Evaluation (iTIE) system addresses these concerns by inexpensively and accurately identifying which contaminants are contributing the most toxicity in an aquatic environment. The iTIE system works by pumping polluted water through chemical sorptive resins to fractionate it into classes of chemicals (metals,

PFAS, PAHs, etc.) before exposing the water to sensitive invertebrates and fish. These organisms are cultured and monitored for death and deformity compared to organisms exposed to unfractionated water and control groups. This study verifies the iTIE system's compatibility with fish embryo-larval stage teratogenicity tests, a class of toxicity test protocols with wide applicability. Organism groups that experience high rates of death and deformity indicate exposure to contaminants of the greatest concern which is invaluable insight when creating a restoration plan. For this study three sites in southeastern Michigan were analyzed for restoration potential using the iTIE system: the Rouge River in industrial metro Detroit, a drainage stream by the Detroit Metro Airport, and Fleming creek within the Matthaei Botanical Gardens as a control site. These diverse urban and reference ecosystems provided strong case studies to validate the iTIE approach.

2.04.P-Tu-081 Effects of Acclimation to UV-B on Daphnia magna in PAH Photo-Induced Toxicity Exposures

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Polycyclic aromatic hydrocarbons (PAHs) are hydrocarbon ring structures produced from anthropogenic sources (e.g., vehicle emissions, industrial processes, leaching), and natural events (e.g., volcanic eruptions, forest fires). Once these compounds are released into the environment, they enter the aquatic system through run off or atmospheric deposition. Most aquatic organisms are also exposed to environmental stressors, such as ultraviolet radiation (UV), leading to multi-stressor exposures. Certain PAHs are known to have increased toxicity in the presence of UV. However, some organisms, such as zooplankton, have been shown to produce pigmentation when exposed to high levels of UV. Previous studies suggest that these pigments can play a protective role when organisms are co-exposed to UV and contaminants such as PAHs. Our research seeks to determine whether acclimation to UV and production of pigmentation will offer protection against photo-induced toxicity in *Daphnia magna*. To test this, *Daphnia* were cultured under 6-12 µWatts/cm^2/nm of UVB (320 nm) for 3 hours a day, or lab lighting. Neonates (<48 hours) were collected from both cultures for a 48-hour single PAH-UV exposure. Mortality was assessed at the 24- and 48-hour time point. This study provides a better understanding of how acclimation to environmental conditions can protect aquatic organisms from multiple stressor exposures.

2.04.P-Tu-082 Exploring the Importance of Dietary-Based Stressors on the Health and Survival of Juvenile Chinook Salmon (Oncorhynchus tshawytscha) in Puget Sound

Melissa Driessnack, *Jenifer McIntyre and John D. Stark, Washington State University*

Ongoing efforts are being conducted to improve the survival of fall run juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Pacific Northwest. Despite all these improvements made to the rivers as well as surrounding watersheds, fall run Chinook continue to show low survival and rates of return in the Pacific Northwest. This has led to an increased focus on the health and survival of juvenile Chinook preparing to migrate into Puget Sound. To undergo that migration salmon must successfully complete the physiologically stressful process of smolt. However, juvenile salmon undergoing smolt are impacted by multiple stressors including exposure to legacy chemicals (*e.g.*, PCBs), emerging contaminants of concern, and altered prey availability. Current toxicological effect data, specific to fall run Chinook salmon for the region, are insufficient to determine if contaminant exposure, alone or in combination with other relevant stressors, is contributing to the low survival of estuarine-reared Chinook. As such, three planned projects are underway that evaluate the importance of restricted and contaminated diets on the health and survival of juvenile Chinook undergoing smolt. The first study used a simplified PCB (Aroclor 1254) spiked diet at an environmentally relevant concentration of 200 ng/g to evaluate the health and survival of juvenile Chinook over a 30-day exposure period. That exposure noted increased mortality in both the feed restricted and contaminated feed treatments as well as changes in condition factor and whole-body PCB accumulation. The second study has focused on the timing of feeding rates in relation to smolt status followed by a saltwater tolerance test, with survival as one endpoint of assessment. Initial results of this three-part research project support that food availability is a valid factor to consider in contributing to the sustained high mortality rates for Chinook in the Duwamish and likely impact the timing and success of smolt in the juveniles.

2.04.P-Tu-083 Toxicity of Ammonia to Threatened, Endangered, and At-Risk Freshwater Mussels with a Co-stressor of an Elevated Temperature or Metal Mixture

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Current chemical toxicity thresholds and underlying chemical toxicity databases have largely been established using laboratory-based single contaminant toxicity tests. However, single-stressor tests do not consider effects of co-stressors, and subsequent synergisms or antagonisms that organisms and larger populations are likely to be exposed to in their native habitats. Thus, it remains critical to conduct environmentally relevant multi-stressor exposures to evaluate cumulative risks for individuals or their populations. Freshwater mussels are one of the most imperiled faunal groups in the world. In an ongoing research project, we use U.S. federally threatened, endangered, or at-risk (TEA) freshwater mussels to evaluate the toxicity of ammonia, one of the most common violators of environmental guideline values across the world, with a co-stressor of elevated temperature, which likely occurs in summer during low flow and drought conditions, or a mixture of metals (copper, nickel, and zinc) that commonly have elevated concentrations in industrial wastewaters, effluents, and surface water runoff. Specifically, we use ASTM E2455-22 standard methods to conduct acute 96-h and short-term chronic 7-day toxicity tests with juveniles of two TEA mussel species (Green Floater, *Lasmigona subviridis*; Pink Mucket, *Lampsilis abrupta*) and a commonly tested mussel (Fatmucket, *Lampsilis siliquoidea*) in ammonia exposures at a standard test temperature and two elevated temperatures or with a mixture of metals at a standard test temperature. The elevated test temperatures are based on temperature regimes in the mussel species native ranges and on temperatures that have been previously shown to be stressful for juvenile mussels. The concentrations of the three metals for creating the metal mixture are selected as 1/3 of previously established acute EC50s and chronic EC20s for juvenile Fatmucket in test waters with similar water quality characteristics to the test water and temperature used in the present study. This study will provide data to evaluate the interactions and effects of multiple stressors on juvenile mussels. In addition, the study aims to gauge the relative sensitivities of the TEA species for protection under existing environmental guideline values for ammonia.

2.04.P-Tu-084 How Land Use Influences Aquatic Ecotoxicity

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A major stressor complex to aquatic ecosystems is the steady increase of micropollutants over the last decades. Micropollutants are particularly problematic for the environment because they can cause toxic effects even at very low concentrations. They have the potential to affect both the environment and human health as they are often ubiquitous and persistent. Micropollutants are anthropogenical organic trace substances and comprise, for example, pharmaceuticals, pesticides, biocides and a wide range of household and personal care products and therefore have multiple pathways into the environment. In this study field samples were analysed using different *in vivo* and *in vitro* effect-based methods (EBM). The overall aim was to attribute the measured effects of the different EBMs to specific pollutant sources. This knowledge should allow a more targeted prior test selection for the evaluation of field samples. 30 water and sediment samples from three different streams in a heavily anthropogenically impacted area in central Germany were analysed. All samples were tested *in vitro* for their endocrine and dioxin-like activity, mutagenicity, baseline toxicity and oxidative stress. For *in vivo* assessment the snail *Potamopyrgus antipodarum*, the crustacean *Gammarus fossarum* and differend macrophytes were tested in chronic exposure. For all sampling sites the surrounding land use (arable land, forest, pasture, urban area) was recorded based on the Corine Land Cover 5 dataset and upsteam discharger of waste water treatment plants (WWTP) were identified. To evaluate the samples, the ecotoxicological effects were first compared with the main land use type. A PCA was then used to identify in more detail the main influencing factors at each sampling site and again compared with the results of the EBM. Overall, the selected test systems were well suited for an effect-based quality assessment of the rivers in general and thus for a first assessment of the hazard potential of existing pollutants. It was difficult to link the effects of a given EBM to one particular type of land use, as the causes of water pollution appear to be multi-causal. Agricultural pollutants may have been underestimated, as they are often not captured by grab samples. However, there were clear correlations between the effects of EBM and the proportion of the pollution load from upstream WWTP. *This project is funded by the German Federal Environmental Foundation (DBU AZ 35663/01).*

2.04.P-Tu-085 Richland Creek, Illinois: Assessment of Land Use Impacts on Microbial Activity, Fecal Contamination, and Pathogen Presence

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Richland Creek in Illinois has long been a focal point of local environmental concern due to persistent water quality issues, including many violations of Illinois surface water standards. The creek hosts multiple municipal wastewater treatment centers, recycling plants, combined sewer systems, and twenty stream miles of bordering agricultural land. Despite this, a significant data gap exists regarding the creek's current health status. Our research narrows this gap by evaluating spatial and temporal differences in microbial activity, fecal contamination, pathogen presence, and general water quality conditions. Ten sample sites were selected based on land use characteristics and stream reach considerations. Over four months, 116 water samples were collected for analysis. Laboratory analyses included microbial community-level physiological profiling, enzyme activity assays, fecal indicator bacteria tests, and standard pathogen plate counts. Hardness levels ranged from 109.0 to 358.0 mg/L $CaCO_3$ and alkalinity levels from 73.0 to 255.0 mg/L $CaCO_3$. pH values ranged from 7.15 to 8.63. *Escherichia coli* (*E.coli*) testing of the four sampling events returned geometric means ranging from 141.70 to 953.02 MPN/100 mL, with three event's means exceeding the State of Illinois standard of 235 MPN/100 mL. Overall, 31 of 39 sites sampled exceeded the state *E.coli* standards, with maximum values of >2419.6 MPN/100 mL occurring at 7 of 39 sites. Mean *E.coli* concentrations (MPN/100 mL) by land use were: Developed = 424.09, Park/Forest = 417.07, Agricultural = 800.94, Wetlands = 369.68. 1285 *Pseudomonas aeruginosa*, 672 *Enterococcus*, and 2 *Salmonella* species were detected. A 100% positive detection rate was found for total coliforms, *E.coli, Pseudomonas aeruginosa,* and *Enterococcus* species at all sites. A positive linear relationship ($\rho = 0.12$) between *E.coli* and *Enterococcus* was observed. Community-level physiological profiling determined that wetland settings had the highest microbial species diversity, richness, and evenness throughout the creek, while parks had the lowest. Enzyme activity was highest near developed and wetland settings, and lowest near agricultural. Eight more sampling events will be conducted; however, our preliminary findings highlight and demonstrate the degradative impacts of current land usage on Richland Creek's aquatic ecology, emphasizing the need for targeted and adaptive management strategies for different land uses interacting with the creek.

2.04.P-Tu-086 Dealing with Sulfide in Sediments for In-Situ Toxicity Identification Evaluations (iTIE)

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The In-situ Toxicity Identification Evaluation (iTIE) system was developed as a cost-effective, highly realistic technology for evaluating contaminated sediments, assessing the success of remediation efforts, and identifying recontamination. In the current iTIE deployment, water is collected from site porewater, aerated with oxygen canister, and filtered with various sportive resins, then the water is examined with selected test organisms. The iTIE system was previously proven effective at multiple freshwater sites without dissolved sulfide content. However, our most recent iTIE verification test at Paleta Creek, an industrial estuarine waterway in San Diego Bay, CA, was confounded by persistently low dissolved oxygen content and dissolved sulfide content in the site porewater. These two factors were the most probable causes of widespread mortality in the test organisms (Eohaustorius estuarius). We hypothesized that replacing the interior oxygenation coil with tubing that increased oxygen diffusion the sulfide would be reduced. A silicone and Fluorinated ethylene-propylene tubing were compared using sulfide concentrations of 10, 50, and 100 mg/L. Sulfide concentrations before and after each test were measured using iodometry. Our results indicate that silicone tubing performs better than FEP tubing in oxygenating water with dissolved sulfides and lowers dissolved sulfide content. As a result, this new system will be tested in August 2024. We hypothesize the iTIE technology will be able to successfully identify the main causes of toxicity at marine sites with high porewater dissolved sulfide concentrations.

2.04.P-Tu-087 Using Weight of Evidence as a Conceptual Framework for Assessing Ecological Risk Across Sites

***Matthew LeFauve**, Abigail DeBofsky, Detbra Rosales and Chuck Nace, U. S. Environmental Protection Agency*

In order to protect wildlife at hazardous waste sites, and Superfund sites in particular, a risk assessment must be conducted to demonstrate the potential for risk. Bringing together multiple lines of evidence is critical to determine the best way to assess potential risk and remedy completeness at a contaminated site. However, assigning value and importance to each line of evidence can be difficult as conclusions might vary. One method to evaluate these lines of evidence is to employ a Weight of Evidence (WoE) approach, as outlined by the US EPA in the Weight of Evidence in Ecological Assessment guidance. The process is a collaborative approach, where a list of considerations to define the WoE is used to create defensible results and has been used successfully at Superfund sites across the country. The WoE framework includes three steps: assemble the evidence, weight the evidence, and weigh the body of evidence. Lines of evidence can have a variety of sources including literature and site-specific studies to help infer a relationship between exposure and outcome to a given ecological receptor. Contaminated sites have a wide array of ecosystems across the United States, but frequently similar lines of evidence are collected. Additional lines of evidence directly relating to the ecological receptors potentially impacted by contaminant exposure should be incorporated into the weight of evidence approach as needed. Evaluating these lines of evidence and the surrounding ecosystem to weigh how the data should be assessed allows for more accuracy and transparency in uncovering the potential for unacceptable risk.

2.04.T Assessing Contaminant Effects in Ecosystems with Multiple Stressors

2.04.T-01 Forty Years Since the Redbook, a Review of the Development and Future Directions of Ecological Risk Assessment for Multiple Stressors, Endpoints and Management Goals

***Wayne G. Landis**, Western Washington University*

In 1983 the National Research Council published “Risk assessment in the Federal Government: Managing the Process. The report has stood as a landmark in the recognition of the importance of risk assessment in the decision making for chemical management. In 2003 I wrote reviewed the developments in risk assessment over the 20 years, specifically in ecological risk assessment at multiple scales with multiple stressors and with multiple endpoints. Incredibly another 20 years have passed, and it is time to take stock and look to the future. My prediction was that in 2023 that ecological risk assessment would be a universal framework for ecological management. I said that “Risk assessment can provide an organizing framework, testable hypothesis and eventually a method for evaluating management scenarios.” This statement has turned out to be only partly fulfilled. Although the tools for accomplishing such goals now exist, there has been an inability to leave the paradigms of the 1990s behind and to apply modern analysis of stressor-response relationships, non-equilibrium complex systems theory and probabilistic risk assessment to design long-term studies, to establish appropriate research programs, to evaluate management alternatives and to effectively manage the environment. I will point to examples of risk assessment case studies that meet these requirements from across the world and contrast them to the “standard” approach and the clear advantages of the application of Bayesian networks and similar approaches to assessment and management.

2.04.T-02 Land Use-Based Mapping of Lethal Stormwater Threats to Wild Coho in Puget Sound

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Urbanization threatens virtually all ecosystems, creating a species conservation challenge. Our understanding of urbanization impacts has focused on physical habitat, as agricultural and forested lands are replaced with human infrastructure. However, aquatic habitats are also chemically degraded by urban development, often in the form of toxic stormwater runoff. Since the late 1990s, coho salmon adults returning to their natal urban streams in Puget Sound experience high rates of spawner mortality syndrome. Evidence to date identifies toxic urban stormwater runoff as the causative agent, which poses a population level threat to wild coho. In addition, steelhead are also vulnerable to this toxic mix of stormwater. Identifying stream basins currently at risk for this syndrome is critical to conservation efforts. This presentation summarizes our understanding of the landscape ecology of this syndrome across an urban gradient in the Puget Sound Lowlands, based on nearly two decades of research that uses spatial analyses to identify relationships between *in situ* spawner mortality time series, and climate and landscape scale characteristics of the built environment. The resulting predicted coho spawner mortality heat-maps that encompass the Puget Sound region have already been leveraged for conservation planning and numerous related studies. For the next generation of landscape ecotoxicology modeling, we will revise and update our analyses using a longer time series of data, processed through state-of-the-art Bayesian statistical modeling frameworks. Salmon restoration spatial patterns can be combined with predictive heat-maps to minimize the risk of creating ecological traps associated with these projects. These analyses improve our understanding of the interplay between urbanization and climatic drivers of the mortality syndrome, are easily transferable to other regions that have similar available data, and can be used for planning restoration and conservation projects in the current built environment and in future development scenarios.

2.04.T-05 Combined Effects of Temperature Increases and Salinization on Freshwater Diatoms

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Salinization and warming are of increasing concern for the health of freshwater ecosystems. Interactive effects of environmental stressors are often studied in bifactorial, two-level experimental setups (all combinations of control vs. stressed for two stressors). The shape of environmental reaction norms and the position of the "control" conditions along them, however, can influence the sign and magnitude of individual responses as well as interactive effects in such comparisons. We consider the problem of empirically modelling binary-stressor effects in the form of three-dimensional reaction norm surfaces. We monitored the growth of clonal cultures of six freshwater diatoms, *Cymbella cf. incurvata*, *Nitzschia linearis*, *Cyclotella meneghiniana*, *Melosira varians*, *Ulnaria acus*, and *Navicula gregaria*, at various temperatures (up to 28 °C) and salinity levels (until the growth ceased). The data were then used to empirically model the dependence of diatom growth on temperature, salinity, and, optionally, terms

depicting their interactive effects. Fitting a broad range of models and comparing them using the Akaike information criterion revealed a large heterogeneity of interactive and non-interactive effects. A bell-shaped curve was often observed in the response of the investigated diatoms to temperature changes, while their growth tended to decrease with increasing electrical conductivity. Limited variations in the optimum temperature (15.8–18.4 °C) were revealed. Among the diatoms, *C. meneghiniana* was more tolerant to temperature as shown by its wide thermal range, whilst *C. incurvata* and *C. meneghiniana* were the most sensitive to salinity changes. Their growth ceased at electrical conductivity above 1.90 mS/cm. Results from empirical modelling revealed interactive effects of temperature and salinity on the slope and the breadth of response curves. Contrasting types of interactions were predicted for the same species, indicating uncertainties in the estimation by empirical modelling. In other words, uncertainties in simulating freshwater diatom growth could be increased by complicated interactions between temperature and salinity.

2.04.T-06 Assessing the Impacts of Metal Exposure in Fathead Minnows Experiencing Hypoxia

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Due to climate change, eutrophication in lakes is expected to increase. This increase in nutrient pollution can result in hypoxic waters. While species like fish have adapted strategies to overcome singular abiotic stressors such as hypoxia, less is known about the capacity of animals to respond to multiple stressors. This is a particular concern in Canada, where mining practices can lead to the presence of metals in aquatic habitats. This study tests the hypothesis that the impacts of metal exposure are augmented in the presence of hypoxia. To test this, fathead minnow (*Pimephales promelas*), a temperate fish species commonly found across North America, were exposed to nickel (Ni) and lead (Pb) for 48 h. Markers of disruption, including loss of equilibrium, changes in ventilation, metal accumulation, and activity levels of fathead minnows were assessed. The results collected to date demonstrate that nickel accumulation in the gills increases in the presence of hypoxia. Levels of activity are altered by exposure to metals, hypoxia, and their combination. Although there was no change in ventilation from either metal, Ni reduces the time to loss of equilibrium in hypoxia. Together, these results suggest that exposure to multiple stressors imposes compromises in animal responses that may imply significant disruptions to the performance and survival of native fish species. Given that Pb and Ni have considerable economic value to Canada, continued work understanding the effects of exposures to these metals in combination with other abiotic stressors are necessary to continue to develop Water Quality guidelines to protect our aquatic ecosystems.

2.05.P-Mo Canada's Oil Sands Mining and Dilbit Pipelines

2.05.P-Mo-034 Assessing the Bioenergetic and Oxidative Impact of Bitumen-Contaminated Water on Mammalian Hepatocytes

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The effects of bitumen extraction on water quality and wildlife health in the Alberta Oil Sands Region (AOSR) is not fully understood. Our group has previously shown altered bioenergetics in mammalian cells following exposure to constituents found in oil sands process-affected water (OSPW). However, the effects of bitumen-influenced groundwaters have not yet been investigated. The goal of this study was to (1) determine the effects of exposure to groundwater extracts on mitochondrial function and (2) compare these effects to naphthenic acid fraction components (NAFC), a subgroup of bitumen-derived organics often cited as primary toxicants in bitumen-influenced waters. McA-Rh7777 cells, a rat hepatocellular carcinoma cell line, were exposed to whole water equivalences [0, 0.0001, 0.001, 0.01, 0.1, 1, 10, 20%] of three chemically distinct fractions (F1, F2, F3) of bitumen-influenced groundwater samples from two sources: one associated with industry-sourced bitumen and the other with naturally occurring bitumen for 24 and 48h. Given that the abundance of O2 species, including naphthenic acids, is confined to F1 (Frank et al., 2021), we further exposed McAs to NAFC for 24 and 48 h at environmental concentrations [0, 0.25, 1.25, 25, 125 mg/L]. We assessed cytotoxicity, ATP levels, and cellular ROS. Cytotoxicity assessments for the bitumen-influenced groundwater exposures showed cell viability above the 80% threshold. Evaluating dose effects within fractions and fraction effects within doses revealed most changes within cells treated with the natural bitumen-influenced source. The 10% and 20% water equivalences for Fractions 2 and 3 from both groundwater sources resulted in increased ATP levels at 48 h, while the 20% equivalence in Fraction 1 led to a significant decrease in ATP levels. Interestingly, at 48 h, 0.25, 25, and 125 mg/L NAFC increased ATP levels. This increase in ATP levels in NAFC exposures was associated with a decrease in cellular ROS. Together, these data suggest that both NAFC exposure and exposure to bitumen-influenced groundwater can alter bioenergetics within mammalian hepatocytes. Specific fractions and doses significantly affected cellular ATP levels and oxidative stress responses, highlighting the complex interaction with cellular energy pathways. Given that altered energy homeostasis is critical to organism health, this study provides a potential mechanism by which bitumen-derived organic compounds may be impacting wildlife health.

2.05.P-Mo-035 Treatment Mitigation of Oil Sands Process-Affected Water Toxicity: Hybrid Wetland Mesocosm Study

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Oil sands process-affected waters (OSPWs) comprise complex mixtures of multiple contaminants of potential concern (COPCs), from dissolved organics such as naphthenic acid

fraction components (NAFCs) and polycyclic aromatic hydrocarbons (PAHs) to inorganics such as trace elements, and total suspended/dissolved solids (TSS, TDS). Passive treatment approaches based on natural processes, such as constructed treatment wetlands (CTWs) have been a mainstay of the mining industry's remediation repertoire and reclamation planning. Solar photocatalysis (PC) is another promising semi-passive advanced oxidation treatment process capable of detoxification and degradation of OSPW organics otherwise recalcitrant to natural biodegradation, while CTWs enable treatment of biodegradable organics and trace elements. It was hypothesized that a combined solar PC-CTW hybrid sequential treatment process could provide a more comprehensive treatment of both organic and inorganic COPCs in OSPW.
We report results from our study of hybrid sequential photocatalytic-wetland OSPW treatment, starting from small-scale indoor PC-CTW mesocosm trials under grow lamps, to a larger continuous-flow outdoor mesocosm trial operating under natural sunlight and weather. Numerous water quality analytical parameters were tracked through each stage of treatment, including whole effluent toxicity (WET) fish bioassays, NAFCs, PAHs, and trace elements, as well as CTW ecosystemic endpoints such as soil microbial communities by next-generation sequencing (NGS) and plant growth metrics. Our results demonstrate the solar photocatalysis detoxified OSPW to WET endpoints and treated organic COPCs as well as oxidizable trace elements. The secondary CTW stage further eliminated remnant organic and inorganic COPCs, validating a hybrid PC-bio approach can provide comprehensive OSPW treatment. This work offers a unique perspective on ecosystemic and phytotoxicity amelioration in CTW mesocosms, as well as phytoremediation by trace element bioaccumulation. Overall, these results provide fundamental insights into toxicity mechanisms in OSPW-influenced wetlands, and promise to significantly influence treatment strategies to successfully manage OSPW toxicity.

2.05.P-Mo-036 Biomimetic Extraction and Passive Sampling Tools for Monitoring Oil Sands Process-Affected Water (OSPW) Treatment

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Oil sands process-affected waters (OSPWs) are complex mixtures of organic and inorganic contaminants of potential concern (COPCs), including naphthenic acid fraction components (NAFCs). Literature results indicate diverse aquatic toxicity of different chemical classes of NAFCs: many appear toxicologically benign, and the aquatic toxicity of OSPW may be driven by only a small subset of bioaccumulative compounds. Given the high latency and ethical concerns with vertebrate animal testing of whole effluent toxicity (WET), for effective risk assessment and management it is imperative to develop water quality monitoring tools to serve as toxicity surrogates, with a priority focus on bioavailable COPCs. We present a compendium of studies – compiling results collected over 7 years from 12 different OSPW samples from 3 oil sands operators – evaluating the use of passive samplers and "biomimetic" analytical techniques for monitoring OSPW toxicity, and toxicity dynamics through water treatment processes: (1) biomimetic acid extraction to solid phase microextraction fibers (BE-SPME), simulating target lipid bioaccumulation in aquatic organisms; (2) characterization of bioconcentrating (SPME-bound) organics by GC-Orbitrap-MS; (3) biomimetic liquid chromatography with immobilized artificial membrane (IAM) columns/Orbitrap-MS to rapidly measure lipid partition coefficients of thousands of NAFCs simultaneously, toward improved quantitative structure-activity relationship (QSAR) risk assessment models for OSPWs; (4) passive samplers for bioavailable metals and trace elements; (5) a novel colorimetric NAFC-labelling technique as a simple visual indicator for rapid field assessment of OSPW toxicity. These data are indexed versus WET bioassays conducted on the same samples, providing a rich dataset demonstrating the potential of biomimetic approaches to predict and monitor OSPW toxicity throughout water treatment. Together, these results contribute important new toxicological findings and practical tools toward the ongoing scientific discourse on environmental monitoring of oil sands impacted waters in the Athabasca region, and the development of focused OSPW treatment methods.

2.05.P-Mo-038 Assessing the Effects of Sulfur-Containing Polycyclic Aromatic Compounds on Estrogen Synthesis and Metabolism in Granulosa Cells

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The expansion of oil and gas extraction activities in Northern Alberta has led to increasing concerns regarding the reproductive effects of exposure to petroleum-derived chemicals on local wildlife. Of these, polycyclic aromatic compounds (PACs) are considered to be one of the major determinants of toxicity. Current evidence regarding the reproductive toxicity and carcinogenic potential of substituted PACs like sulfur-containing PACs and alkylated PACs remain largely unexplored despite their prevalence in the environment and exposed biota. Few studies report that substituted PACs possess endocrine disrupting properties that may impact ovarian function and fertility. Given that estrogens may increase cancer risk, with some metabolites demonstrating higher carcinogenic potential than their parent counterparts, this study aimed to determine the effects of sulfur-containing PACs, dibenzothiophene (DBT), and its alkylated congener, 2,4,7-trimethyldibenzothiophene (2,4,7-DBT), on estradiol synthesis or metabolism and apoptosis in granulosa cells - key cells important for proper ovarian function. Spontaneously immortalized rat granulosa cells were exposed to DBT or 2,4,7-DBT for 24h at concentrations detected in wildlife tissues collected from the Alberta oil sands region [0, 0.1, 1, 10nM]. We assessed mRNA expression of key genes involved in estrogen synthesis [*Cyp19a1*, *Hsd17b1*], signaling [*Esr1*, *Esr2*] and metabolism [*Cyp1a1*, *Cyp1a2*, *Cyp1b1*]. Estradiol (E2) output and the production of E2 metabolites [2-OHE2, 4-OHE2] were measured via ELISA and HPLC-MS/MS, respectively. We assessed apoptosis via TUNEL. Exposure to both DBT and 2,4,7-DBT significantly increased the expression of all markers for estrogen synthesis, signaling and metabolism. While DBT increased E2 output, there was no significant effect on 2-OHE2 or 4-OHE2 output. Contrastingly, 2,4,7-DBT did not alter E2 synthesis, but significantly increased the production of 4-OHE2, a carcinogenic metabolite of E2. Exposure to both DBT and 2,4,7-DBT increased apoptosis. Given the critical role of granulosa cells in ovarian steroid hormone synthesis and ultimately female reproductive health, these results provide a proof-of-concept that sulfur-containing PACs and alkylated PACs have the potential to function as endocrine disrupting chemicals. This study will further our understanding of how petroleum-derived chemicals can cause reproductive toxicity and may affect carcinogenic risk in mammals.

2.05.T Canada's Oil Sands Mining and Dilbit Pipelines

2.05.T-01 Bioavailability and Bioaccessibility of Pb in Atmospheric and Aquatic Particles of the Lower Athabasca River Watershed in Alberta, Canada

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Although atmospheric Pb deposition has decreased dramatically after the ban of Pb addition to gasoline, it is still of global concern due to its deleterious effects on living organisms. However, not all chemical forms of Pb display the same mobility, bioaccessibility, bioavailability and toxicity. The lower Athabasca River (AR) watershed in northern Alberta has been the focus of attention regarding possible contamination by trace elements (TEs), including Pb. In addition to natural inputs of TEs, industrial activities such as open-pit mining and upgrading of bitumen in the Athabasca Bituminous Sands (ABS) region have been viewed as potentially important anthropogenic sources. To date, surface waters, peat bog porewaters, and snow collected in the ABS region were used to study the spatial variation in Pb concentrations. The size distribution of Pb was determined as particulate (> 0.45 µm), dissolved (<0.45 µm), colloidal (1KDa to 0.45 µm), and truly dissolved (< 1KDa) fractions. Among these, the truly dissolved fraction has the greatest potential to directly affect aquatic organisms. In addition, to mimic the digestion of sediments by fish, an *in-vitro* test was conducted using synthetic gastric fluid in order to determine Pb bioaccessibility. Potential sources of dusts in the ABS region such as soils, tailings and road construction material, were leached using this fluid. All of these analyses and experiments were performed in a metal-free, ultra-clean laboratory. Our results indicated that total Pb concentrations were generally less than 1 µg/L in peat porewaters, 2 µg/L in snow, and < 40 ng/L in surface waters. Spatial variations, with increasing concentrations toward the centre of industrial activities were more evident in surface waters and snow than in peat bog waters. Lead was mostly found in the particulate fraction in surface waters (96%) and snow (99%). Dissolved Pb in surface waters is more abundant in the inorganic colloidal form (73%), which are expected to have limited bioavailability. By contrast, Pb in snow is primarily present in ionic form (97%), and is potentially bioavailable. However, these concentrations are as low as those observed in ancient ice samples from a core collected in Nunavut (~ 5 ng/L). From the *in-vitro* gastric leaching, Pb bioaccessibility increases as particle size decreases (from <2 mm to <10 µm). However, only 1-30% of total Pb in natural and industrial particles may be bioaccessible.

2.05.T-02 Non-Target Analysis for Oil Sands Toxicology: Beyond Naphthenic Acids

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Oil sands process-affected waters (OSPWs), comprising complex mixtures of chemical constituents, may exhibit toxicity to aquatic biota. Much of this toxicity has been ascribed to the waters' naphthenic acids (NAs) content. However, NAs alone do not reflect the full chemical diversity of OSPW dissolved organics, and recent research suggests NAs do not fully account for all toxic effects associated with these waters (in some cases, not even correlating with acute lethality endpoints). An overemphasized predilection with NAs may neglect more complex, but ultimately more important, causal mechanisms driving OSPW toxicity. Reflecting on the historical underpinnings of OSPW toxicology, preponderant scientific focus on NAs stemmed from limited-resolution analytical methods of the 1980s, further reinforced by toxicity identification evaluation (TIE) and effects-directed analysis (EDA) studies on isolated NA extracts separated from their original OSPW matrices. While much remains to be understood as to interactive chemical toxicity in OSPW, recent studies indicate an important role of complex mixture effects such as chemosensitization. Rather than myopic focus on a single presupposed toxicant, we instead advocate for non-target analysis (NTA)-based toxicology: by embracing the inherent chemical complexity of OSPW and aiming for comprehensive characterization of *all* constituents, NTA enables a "big data" approach to toxicology, capturing higher-order toxicochemical correlations for deeper mechanistic insights. Here, we report on the largest dataset to date of NTA high-resolution liquid chromatography-mass spectrometry (LC-MS) paired with whole effluent toxicity bioassay outcomes, collected over 7 years from 12 different OSPW samples from 3 oil sands operators. We applied open-source workflows from -omics disciplines to OSPW organics for the first time, including (1) comprehensive multiphase extraction; (2) formula and structural annotation by molecular networking; (3) a new approach to standards-free NAFC quantification; and (4) toxicity-relevant physicochemical property inference (pKow, pKa). We hypothesize that a comprehensive NTA approach will enable a more holistic and accurate understanding of OSPW toxicity, contributing to more effective risk management frameworks and focused water treatment solutions. Encouragingly, we found treatment can rapidly detoxify OSPW (to fish endpoints) without the need for complete elimination of NAs.

2.05.T-03 An Improved Stable Isotope Approach for Differentiating Processed and Non-Processed Bitumen Residues in the Presence of Natural Groundwaters

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This presentation describes a study conducted at a naphthenic froth-treatment (NFT) oil-sands operation in the North Athabasca Oil Sands Region, Alberta Canada where isotopes of water, solutes and acid-extractable organics (AEO) were measured in tailings ponds containing oil-sands process water (OSPW), groundwater from the basal McMurray Formation, and a variety of on-lease groundwater monitoring wells and mitigation structures designed to intercept potentially contaminated runoff. Differentiation of the isotopic fingerprints of various waters including those containing residue of processed and unprocessed bitumen is shown to be possible using the method, including identification of likely OSPW influence in a drainage ditch constructed as a mitigation measure for potentially contaminated runoff. Another mitigation structure was found to be unimpacted as were a variety of monitoring wells situated subjacent to the tailings impoundment. The study provides supporting evidence that tailings ponds are well-contained and are not hydraulically connected with monitoring wells in local Quaternary aquifers or underlying formations. The AEO isotope method is employed through measurement of a suite of tracers including: oxygen-18 and deuterium in water ($\delta^{18}O_{H2O}$, $\delta^{2}H_{H2O}$), oxygen-18, deuterium, carbon-13, sulfur-34, and nitrogen-15 in both water solutes ($\delta^{13}C_{DIC}$, $\delta^{13}C_{DOC}$, $\delta^{34}S_{SO4}$, $\delta^{18}O_{SO4}$, $\delta^{15}N_{NO3}$) and bulk AEOs ($\delta^{18}O_{AEO}$, $\delta^{2}H_{AEO}$, $\delta^{13}C_{AEO}$, $\delta^{34}S_{AEO}$, $\delta^{15}N_{AEO}$) as well as the AEO decarboxylation fraction ($\delta^{13}C_{PYR}$). Supporting analyses include mole fractions of carbon, nitrogen and sulfur in AEO. Visualizations are provided using principal component analysis. The method is compared and shown to be superior to a number of other approaches advocated for differentiating OSPW influence including per-and polyfluoroalkyl substances (PFAS), artificial sweeteners (saccharin and acesulfame K), radon-222, O_2:O_4 ratios of OSPW. Other complimentary techniques such as orbitrap naphthenic acid (NA) concentration and speciation are also compared as fingerprinting tools for tracking OSPW seepage.

2.05.T-04 Can the Kynurenine-Tryptophan Ratio Serve as a Marker for AhR Activation in Response to PAC Exposure?

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Many polycyclic aromatic compounds (PACs) identified in the oil sands region are known ligands of the aryl hydrocarbon receptor (AhR). Recent evidence has suggested that AhR-ligands can alter tryptophan metabolism through the kynurenine pathway. Given that the kynurenine pathway is conserved across vertebrate species, this study aimed to explore the relationship between AhR activation and the Kynurenine-Tryptophan Ratio (KTR) as a marker of toxicant exposure and physiological disruption *in vitro* and *in vivo*. McA-RH7777, a rat hepatoma cell line was exposed to Benzo[a]pyrene [10uM], a prototypical PAC; a Bitumen Water Accommodated Fraction (BitWAF) at [0, 0.01%, 0.1%, 1%, 10%], a mixture chemically characterized for PACs; and Naphthenic Acid Fraction Components (NAFC) at [0, 1.25, 25, 125 mg/L], an organic acid mixture derived from oil sands process-affected water for 48h. We assessed the mRNA expression of *Cyp1a1*(indicative of AhR activation) and tryptophan metabolism genes (*Tdo2*, *Ido2*, *Tph1*). KTR levels were quantified in spent media using HPLC-MS/MS. Further environmental analyses were conducted on liver tissues from Longnose (*Catostomus catostomus*) and White Suckers (*Catostomus commersonii*) from the Athabasca River. We assessed ethoxyresorufin-O-deethylase (EROD) activity and KTR levels in the liver samples. Pearson's correlation analyses were performed to assess the relationship between AhR activation (*Cyp1a1* expression or EROD) and KTR *in vitro* and *in vivo* samples, respectively. Benzo[a]pyrene increased KTR in a time-dependent manner, showing a significant and proportional correlation with AhR activation and the induction of *Cyp1a1* mRNA at 48h. Exposure to BitWAF resulted in dose- and concentration-dependent changes in KTR. However, BitWAF affected KTR through multiple pathways, including cell metabolism, cellular stress responses, and receptor (AhR (p=0.051), GR, PPARa and PPARg) activation. While not a PAC, NAFCs similarly induced *Cyp1a1* and influenced KTR transcriptionally through some of the same pathways identified following exposure to BitWAF, including AhR activation (p=0.001). In fish, EROD activity and KTR were significantly correlated in a species-specific manner. Collectively, these findings reveal that KTR, activated by AhR ligands, may serve as a novel marker for assessing metabolic responses in hepatocytes and may be a novel mechanism through which bitumen-derived contaminants alter mammalian and fish health.

2.05.V Canada's Oil Sands Mining and Dilbit Pipelines

2.05.T-05 The Impact of Naphthenic Acid on Rainbow Trout Liver Mitochondrial Reactive Oxygen Species Metabolism

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Numerous toxicants induce oxidative stress, a major cellular toxicity mechanism. The mitochondrial oxidative phosphorylation pathway is a major cellular source of reactive oxygen species (ROS), contributing to oxidative stress. Naphthenic acids (NAs) are present in oil sands process-affected water. These chemicals possess both toxicity and persistence, rendering them a significant issue for the remediation of tailings ponds in the oil sands regions of Alberta. Recent research shows that 3,5-dimethyladamantane-1-carboxylic acid, a model quaternary naphthenic acid, increases ROS emissions in isolated mitochondria. The study's goal was to find out if a primary carboxylic acid, specifically 3,5-dimethyladamantane-1-acetic acid, which is a moderately hydrophobic naphthenic acid, would cause mitochondria to produce hydrogen peroxide (H_2O_2) in different bioenergetic states. The hypothesis was that NA would increase ROS production at particular mitochondrial electron transport complex sites. Mitochondria isolated from rainbow trout (Oncorhynchus mykiss) liver were exposed to commercially available 3,5-dimethyladamantane-1-acetic acid. ROS generation was measured fluorometrically by the O_2k-fluorometer (Oroboros) by using the Amplex UltraRed-horseradish peroxidase (AUR-HRP) detection system during bioenergetic states 3 and 4. Subsequently, each ROS emission site in the mitochondrial complexes was isolated using inhibitors, and the ROS emission of each site was measured in multiwell plates using the AUR-HRP system. The compound 3,5-dimethyladamantane-1-acetic acid caused ROS emission in states 3 and 4 with EC50s of 0.99 and 1.21 mM, respectively, as compared to IC50s of 0.77 and 1.01 mM for oxygen consumption (Kalvani et al., 2024 Env. Tox. Pharm 107:104386). The test compound increases ROS emission in a dose-dependent manner at all ROS production sites: OF, IF, IQ, IIF, and IIIQo. Some sites, however, were more responsive to ROS production than others. Overall, the research shows that the mitochondrial H_2O_2 metabolism in rainbow trout liver is highly responsive to stressors like naphthenic acid.

2.06.P-We Contaminant and Trace Element Biogeochemical Cycling in Aquatic Ecosystems

2.06.P-We-055 Silver Nanoparticles as a Potential Driver of Mercury Transformation in Water

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One of the global pollutants that is reckoned with is mercury (Hg). It is present in environmental media and can be released naturally and anthropogenically through different sources. Photochemical redox reaction is a major process that leads to transformation of Hg species in water from one form to another via redox reactions to generate dissolved gaseous Hg (Hg(0). This study focuses on the potential role of silver nanoparticles (AgNPs) in photoredox reactions of Hg in water. To this end, it is important to first study the interactions between AgNPs and divalent Hg(II) under dark conditions because of tendency of AgNPs to reduce Hg(II) electrochemically and also due to amalgam formation between AgNPs and Hg(0). A batch experiment was carried out using different mercuric acetate (Hg(II)) concentrations to interact with AgNPs in the presence of 0.001 M acetate buffer to control the suspension pH against AgNPs aggregation. Inductively coupled plasma mass spectrometry was used to determine purgeable Hg(0) (trapped with $KMnO_4$ solution), Hg(0) amalgamated and Hg(II) bound to Ag-Hg amalgam (after thermal desorption at 508°C and 302 °C, respectively, and trapped with $KMnO_4$), to reveal Hg transformation through interacting with AgNPs.The results showed that there were quantifiable amounts of purgeable Hg(0) from the suspensions containing 0.49, 1.22, 2.45 and 6.12 mg/L Hg(II), revealing the potential of AgNPs to mediate in Hg(II) transformation in water. From thermal analysis, it was evident that there was amalgamation between Hg(0) generated and AgNPs in suspension, as shown by a thermal release of amalgamated Hg(0) from the Ag-Hg amalgam obtained after interaction. It was also noticed that the percentage of Hg(0) that formed amalgam (6.0-16% of spiked Hg) with AgNPs far exceeds the purgeable Hg(0) (0.19-0.21%), indicating the potential of AgNPs to immobilize Hg(0) in water. This study will serve as preliminary understanding of the role and/or behavior of AgNPs in Hg cycling in water.

2.06.P-We-056 Exploring the Diurnal Variations in Dissolved Elemental Mercury Distribution from Surface Water to Sediment-Water Interface in Wetlands

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Mercury (Hg) undergoes complex transformations in aquatic ecosystems, transitioning between its gaseous elemental state (Hg(0)), divalent form (Hg(II)), and the neurotoxic methylated state (MeHg). MeHg, primarily produced in the sediment-water interface (SWI), bioaccumulates and biomagnifies along the food chain, posing a significant threat to humans. One process altering MeHg formation at the SWI is the reduction of Hg(II) to Hg(0). The processes responsible for and the implications on aquatic Hg cycling of this Hg(0) production at the SWI remain poorly understood. Therefore, a diurnal variation experiment was conducted in the Florida Everglades, to examine the influence of photo-reduction and Hg reduction at the SWI on Hg(0) distribution in the water. Here, we invented a novel sampling device to preserve the volatile Hg(0) in water at the SWI without disturbance or bubble formation, where the position-adjustable 1L sampling bottle was equipped with two ports separating water intake inlet and air release outlet. Samples collected every 3 hours between 9 AM and 6 PM were purged with Ar gas and trapped in an acidic KMnO4 solution and the trapped Hg(0) were analyzed using Cold Vapor Atomic Fluorescence Spectroscopy. The mean Hg(0) levels in the surface water across all four locations were 0.09±0.05 ng/L, whereas at the SWI, mean levels were 0.06±0.05 ng/L. While Hg(0) levels consistently declined in the surface water and SWI across all locations throughout the day, no significant variation were observed. However, Hg(0) pool were consistently higher at the surface water compared to the SWI at all times of the day. The Hg(0) concentrations observed in the surface water ranged from 0.20±0.00 ng/L to 0.02±0.02 ng/L while SWI concentrations ranged from 0.17±0.05 ng/L to 0.01±0.01 ng/L. Remarkably, in the two locations exhibiting the lowest overall Hg(0) content, Hg(0) persisted in the surface water throughout the day, whereas after 12 noon, its presence was no longer observed at the SWI. These findings suggest the influence of photo-reduction of Hg(II) on the Hg(0) pool and/or evasion of Hg(0) formed at the SWI of wetlands to the surface, thereby increasing Hg(0) concentration in the surface water. This study emphasizes the need for further research into Hg(0) production processes at the SWI in wetland environments.

2.06.P-We-057 Comparative Study of Analytical Methods to Estimate the Reliable Conditional Stability Constants of Mercury-Dissolved Organic Matter Binding

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Mercury (Hg) is widely acknowledged as a highly toxic element, particularly in the form of methylmercury (MeHg), which has raised significant concerns regarding its potential to pose a serious human health risk worldwide. Dissolved Organic Matter (DOM) has a strong affinity to bind Hg and thus serves as the primary factor controlling the kinetics and thermodynamics of aquatic Hg biogeochemical processes. The quantification of the Hg-DOM conditional stability constants (logK) enhances the understanding of aquatic Hg transformation. However, the reported logK values vary widely due to differing methodologies and conditions, making it challenging to select reliable constants for accurately describing DOM-influenced Hg speciation. In this investigation, the complexation of DOM isolate - Suwannee River Humic Acid (SRHA) and Hg (II) (mercuric chloride, $HgCl_2$) was studied using different analytical methods such as cyclic voltammetry (CV), fluorescence titration spectroscopy, and isothermal titration calorimetry (ITC) under identical experimental conditions. The CV analysis of 0.1 mM Hg(II) with 0.00625 mM SRHA in a supporting electrolyte (0.1 M NaCl at pH 8, buffered with 1 mM $NaHCO_3$) at a scan rate of 0.03 V s^{-1} employing a glassy carbon electrode as the working electrode revealed the formation of 1:1 and 1:2 metal-ligand complexes. The logK values for the 1:1 and 1:2 species were determined to be $logK_{1:1} = 2.1039$ and $logK_{1:2} = 4.1558$, respectively. Fluorescence titration spectroscopy revealed logK values of SRHA with Hg(II) to be 1.9476 ± 0.2222 and 3.8309 ± 0.0278 for 1:1 and 1:2 stoichiometries, respectively. ITC investigations indicated the presence of two sequential binding sites with logK values of 1 ± 0.2703 and 1.4905 ± 0.3868 for the first and second binding events, respectively. The formation reactions of Hg-SRHA complexes were found to be driven by both enthalpy and entropy. A comparison of the results obtained from the different analytical methods demonstrated good agreement between fluorescence titration and cyclic voltammetry, while ITC exhibited varying values, which could be attributed to the various heat reactions considered during the ITC analysis. Therefore, it can be deduced that the variation of the stability constant is influenced by the underlying principles and assumptions of analytical methods, as well as the specific experimental conditions necessary for their validity.

2.06.P-We-058 Assessing the Presence, Concentration, and Biological Uptake of Trace Metals and PFAS Near the Dahlgren Naval Support Facility (Dahlgren, Virginia)

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The Naval Surface and Warfare Center Dahlgren Division (NSWCDD) was first established in 1918 for use in testing naval weapons. Since its inception, NSWCCD has launched an estimated 33 million pounds of munitions into the Potomac River which are enriched in per and poly fluoroalkyl substances (PFAS) and trace metals including zinc, lead, and copper. While modeling has suggested that hazardous materials introduced from this activity are captured and isolated in sediments, little to no research has been done to assess the actual presence and concentrations of these known contaminants in the field and confirm these predictions. Surface water, deep water, and sediment samples were taken upstream, adjacent, and downstream of NSWCDD and analyzed for 31 PFAS using EPA draft method 1633 and trace metals (Al, Cd, Cr, Cu, Fe, Pb, Mn, Zn, Ca, As, Se, B) using ICP-OES. PFAS water samples were collected in methanol rinsed Nalgene bottles that were rinsed three times with river water before collection at each location. PFAS sediment samples were collected using a dedicated stainless-steel van veen grab sampler treated with PFAS free soap, rinsed with methanol, and wrapped in PFAS-free plastic until use in the field. Deep water samples were obtained using a stainless-steel Kemmerer sampler. Sediment samples for trace metal analysis were collected using a separate van-veen grab sampler and stored at -8oC for further digestion, extraction, and trace metal analysis using ICP-OES. While this study is still ongoing, we expect to observe 1) increased concentrations of both PFAS and trace metals in sediments compared to surface waters and 2) elevated concentrations of PFAS and trace metals at sites downstream of NSWCDD in locations where testing is conducted. The results of this study will give insight into the fate and transport of munitions-associated contamination at NSWCDD and elucidate any potential risks to aquatic organisms.

2.06.P-We-059 Trifluoroacetic Acid (TFA) Still Dominates in Great Lakes Aquatic Ecosystems

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Per- and polyfluoroalkyl substances (PFAS) have been widely used in various applications for over 70 years, leading to ubiquitous distribution in the environment. As the shortest perfluorocarboxylic acids (PFCAs), trifluoroacetic acid (TFA) can be generated from the photodegradation of PFAS precursors and hydrofluorocarbons. Atmospheric deposition is an important source of PFAS to the aquatic ecosystems. ~10% of the U.S. population and 30% of the Canadian population lives in the Great Lakes basin. However, data on PFAS, especially TFA levels in the Great Lakes aquatic ecosystems was reported only ~20 years ago, so it is time to revisit the TFA levels in this region. Precipitation samples were collected every two weeks from five sites in the Great Lakes region in 2021-2023 and open lake water samples were collected from each of the Lakes in 2022-2023. All samples were analyzed using a liquid chromatograph coupled to tandem mass spectrometer (LC/MS/MS) for a total of 42 targeted PFAS including TFA. TFA was the most dominant compound, contributing >90% to the total targeted PFAS concentrations. Among the five IADN sites, TFA concentration ranged from 89.1 –1897 ng/L with a median of 474 ng/L in precipitation. TFA median concentration was highest at the two urban sites of Cleveland (780 ng/L) and Chicago (489 ng/L), followed by the rural and remote sites at Eagle Harbor (476 ng/L), Sleeping Bear Dunes (413 ng/L), and Sturgeon Point (407 ng/L). For lake water, TFA concentration ranged from ND to 1529 ng/L with a median of 422 ng/L. TFA median concentration was highest in Lake Erie (498 ng/L) and lowest in Lake Superior (133 ng/L). Compared to previous literature data from two decades ago, the levels of TFA increased about 3-4 folds in Lake Erie and Ontario water, and ~10 times in Lake Superior water and in precipitation. Perfluoropropionic acid (PFPrA), short-chain PFCAs (C4-C6), perfluorooctanesulfonic acid (PFOS), 6:2 fluorotelomer sulfonic acid (FTS) and perfluorobutane sulfonamide (FBSA) were also widely detected in precipitation and lake water. These results highlight the importance of atmospheric deposition as an important source of PFAS to the lakes and the need to include TFA in the assessment of PFAS in the Great Lakes aquatic ecosystems.

2.06.P-We-061 Selenium Impacts on Methylmercury Retention Across Mayfly Life Stages Depend on Dietary Methylmercury Exposure Levels

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Though high concentrations of mercury (Hg) and selenium (Se) are individually toxic to organisms, it is hypothesized that high levels of environmental Se can reduce Hg bioaccumulation and biomagnification in aquatic food webs. This potential interaction between mercury and selenium has been under-studied in aquatic macroinvertebrates, particularly in relation to their complex life histories. We examined the proposed effect of selenium on methylmercury accumulation, along with the transfer of methylmercury, between four life stages for a parthenogenetic mayfly (*Neocloeon triangulifer*). We found support for the mercury-selenium interaction hypothesis, but it is context-specific. At high dietary methylmercury, elevated aqueous selenium lowered mayfly methylmercury concentrations; however, at low methylmercury treatments, there was no effect of selenium on mayfly methylmercury accumulation. Additionally, though we found higher methylmercury concentrations in terrestrial adult stages compared to aquatic larval stages at both methylmercury treatment levels, the cumulative life history transfer factor (LHTF; the ratio of methylmercury in the final adult imago stage compared to late instar larvae) differed by treatment. The LHTF was constant for all aqueous selenium exposure levels at high dietary methylmercury treatment (likely due to impacts of selenium on methylmercury uptake and loss) but increased with aqueous selenium exposures at low dietary methylmercury treatments (likely due to impacts of selenium on methylmercury uptake only). These results suggest predators of adult mayflies would be exposed to greater methylmercury than predators of larval mayflies for all selenium and methylmercury levels, but that the ameliorative effect of selenium on methylmercury accumulation only occurs at high dietary methylmercury levels.

2.06.P-We-062 Defining Subsidy-Stress Gradients for Metals and Relevance for US Surface Waters

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Many metals play dual roles as essential nutrients and potent toxicants, influencing biology significantly depending on their availability in the environment—whether too scarce (i.e., subsidy) or excessively abundant (i.e., stress). Presently, the exploration of biological responses to metals diverges between ecologists and physiologists investigating metal limitation and ecotoxicologists examining metal toxicity. The concentration of metals in surface waters exhibits considerable spatial and temporal variability, exacerbated by human activities altering metal availability in freshwater ecosystems, underscoring the imperative for an integrated research approach. Our assessment scrutinizes various physiological and population-level models pertaining to metal limitation and toxicity to develop an integrated subsidy–stress model. We aim to pinpoint data deficiencies and outline avenues for heightened research endeavors to bridge these gaps. We aim to integrate this dose–response model with a comprehensive continental-scale dataset on metal concentrations in streams of the US. This synthesis should pinpoint intersections between subsidy–stress thresholds and environmental metal levels, signaling instances where metals could impact organisms, either as essential nutrients or toxic substances. Projections indicate a twofold increase in industrial metal demand over the next five decades, emphasizing the necessity for enhanced predictive models to anticipate environmental repercussions. This approach promises a more comprehensive understanding of potential metal effects—whether fostering growth or inducing toxicity—on freshwater organisms.

2.06.T-03 Spatiotemporal Trends and Mass Fluxes of PFAS in Major Pan-Arctic Rivers

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Per- and polyfluoroalkyl substances (PFAS) are a diverse class of thousands of fluorinated compounds that have been abundantly used by industry and in consumer products. While much research has focused on PFAS contamination next to point sources of pollution, they have now been detected in the environment and wildlife from the most remote ecosystems globally. For example, PFAS have been detected in seawater and ice cores throughout the Arctic. However, few studies have reported concentrations in major Arctic rivers, which contribute significantly to the Arctic Ocean. Here, we leverage from surface water samples collected by the Arctic Great Rivers Observatory program. We analyzed 15 targeted PFAS from the major pan-Arctic rivers: Mackenzie, Yukon, Ob, Kolyma, Lena and Yenisey. PFAS profiles for five of the six rivers show similar compositions with perfluoroheptanoic acid (PFHpA) as the most abundant compound. We find higher relative abundance of shorter chain perfluorocarboxylic acids (PFCA), likely reflecting their greater potential for long range atmospheric transport and higher mobility compared to longer chain homologues. The highest targeted PFAS concentrations (~3x other Arctic rivers) were observed in the River Ob. Past research has reported higher relative concentrations of heavy metals and other organic contaminants in this river. We use these data to

assess annual and seasonal PFAS fluxes across the six rivers, and implications for PFAS discharges into the Arctic Ocean and also screen a subset of samples for novel PFAS.

2.06.T Contaminant and Trace Element Biogeochemical Cycling in Aquatic Ecosystems

2.06.T-02 Seasonal and Annual Mercury Exports Across the Physiographically Diverse Subarctic Yukon River Basin

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Mercury is a trace element and priority pollutant whose biogeochemical cycling, conversion to neurotoxic methylmercury, and accumulation in Arctic and subarctic food webs is strongly influenced by climate and landscape change. The Yukon River—one of the largest watersheds in North America and the panarctic region—has mercury concentrations and area-normalized annual mercury exports nearly twice as high as other large panarctic rivers. Yet the source of elevated Yukon River mercury remains unclear. Earlier studies suggested mercury derived from vegetation and soils, permafrost degradation and associated erosion of organic soils, placer mining disturbance, and atmospheric pollution loading to vegetation, snow, and glaciers. We recently showed that glacial erosion of naturally mercury-bearing sediments was a previously underappreciated component of summer Yukon River mercury exports. However, to predict how Yukon River mercury exports may respond to climate and landscape change, it is necessary to understand how mercury sources vary seasonally in response to regional hydrologic and landscape controls. Thus, we sampled major Yukon River tributaries during the open-water season—in landscapes draining glaciers, permafrost, mining regions, and deep lakes across the Canadian Yukon River basin—to investigate the chemical association, speciation, and seasonality of mercury exports. We found that mercury was predominantly transported in the particulate phase in association with inorganic sediment rather than organic carbon, and that methylmercury concentrations were low across the basin. Mercury derived from two key sources: boreal rivers eroding mercury-rich sediment during spring snow melt, and glacierized rivers eroding mercury-poor sediments during summer glacier melt. In contrast, permafrost thaw following rainfall and runoff were minor sources of mercury and organic carbon, and large deep lakes that receive atmospheric inputs had overall minimal mercury exports. Our results suggest that Yukon River mercury associates with low-bioavailability sediments, and that annual mercury export will decrease as glaciated catchments pass peak water. Our study underscores the utility of basin-wide sampling, and the importance of considering geomorphic, physiographic, geologic, and hydroclimatic influences on mercury cycling in a warming arctic.

2.06.T-05 Bioaccumulation and Transfer of Per- and Polyfluoroalkyl Compounds in a Contaminated Stream Food Web

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Per- and polyfluoroalkyl substances (PFAS) in freshwater ecosystems are a global concern due to their persistent and bioaccumulative nature and potential toxicity to aquatic life. We

investigated the fate of PFAS in a contaminated agricultural stream receiving treated wastewater discharge from a food processing plant. We assessed accumulation of PFAS and their degradation products in the aquatic food web by sampling abiotic compartments (i.e., water, bed sediment), algae, benthic macroinvertebrates, and fish. In addition, we sampled adult aquatic insects and riparian spiders (tetragnathids) to assess the potential transfer of PFAS to the riparian food web. Downstream PFAS concentrations in water were up to three orders of magnitude higher than upstream and reflective of the PFAS composition in the effluent. Fish bioaccumulated PFAS both upstream and downstream of the effluent, but downstream fish accumulated relatively higher concentrations and more PFAS compounds. Accumulation in larval aquatic insects was highly variable, with caddisflies accumulating more PFAS compounds (n=11) compared to odonates and craneflies (n=1 to 3). Evaluation of the relative concentrations of PFAS in larval and adult caddisflies suggested compound-specific metamorphic retention. In addition, transfer of PFAS to riparian consumers was observed, with spiders showing elevated PFAS concentrations that generally reflected the composition of PFAS observed in their prey (i.e., adult aquatic insects). Our research demonstrated PFAS uptake in the stream food web and trophic transfer to riparian consumers. Variation among PFAS (including precursors and chain length), and trophic position of aquatic taxa, were important drivers of contaminant transfer.

2.07.P-Th Late Breaking Science: Wildlife Toxicology, Ecology and Stress Response

2.07.P-Th-122 Putting Pedal to the Metal: Discovering Fate and Transport of Heavy Metals From Tire Road Wear Particles in Stormwater

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Tire Road Wear Particles (TRWPs) containing heavy metals, microplastics, and exotic inorganic chemicals are of great environmental concerns, especially in coastal areas of the US where about 40 percent of the total national population reside in <10% of land mass. We aim to study the fate and transport of Tire Road Wear Particles (TRWPs) and associated heavy metals in stormwater retention ponds of a coastal urban watershed in northwestern Florida. After a preliminary evaluation of 30 sites, we selected 9 sites representing typical stormwater treatment techniques and varying traffic conditions. We collected sediment samples from Vortech upflow filters, Bay Savers filters, Baffle Box, and one storm water retention pond with a vortech upflow filter. Total Zn, Cu and Pb concentrations were analyzed via ICP-MS, mobile XRF, and Electron Microscopy. The average metal concentrations of all sites were 150, 68.3, 43.9 mg/kg for Zn, Cu and Pb, respectively. Comparatively, total Zn, Cu and Pb concentrations in Vortech filters were 82.1, 50.8, 23.6 mg/kg, lower than in Bay Saver filters, which were 360.9, 155.5, 58.93 mg/kg, respectively. The baffle box system demonstrated the highest Zn, Cu and Pb concentrations, which were 525, 159, 102 mg/kg, respectively, possibly due to the high level of traffic in this location (downstream of a bridge). The retention pond had Zn, Cu and Pb concentrations of 106.4, 62.2, 45.6 mg/kg, respectively. This is lower than the filter sample for this site, which is 159.5, 67.7, and 20.4 mg/kg, respectively. This suggests that upflow filters are effective in trapping TRWPs. To further characterize the samples, we performed a density separation to isolate TRWPs in sediments and performed qualitative analysis with an electron microscope to examine the presence of Zinc Oxides and Sulfur Oxides, which are associated with TRWPs. High counts of Zinc were noted in sediments near high traffic density locations. Future work will

examine the associations of heavy metal concentrations with sediment properties, proportion of Tire Road Wear Particles, traffic density, and antecedent rainfall conditions. The results of this research will provide the needed technical basis for the design of effective stormwater treatment facilities in terms of sediment remediation of TRWPs.

2.07.P-Th-123 Trends and Limitations in the Reporting of Aquatic Toxicology Experiments Examining Transcriptomic Endpoints According to the OECD Omics Reporting Framework

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Due to increasing affordability, and the necessity to uncover and understand transcriptional responses of organisms to biotic and abiotic stimuli, transcriptomics has become a tool with significant impact on toxicological investigations and regulatory sciences alike, especially in the field of new approach methodologies (NAMs). Data detailing the molecular mechanisms of action of toxicants have been collected for various organisms, directly informing adverse outcome pathway frameworks, informing read across, and identification of points of departure. While the use of molecular data via omics-based approaches in human and ecological risk assessment of industrial and agrochemicals has been anticipated by the scientific community for decades, change has been slow. Their absence in decision making has been attributed to various factors including standardization and reporting guidance, despite various stakeholders collaborating to develop both guidance documents and frameworks to improve their adoption. Employing these reporting frameworks for transcriptomics data offers an opportunity to identify barriers towards the adoption of these NAMs, especially in ecotoxicological applications.

Here, we employ a standardized form of data reporting for ‘omics studies via the OECD Omics reporting framework (OORF), which specifically reports on a list of parameters that should be included in ‘transcriptomics’ studies used in a regulatory context within an aquatic toxicology context to examine existing aquatic toxicology literature. Relevant studies focused specifically on those employing RNA-Seq/microarray technologies were systematically characterized on these fundamental reporting guidelines, including the reporting of the experiment. In both approaches, inconsistencies in reporting and methodologies among the experimental designs (toxicology versus molecular characterization) were observed, in addition to foundational differences in reporting of sample concentration or preparation or quality assessments, which can affect the reproducibility and read across of experimental results, confidence in the data and contribute substantially to variability in understanding of molecular mechanisms of toxicant responses. While the framework provides an opportunity for improved reporting of experiments within a research context, this study also outlines several recommendations as logical steps to reduce barriers in the adoption of this approach within ecotoxicological applications.

2.07.P-Th-124 Acute Exposure to an Environmentally Relevant Mixture of Road-Related Contaminants Does Not Lead to Interactive Effects

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Understanding biological responses to environmental toxicants, both in isolation and as a mixture, is an important step in determining risk to humans and organisms. One contaminant mixture of concern is stormwater runoff, which can impact aquatic organisms in a variety of ways such as mortality, growth, and elevated stress hormones. In our study, we assessed the individual- and combined impacts of road-related contaminants to a common freshwater invertebrate in a modified standard acute toxicity test. We exposed *Daphnia magna* neonates (<24 hr), to fifteen binary, tertiary, and ternary combinations of road salt (NaCl;1.0 – 7441.5 mg/L), metal (copper; 1.5 – 121.5 /L), polycyclic aromatic hydrocarbon (pyrene range:1.0 – 507.9 mg/L) and microplastics (tire wear particles range: 0.0014 – 220.54 mg/L) at five concentrations previously measured in sediments (copper, pyrene, tire particle leachate) and surface waters (NaCl) in an urbanized stream (Humber River, Toronto, Canada), using a modified (i.e., miniaturized) standardize acute (i.e. 48-hours) toxicity test protocol. Endpoints included survival, and tissue collection for transcriptomic analysis to assess the mechanism of impact. Survival results demonstrate that copper and road salt had a significant impact on *Daphnia* survival, with 100% mortality observed at the highest concentration of road salt (NaCl: 7441.5 mg/L) and all concentrations of metals (copper: 1.5 – 121.5 mg/L) However, no interaction effects were observed; instead, *Daphnia* survival was driven by the most toxic stressor in the mixture. RNA-seq was performed on tissue from a subset (i.e., pyrene and tire particle leachate) of surviving organisms for transcriptomic analysis, and Weighted Gene Correlation Network Analysis (WGCNA) is being conducted to explore transcriptomic patterns and investigate molecular mechanisms impacting survival. Preliminary results suggest that for *Daphnia magna* and this set of contaminants, interactions are not common amongst contaminants with different modes of action. This study also highlights the utility of using high throughput testing as a rapid screening tool to better understand the impacts of locally relevant chemical mixtures, potentially aiding in management and decision making. We measured copper in sediments since we planned to work with mussels who are exposed to sediment. We didn't make any adjustments when switching to daphnia who are exposed to water rather than sediment.

2.07.P-Th-125 Assessing the Impact of Water Temperature on Microplastic Uptake and Depuration in the Eastern Oyster, *Crassostrea virginica*

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Microplastics, defined as plastics 5mm or smaller in size, are ubiquitous in marine and estuarine systems. Because of their small size, microplastics may be easily consumed by organisms. This is especially true of filter feeders, which must intake particulate matter to feed. In addition to microplastic exposure, increased seawater temperatures represent a significant risk to these organisms. Despite this, it is so far unclear how water temperature might impact the uptake of microplastics by filter feeders. The objective of this ongoing study is to characterize the effect of

temperature on microplastic uptake and depuration in the Eastern oyster, *Crassostrea virginica*. Oysters will be exposed to 5000 particles/L of microplastics at one of three temperatures (20°C, 24°C, or 28°C) and then sacrificed at 0, 24, 48, and 96 hours to assess microplastic uptake. The oysters will then be transferred to clean, microplastic-free seawater and sacrificed at 24, 48, and 96 hours to assess microplastic depuration. It is hypothesized that oysters exposed to higher water temperatures will accumulate a greater number of microplastics. This study will quantify the impact of temperature on microplastic accumulation in Eastern oysters, thereby helping to define the risk posed by the combination of microplastic pollution and increased sea temperatures to this keystone species.

2.07.P-Th-126 Machine Learning and Deep Learning for the Identification and Enumeration of Early Developmental Stages of Larval Fish

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Larval fish are a key component of biomonitoring to assess the health of aquatic systems but are notoriously difficult to identify. Recent advances in optical imaging and artificial intelligence (AI) have led to testing these technologies to identify organisms. The purpose of this research is to test the capabilities of AI, specifically machine learning and deep learning techniques, for identifying early developmental stages of freshwater fish. The Small Aquatic Organism optical imaging system (SAO) is a high-throughput optical imaging device that incorporates AI techniques to identify organisms. We constructed an image training library of early developmental stages of three fish species, the channel catfish (CCF), the Florida largemouth bass (FLLMB), and koi that included eggs and at least 2 stages of larval development. We conducted enumeration trials with each stage of each species by adding a known number of specimens into the SAO. We compared the identification accuracies of a support vector machine (SVM) classifier to a convolutional neural network (CNN) classifier. We ran different experiments by modifying the number of classes in the training library to determine identification accuracies. The full training library included all three species and all 10 stages of development and saw an accuracy of 0.915 for the SVM and 0.9422 for the CNN when bubbles and detritus classes were included. With bubbles and detritus removed, the SVM accuracy was 0.343 and the CNN accuracy was 0.6505. We also reduced the training library to include each species individually. With bubbles and detritus included, the SVM accuracy was 0.9698 for CCF, 0.9521 for FLLMB, and 0.8840 for Koi while the CNN accuracy was 0.8748 for CCF, 0.9638 for FLLMB, and 0.9420 for Koi. With these classes removed, the SVM results were 0.5323, 0.5749, and 0.6545 for CCF, FLLMB, and Koi respectively, and 0.6629, 0.7198, and 0.6707 for the CNN for CCF, FLLMB, and Koi. In every experiment except the CCF by itself, the CNN outperformed the SVM. It is important to note that the accuracies were being inflated by large number of bubbles and detritus images, which is why we also chose to report the accuracies without those classes. These accuracies are still too low to be biologically useful for replacing manual identification with AI identification; however, as these imaging technologies and AI classifiers continue to improve, we could see a transition to AI identification of larval fishes.

2.07.P-Th-127 Deploying Juvenile Unionid Mussels As Biomonitors of Chemical Stressors Within a Biodiversity Hotspot

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Mussel populations in portions of the Clinch River in Virginia and Tennessee have declined substantially in recent decades, but the causes of the decline remain unknown. To investigate this "zone of decline" (ZOD), we deployed juvenile freshwater mussels (*Villosa iris* in 2012 and *Lampsilis fasciola* in 2013) in both cages and silos to sites within the Clinch River System. We analyzed mussel tissues for trace element and organic contaminant concentrations, shells for trace elements, and environmental media (total water, dissolved water, particulate sediment and bedload sediment) for both inorganic and organic contaminants. We found few differences between mussels deployed in cages and those deployed in silos: survival was lower in cages due largely to sedimentation. Our results identified the ZOD based on accumulation of trace elements (notably As, Cu Fe, Mn, Ni and Sr), PAHs, and δ^{15}N enrichment, with especially high concentrations found in the human-impacted tributaries, Dumps Creek and Guest River. Some correlations were found between environmental media and both mussel tissues and shell. In particular, PAHs and Mn had several significant relationships between bioaccumulated concentrations and environmental concentrations. Finally, several elements (Co, Cu, Fe, V) had negative correlations between the concentrations in soft tissues and mussel growth, whereas bioaccumulated PAH concentrations correlated with resident mussel densities. This study further confirms the existence of a chemical component to the Clinch River ZOD and links bioaccumulation of contaminants to mussel growth and population density.

2.07.P-Th-128 Mercury Concentrations in Fur of Adult Female Northern Fur Seals From the Pribilof Islands and Bogoslof Island

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Northern fur seals (NFS, Unangax̂: laaqudan; *Callorhinus ursinus*) have been an important resource for the indigenous communities of the Pribilof Islands for many generations. The Pribilof Islands support the largest breeding colony of NFS, which has been in decline since the 1970s. Currently, the Pribilof/Eastern Pacific Stock of NFS are listed as depleted. NFS are opportunistic foragers, feeding on a variety of fishes and invertebrates, including pollock, herring, salmon, mackerel, and squid. For the indigenous communities that harvest NFS for subsistence use, chemical pollutants such as mercury are a stated concern. Natural and anthropogenic mercury biomagnifies in marine food webs and bioaccumulates in long-lived, upper trophic-level species, such as NFS. Other pinnipeds in nearby regions have been shown to have increasing fur total mercury concentrations ([THg]) during the past decade. In other studies [THg] in fur has been positively correlated to [THg] in tissues that are commonly consumed, such as liver, kidney, and muscle. We investigated [THg] in fur of live-captured adult female NFS (n=335) at seven rookeries on St. Paul Island, St. George Island, and Bogoslof Island between 2015-2023. Fur was washed using Triton-X^{TM} detergent and analyzed on a Nippon direct mercury analyzer. We found a median [THg] among all years and rookeries of 5.98ppm (SD: 1.57ppm). We found differences in [THg] by rookery and by year. Further, an interaction between year and rookery was significant ($p < 0.001$) in 49 of 190 pairwise comparisons. Maximal median [THg] was observed during 2022 at the Zapadni Reef rookery (8.76ppm) and during 2019 at Bogoslof and Vostochni (8.00ppm and 7.99ppm, respectively) rookeries. Although mercury was detected in all fur samples, none were above the 20-ppm threshold for potential adverse effects in pinnipeds. This study contributes valuable baseline [THg] values for the continued monitoring of this regionally-important pinniped species.

2.07.P-Th-131 Determination of 6PPD-Quinone in Aqueous Matrices Using SPE With Various Polymeric Sorbents and LC/MS/MS

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The degradation of tires on roadways is known to release numerous chemicals into the environment, posing ecological and health risks. To combat this, 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine) is used in tire production due to its role as a rubber antioxidant. However, studies now show that through reactions with environmental oxidants such as ozone, this compound forms 6PPD-quinone (6PPD-Q), a persistent and toxic compound that can pose risks to ecosystems and animal health. It has popularly been linked to the mortality of coho salmon as they migrate upstream to spawn, so much so that only a few hours of exposure of the salmon to contaminated stormwater has proven lethal. Considering these recent discoveries, the EPA released an official Draft Method 1634 in January 2024 to extract and analyze 6PPD-Q in water. This method uses an extracted internal standard (EIS) to measure the concentration of 6PPD-Q by isotope dilution and a native internal standard (NIS) to measure the efficiency of the extraction procedure. This application outlines solid phase extraction and analysis of water samples using quality control protocols outlined in draft method 1634, comparing both UCT’s Enviro-Clean® polymeric highly-crosslinked divinylbenzene SPE cartridges and UCT’s Enviro-Clean® polymeric hydrophilic-lipophilic-balance SPE cartridges. A sample preparation method was optimized by modifying the procedures outlined in the draft method. Both cartridges demonstrated efficient recoveries over a 3-day method detection limit study and mid-level demonstration of capability studies, with the divinylbenzene cartridges achieving cleaner extracts due to reduced signal-to-noise on the instrument. Both cartridges were also tested using unfiltered tap water, and with these tests, it was discovered during the experiment that maintaining a pH near 5 in the sample was crucial to the extraction procedure. Using UCT’s SelectraCore® C18 HPLC Column, an optimized analysis method was developed via LC-MS/MS. The core-shell technology of the column reduces backpressure and system downtime on the HPLC and improves chromatographic resolution. The LC method is shortened from the EPA’s outlined run time & injection volume to a 5-minute run time and 10 microliter injection volume. The MS/MS method reached sensitivity levels below the EPA’s lowest suggested standard and met all method criteria for linearity and reproducibility.

2.07.P-Th-132 An Epigenetic Evaluation of Toxicity Responses in Two Salmonids at a Mine Tailing Impacted Spawning Reef

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Lake Superior lake trout and whitefish are a conservation success story in being the only predominantly self-sustaining stocks of these native species across the Great Lakes. However, legacy mine tailing (‘stamp sands’) contamination of Buffalo Reef, a large natural spawning reef, poses a substantial hazard to these populations. In this research, we used reduced representation bisulfite sequencing to compare and contrast the epigenomes of Buffalo Reef lake trout and whitefish eggs against those from populations unexposed to stamp sands. Epigenomes of Buffalo Reef eggs indicated a contrasting degree of genome hypomethylation relative to Lake Huron and Michigan outgroups. Importantly, the methylation pattern for Buffalo Reef individuals correlated to pathways related to DNA damage, immune responses, stress, and altered nutrient metabolism relative to Lake Huron and Michigan outgroups. These results highlight adverse effects associated with stamp sand exposure at Buffalo Reef and help inform a multi-billion dollar effort to rehabilitate and conserve this natural resource.

2.07.P-Th-133 Evaluating Methane Dynamics in Base Mine Lake: Implications for Oil Sands Reclamation With Water-Capped Tailings Technology

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Canadian oil sands are among the largest oil deposits in the world, with reclamation and tailings management posing significant challenges to industrial and local ecosystems. A promising approach in oil sands reclamation is the Water-Capped Tailings Technology (WCTT). WCTT is a method of tailings reclamation involving the deposition of tailings below grade into a mined-out pit, placing a layer of water of sufficient depth to prevent wind-driven re-suspension of fines, and allowing the densification of the tailings to occur without mechanical or chemical treatment. The resulting end-pit lake features a layer of water cap on the tailings deposit that forms a lake habitable for plants and animals. Base Mine Lake (BML) is the first commercial-scale demonstration of WCTT in the oil sands industry, and results from the research and monitoring programs have highlighted methane as a key factor in the biogeochemical cycling of petroleum contaminants. Methanogenesis occurs within the fluid fine tailings (FFT), with methane transported to the water column via diffusion, advection, and ebullition. Methane ebullition can facilitate bitumen related organics transfer from FFT to the water column. Understanding methane dynamics is important for understanding BML performance. Microcosm studies in this research have demonstrated persistent methane production within FFT, with spatial and depth-related variability. The observed methane production rates are closely linked to naphtha content, suggesting that naphtha contributes to methane production by serving as a source of organic carbon. Isotopic analysis of microbial phospholipid fatty acids (PLFAs), specifically C16:1 PLFA, shows marked δ^{13}C depletion in the shallow FFT of certain BML zones. This depletion indicates active methane oxidation by methanotrophs even in anoxic conditions. These isotopic patterns correspond with previous observations in the water cap, suggesting dynamic water exchange at the FFT-water interface, potentially driven by ebullition. These findings can help us

evaluate the potential impact of methane on the evolution of the BML and contribute to enhanced management practices for BML and other proposed pit lakes within the oil sands region of Alberta.

2.07.P-Th-134 Determination of DDT and Its Metabolites in Wetland Soils and Sediments of Cootes Paradise in Hamilton, Ontario, Canada

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Cootes Paradise, located in Hamilton, Ontario, Canada is a 600-hectare wetland and an important habitat for local species of fish, reptiles, mammals, and plants. Recent detection of legacy insecticide dichlorodiphenyltrichloroethane (DDT) within frogs living in Cootes Paradise highlighted the necessity of assessing the distribution and levels of DDT, as well as its metabolites dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyltrichloroethylene (DDE) within the area, as the trio of chemicals are toxic to local wildlife, bioaccumulate through the food-chain, and are persistent in the environment. This ongoing study involves collecting soil and sediment samples from areas of interest determined via GIS analysis of the wetland's topography. DDT and its two metabolites were extracted via solvent extraction, followed by silica gel purification and quantification by Gas Chromatography Mass Spectrometry (GC/MS). Results have shown that DDT is present in all soil and sediment samples analyzed so far, at levels between 0.3 ng/g to 200 ng/g, with some soil samples exceeding soil quality guidelines and probable effect levels defined by the Canadian Ministry of the Environment. Concentrations of DDD and DDE range from below detection limit to 20 ng/g and are less variable than DDT concentrations. Two groups of samples have been identified- one group of samples show the presence of DDT in conjunction with high amounts of DDD and DDE, and a second group of samples that have only DDT present. Results from last year also indicate that concentration is correlated with organic carbon in the sediments for certain sampling locations within the wetland, with some areas being less correlated. The relative abundance of metabolites may be a result of differences in processing of the DDT within source soils and sediments, or they may reflect difference in biogeochemical conditions for the specific sites leading to differing extents of biodegradation on site. Ongoing work seeks to differentiate these potential explanations. Through this work, the improved knowledge on the amount and distribution of DDT and its metabolites within Cootes Paradise allows for better decision making on remediation of the wetland, so it can continue to sustain the ecosystem and serve as an important habitat for local and migratory species.

2.07.P-Th-135 Neurotoxicity and Oxidative Stress of Short-Chain PFAS Mixture in Larval Zebrafish in Comparison With C8 PFAS

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Due to the toxicological and public health concerns on 8-carbon chain PFASs (C8) such as PFOS and PFOA, shorter-chain PFASs (e.g., PFBS, PFPeA, PFHpA, PFHxS) have been increasingly used as alternatives. The 8-carbon chain PFASs (C8) such as PFOS and PFOA have been reported for adverse effects of developmental toxicity and swimming behavior in fish. However, the neurotoxicity of shorter-chain PFASs is not as well understood. This study aims to compare the toxicity of a frequently detected short-chain PFAS mixture with that of PFOS or PFOA.

A mixture of three short-chain PFASs (PFBS, PFPeA, PFHpA) was prepared based on the occurrence data in groundwater. The mixture concentrations were set at 2.45, 24.5, and 245 nM with a mixing ratio of 13:11:12 (C4:C5:C7, 'short-chain mixture') and those of PFOA and PFOS were 245 nM. Following a 7-day exposure of embryo-larval zebrafish (*Danio rerio*), swimming activity and the transcription of key neurological regulating genes were measured. Oxidative stress markers (SOD, GPx, MDA) and neurotransmitters (DA, AChE) were also assessed using ELISA. Locomotor activity under lit conditions in larval zebrafish did not show significant differences following exposure to PFOS, PFOA, or the short-chain mixture. Similar results were observed during the light-dark transition. In the short-chain mixture, some oxidative stress markers (SOD, GPx) and dopamine levels decreased dose-dependently. In both PFOS and PFOA exposures, SOD levels decreased, with dopamine levels decreasing following PFOA exposure. Additionally, in the short-chain mixture, the changes in GPx levels observed were less pronounced than those in the C8 groups, while MDA level was higher than those in the PFOS or PFOA groups. Furthermore, neurogenesis genes (*mbp, gfap, nestin*) were down-regulated across all exposure groups. The transcriptional changes in the C8 PFASs were relatively greater than in the short-chain mixture group. Notably, the genes regulating neurotransmitter release (*syt1a, syn2a*) were downregulated only in the short-chain mixture groups. The current observations demonstrate that exposure to the short-chain mixture did not show significant differences in neurotoxicity (behavior, neurotransmitter levels, gene expression) compared to the long-chain PFASs of the same concentration. The results of this study show that the short-chain PFAS mixture, at comparable concentrations, may exert similar levels of neurotoxicity on larval zebrafish.

2.07.P-Th-136 Leachates of Biodegradable Mulching Films Disrupt Thyroid Function and Neurodevelopment of Larval Zebrafish

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Mulching films, commonly used in agriculture, have been considered as a source of soil contamination. Recently, biodegradable materials such as polylactic acid (PLA), and polybutylene adipate terephthalate (PBAT)have been increasingly used in these films, instead of more conventional plastics like low-density polyethylene (LDPE). The impacts of biodegradable mulching films on the thyroid system remain unexplored. This study addresses this gap by investigating how leachates from mulching films affect thyroid function. We exposed zebrafish larvae (*Danio rerio*) at 7 days post-fertilization to varying concentrations of mulching film leachates: solvent control (DMSO 0.01%) and leachates of different strengths at 0.05, 0.5, and 5 mg/µL. Results revealed significant changes in thyroid hormone levels and gene expression. Specifically, we observed increased levels of triiodothyronine (T3), thyroxine (T4), and/or free thyroxine (FT4), correlating with upregulation of genes mct8, slc16a5, ugt1ab, pax2a, pax8, and trb, which are related to cellular transport, metabolism, and thyroid development. The changes in hormones and related genes suggest that components of mulching films have potential to disrupt thyroid homeostasis. Additionally, transcriptional changes in neurogenesis-related genes, including brain-derived neurotrophic factor(bdnf), were detected, suggesting neurotoxic effects of the mulching films. This pattern implies that metabolic disruptions impacting thyroid hormone regulation also affect neural development and function. Our findings suggest that leachates from mulching films may inhibit both thyroid and nervous system functions. This observation enhances our understanding of the environmental risks associated with biodegradable plastic contamination from agricultural practices and underscores the need for further research on the long-term effects of these pollutants on aquatic ecosystems.

2.07.P-Th-137 Characterization of Water Quality Stressors in an Impaired Midwestern Watershed

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Minnesota has a robust statewide water quality assessment and reporting program, but condition monitoring and Total Maximum Daily Loads (TMDLs) cannot always sufficiently identify or explain causes of biological impairments. The project was conducted in Spring Valley Creek, MN, stream where routine monitoring & assessment approaches could not identify stressors associated with observed fish and invertebrate impairments. To determine whether chemicals could be contributing to the biological impairment we collected water samples during low and high flow runoff events at eight locations throughout the watershed including wastewater treatment effluent (WWTP) and stormwater. Samples were analyzed for circa 90+ chemicals including industrial, wastewater and pesticide derived chemicals. As a part of this study exposure activity ratios (EAR; similar to hazard quotients) were calculated to identify exceedances of the aquatic-life benchmarks for pesticides. Aquatic Life Benchmarks codeveloped by U.S. EPA and USGS are estimates of the concentrations below which pesticides are not expected to represent a risk of concern for aquatic life. Fish, invertebrates, vascular and non-vascular plant benchmarks were used. Assessment of 24 hr exposure to solid-phase extracts of stream waters on larval mortality and behavior was completed. Three toxicity evaluation methods, EAR-based predictions of toxicity, direct in vitro (data not shown), and direct in vivo assessments showed that WWTP and stormwater were important contributors of CECs in this system and that they may be able to initiate adverse biological responses. EAR-based assessment indicated that copper and clothianidin occur at most of the sampled sites at concentrations that exceed benchmarks, and that their quantity varies with hydrological conditions. Additional sampling is underway to develop additional benchmarks relevant to this site and to advance understanding of seasonal variations in chemistry and associated hazard.

2.07.P-Th-138 Characterizing Effects of Quinone Outside Inhibitor Fungicides and Their Metabolites on Mitochondrial Function in Rainbow Trout Cell Lines

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Quinone outside inhibitors (QoIs) are widely used fungicides representing 15% of the fungicide market share. Due to their efficacy, QoIs are used extensively throughout the USA, particularly in the agricultural Midwest. QoI occurrence in water combined with the findings that some cause mortality and developmental malformations in fish has heightened concerns over ecological impacts. The reactions with chemicals used in water and wastewater treatment (i.e., disinfection with free chlorine) often produce new chemical species, or QoI transformation products. Because transformation products can retain structural similarity to their parent QoI compounds, we hypothesize that these QoI transformation products retain biological activity. High resolution, animal-free, and highly controllable testing approaches are needed to rapidly explore mechanisms of cellular toxicity of numerous QoIs and their metabolites. We used two rainbow trout cell lines, RTG-2 and RTgill-W1, to characterize mitochondrial function of three QoIs (kresoxym-methyl, azoxystrobin, pyraclostrobin) and their transformation products. Basal respiration, ATP-dependent, maximum, and proton leak-dependent respiration were measured using a Seahorse XFe Extracellular Flux Analyzer. We demonstrated that exposure of either cell line to a wide range of concentrations of QoIs adversely affected cell viability and mitochondrial function in a dose-dependent manner; results were comparable to those reported for human cell systems. Pilot data indicated that one of the kresoxym-methyl transformation products affected cell respiration. Analyses of additional chlorinated metabolites are underway. Use of fish-based cell lines as a platform for mitochondrial toxicity testing can aid with the assessment of risks that fungicides pose for fish health, as well as with the development of safer fungicide alternatives.

2.07.P-Th-139 Chasing the Contaminant: Integrating Mercury To Examine Juvenile Shark Diets Across Texas Bays.

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Sharks play crucial ecological roles as top predators, influencing food webs and energy pathways across diverse environmental conditions. As higher-trophic level consumers, sharks are also prone to mercury (Hg) exposure through diet and the amount of Hg contamination in their habitats. However, a significant knowledge gap remains in understanding how Hg concentrations in juvenile sharks relate to the spatiotemporal variability in their prey. Typically, stable isotope tracers ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) are employed to study shark diets and food web dynamics, however, any chemical tracer that: bioaccumulates, is readily retained in tissues, and taken up through the diet can be potentially used as a chemical tracer to assess diet and food-web dynamics. Mercury represents a potential additional chemical tracer for assessing dietary contributions. Mercury bioaccumulates in higher-trophic predators, and its concentrations vary based on environmental contamination and methylation rates. In this study, we analyzed Hg concentrations and estimated diets of juvenile bull sharks (*Carcharhinus leucas*) from three Texas bay systems (Sabine Lake, Aransas Bay, and Corpus Christi Bay) using more traditional stable isotope tracers ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$), as well as total mercury (THg) in muscle tissues of sharks and their putative prey. Shark dorsal muscle and prey items were analyzed for THg using a direct mercury analyzer (DMA-80). To evaluate THg as a tracer, we applied isotope and THg data to Bayesian dietary mixing models, incorporating each tracer singly (e.g., C, N, S, and THg) and all possible combinations to estimate the proportion of different prey to shark diets. Preliminary findings indicate that the effectiveness of each chemical tracer ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and THg) in reflecting juvenile shark diets varies among tracers and by site. Our results suggest that using THg as a chemical tracer can help to improve dietary mixing model interpretability, but isotopic data often outperforms THg.

2.07.P-Th-140 Spatial Variation and Environmental Drivers of Mercury in Caddisflies (Trichoptera) in a Complex River Basin

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Mercury (Hg) is a global contaminant with its toxic organic form (methylmercury; MeHg) biomagnifying in food webs. Inputs of Hg to aquatic systems are predominately atmospheric, and ecotoxicologists have identified factors (e.g., water chemistry, extent of watershed forest cover) that influence river food web sensitivity to MeHg biomagnification. However, the relative strength of factors in determining river food web MeHg contamination across broad spatial scales remains unknown. We assessed spatial variation and environmental factors driving MeHg concentrations in benthic macroinvertebrate (BMI) communities across a complex river basin (Colorado River basin, Texas, USA) which exhibits pronounced spatial gradients in land use and land cover (LULC) and water chemistry. In this study, we specifically targeted the ubiquitous caddisfly genera *Hydropsyche* and *Cheumotopsyche*. Invertebrates and water chemistry were collected from 20 reaches from five rivers in the basin, and LULC composition for each reach was determined at riparian and whole watershed scales. Benthic macroinvertebrates were analyzed for total Hg (THg), MeHg, and stable isotopes of carbon and nitrogen. There were pronounced gradients in LULC and water chemistry across the basin, with reaches in two rivers (Concho and Pedernales) having greater forest cover. In addition, the Concho and upper Colorado river study reaches exhibited higher nutrients and salinity when compared to other study reaches. Preliminary analysis indicates a significant difference in caddisfly THg among rivers ($p<0.001$), while there was a marginally non-significant effect of reaches nested within rivers ($p=0.0548$). However, there was a significant river x reach interaction ($p<0.001$), indicating that caddisfly THg between upper and lower portions of rivers were not consistent among rivers. Elevated THg was found in the San Saba, Llano, and Pedernales compared to the Concho and Colorado. The Colorado and Llano rivers had greater THg concentrations in the lower reaches than the upper, opposite from the remaining three rivers. This study provides a better understanding of the relative strength of local- and watershed-scale factors influencing MeHg contamination of food webs in spatially complex drainages and provides a foundation to assess sensitivity of specific river reaches to MeHg contamination.

2.07.P-Th-141 Variation in Mercury Levels and Proximate Composition of Northwest Atlantic Forage Species

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The ecological significance of forage species lies in their role as key trophic intermediaries, making them critical indicators of environmental contamination and nutritional quality. In this study, we investigated the variation in mercury (Hg) levels and proximate composition (per gram dry weight, lipid, and protein content) across five key forage species from the Northwest Atlantic: Silver Hake *(Merluccius bilinearis),* Butterfish *(Peprilus triacanthid)*, Atlantic Mackerel *(Scomber scombrus)*, Atlantic Herring *(Clupea harengus),* and Alewife *(Alosa pseudoharengus).* Mercury levels were quantified in 120 individuals using the NIC MA-MA-3000 mercury analyzer, while proximate composition was assessed through lipid extraction and

protein quantification via muffle furnace combustion. Statistical analyses revealed significant differences in Hg concentrations among species (ANOVA, $p < 0.05$). Silver Hake exhibited the highest mean Hg concentration (148.94 ± 66.67 ng/g), significantly exceeding that of Alewife (77.67 ± 40.46 ng/g), indicating species-specific Hg bioaccumulation patterns, possibly linked to protein content. Energy density (ED) also varied significantly, with Silver Hake showing the highest ED (33.16 ± 2.33 kJ/g) and Atlantic Herring the lowest (24.87 ± 1.92 kJ/g). A negative correlation between Hg levels and lipid content ($r = -0.280$, $p = 0.002$) suggests that species with higher lipid content tend to have lower Hg concentrations. Conversely, a positive but non-significant correlation was observed between Hg levels and protein content ($r = 0.137$, $p = 0.136$). These findings underscore the complex interactions between Hg accumulation and nutritional composition in forage species, with implications for food web dynamics and human consumption risks. The study highlights the need for continued Hg monitoring in the region and also emphasizes the importance of species-specific considerations in ecological risk assessments.

2.07.P-Tu General: Wildlife Toxicology, Ecology and Stress Response

2.07.P-Tu-088 Effects of Fine-Grain, Calcium Carbonate Sediment on Two Stony Corals, *Montastraea cavernosa* and *Stephanocoenia intersepta*

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Dredging, the removal of sediments and rocks from an aquatic environment, is necessary to ensure that adequate coastal infrastructure is maintained for maritime shipping activities. However, the sediment plumes generated by dredging operations could have adverse impacts on nearby coral reef ecosystems that are already facing numerous local and global stressors negatively impacting their health and resilience. Currently, there is no standardized laboratory reporting procedure for measuring sediment in the water column making comparative effects on coral challenging. In this laboratory study we exposed two Atlantic stony coral species (*Montastraea cavernosa* and *Stephanocoenia intersepta*) to six different treatments of fine-grain sediment concentrations ranging from 0 to 511.7 mg L^{-1} for 30 days. Coral mortality, growth, and three bleaching indicators were documented after 10- and 30-day exposures. No mortality or tissue loss was observed in either species across the experimental period. No statistically significant differences in growth were observed for the 10-day exposure; however, both species displayed significant declines in calcification at the end of the 30-day exposure. Bleaching indicators did not appear to be affected by sediment exposure at day 10 or 30. In the water column of the exposure chambers, reduction in photosynthetically active radiation (PAR), NTU units, and Total Suspended Solids were highly correlated with each other. These data indicate that short-term pulses of sediment to coral ecosystems may minimally impact coral, however if these sediment pulses are longer in duration, they may negatively affect coral growth. As no tissue loss or mortality were observed during this study, we conclude that corals may have the capacity to survive strategically timed dredging operations that suspend sediment near coral reef habitats.

2.07.P-Tu-089 Investigating Endocrine Disruption from Coastal Pollution in *Mytilus edulis* Using RNAi

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The blue mussel, *Mytilus edulis*, is found throughout the North Atlantic, and is a keystone species that resides intertidally and provides economic value to coastal communities. *M. edulis* is a species of environmental importance and has previously been used to evaluate the quality of coastal environments. Sex ratios and development of *M. edulis* are compromised due to coastal contamination that is found to disrupt developmental genes. When exposed to endocrine disrupting chemicals such as 17-a-ethinylestradiol or ketoconazole, *M. edulis* change sex phenotypically and normal expression of sexual development genes is disrupted. Our aim is to understand the role of previously identified sex determining genes (Fox-L, SoxH, DMRT1L, and β-catenin) in endocrine disruption and determine if their suppression is sufficient to alter sexual development and differentiation in *M. edulis*. We used RNA interference (RNAi) to knockdown four sexual differentiation genes. Hemolymph was initially collected from a set of mussels to determine individual sex before exposures. Then, male mussels were exposed to SoxH and DMRT1L RNAi and female mussels were exposed to Fox-L and β-catenin. We exposed mussels to treatments at the beginning of gametogenesis for two weeks. RNAi bacteria cultures were grown overnight for each of the targeted genes and fed to treatments suspended in live algae three times a week. Our controls consisted of a GFP and no treatment for both sexes. After week one, one mussel per treatment was sacrificed, and mantle tissue was collected for RT-qPCR to determine successful knockdown of the genes and confirmation the mussels were consuming the RNAi bacteria. The experiment was followed up by a final sample collection of mantle tissue to determine final expressions of sex determining genes using RT-qPCR, and histology samples to evaluate gonadal development. We expect to see sex change in mussels following knockdown and reduced gonadal development in histological sections. This study will provide valuable insight into the mechanism of endocrine disruption for bivalves, which has eluded researchers because of gaps in our basic knowledge of sexual differentiation and endocrinology in these species.

2.07.P-Tu-090 Wildfire Impacts on Aquatic Ecosystems: Assessing Macroinvertebrate Communities and Metal Concentrations in Impoundments of Northern New Mexico

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In the western United States, catastrophic wildfires are expected to occur more frequently and severely. Alterations to forest and aquatic habitats from severe fires can significantly impact ecosystems, negatively affecting aquatic communities by reducing total abundance and species richness and altering metal concentrations and ecological structures. This study investigates the impacts of the 2022 Hermits Peak and Calf Canyon wildfires on metal concentrations within various aquatic systems and their effects on macroinvertebrate communities in northern New Mexico. Our research's primary objective is to evaluate metal contamination between anthropogenic impoundments and beaver ponds. We will focus on macroinvertebrates as bioindicators to better understand impoundments' protective role against contamination caused by wildfire activity. This approach will offer valuable insights into exposure pathways within these aquatic ecosystems. We will conduct field surveys in 20 impoundments across burned and unburned areas, focusing on high-severity and unburned sites. Standardized D-frame kick net

sampling will be used for macroinvertebrate community samples, with further analysis at ENMU. Metal analysis will be performed on water and sediment samples using EPA techniques. Permutational multivariate analysis of variance (PERMANOVA), canonical correspondence analysis (CCA), and generalized linear models (GLM) statistical analyses will be used to evaluate the influence of metals on community structure. Our multidisciplinary approach aims to increase our understanding of the effects of wildfires on aquatic ecosystems and the potential mitigating role of impoundments, thereby offering crucial insights for conservation and management strategies.

2.07.P-Tu-091 Hey You Mussel, Are You Alive? Aquaculture Therapeutants Effect on the Marine Bivalve *Mytilus edulis*

Davide Asnicar *and Benjamin Patrick de Jourdan, Huntsman Marine Science Centre, Canada*

Around 40% percent of global aquatic products derive from aquaculture, showing an increasing trend of production. Salmon aquaculture sites create a particular environment for the aggregation and reproduction of sea lice (marine ectoparasites) which cause severe skin and tissue damage with consequent fish stress, mortality, and economical loss. Chemical solutions involving the application of drugs and pesticides are commonly used as part of an integrated management plan to maintain fish health and limit loss. To this end, nearly 75% of the active marine finfish aquaculture sites in Canada use at least one drug or pesticide each year. Chemicals that enter the marine environment are expected to disperse following release, potentially harming non-target species. Despite their wide usage, there are still unknowns and concerns regarding the environmental effects of these compounds, as well as the influence of formulation vs active pharmaceutical ingredient (API) and the impact of mixtures. In this context, a series of toxicity tests were performed using the water-soluble formulation Salmosan® and its API Azamethiphos on a non-target species, the blue mussel *Mytilus edulis* using both in vitro (lysosomal membrane stability) and in vivo (mortality, clearance rate, and survival in air) approaches. Azamethiphos did not cause any effect on hemocytes' lysosomal stability up to 1000 mg/L, neither in API nor formulation form. In the in vivo exposure trial (API concentrations tested: 0.19-15.66 mg/L) carried out with juvenile mussels, azamethiphos caused paralysis in the animals, affecting their ability to close, and often showing the overextension of the foot and being not responsive to the touch. Animals in this condition could be considered dead as it increases the risk of predation or exposure to other stressors. The compound most likely affected their adductor muscle, paralyzing them. However, they were still able to filter seawater as seen during the clearance assay and made a full recovery once placed in clean seawater. The effect concentrations observed in this study were several orders of magnitude higher than the proposed environmental quality standard for the compound which is set at 0.1 µg API/L and above the prescribed application rate in aquaculture operations (0.1 mg API/L).

2.07.P-Tu-092 Effects of Salinity on the Toxicity and Real-Time Metabolic Rate Responses of Acute Ammonia Exposure to Juvenile *Macrobrachium rosenbergii*

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Macrobrachium rosenbergii, commonly known as the giant freshwater prawn, has gained significant attention in global aquaculture initiatives. As a catadromous species, *M. rosenbergii* relies on brackish water for early life stage development and can be grown to market size at typical salinities observed for brackish groundwater desalination concentrate or below. This offers an opportunity to use desalination waste to offset the operating and environmental costs of brine disposal. A major challenge in aquaculture is receiving real-time, comprehensive feedback on changing water quality parameters and the health status of organisms. For example, high density recirculating aquaculture systems (RAS) incur the potential for ammonia accumulation which affects the survival, growth, and overall health of aquatic animals. However, the interactive effects of ammonia and salinity on *M. rosenbergii* remain poorly understood, particularly during the juvenile stages that coincide with the transition from brackish water to freshwater. The main goal of this research is to develop an in-line, real-time sentinel respirometry system to promptly detect subtle changes in water quality that might affect growth and survival. To this end, we sought to first determine the acute toxicity of ammonia at different salinities to establish LC50s at various time points out to 48 h. Informed by these data, we then sought to test the acute real-time effects of stepwise increases in sublethal ammonia exposures on the routine metabolic rates of juvenile *M. rosenbergii* using static intermittent respirometry. 48 h ammonia bioassays were performed at 3 salinities (1, 5 and 10 ppt; pH 8.2) and mortality was scored at 3, 6, 24 and 48 h. Acute mortality generally increased with increased exposure duration and was highest at 10 ppt for all time points except 24 h (no significant difference across salinities). Subsequent respirometry trials revealed that metabolic responses were also highest at 10 ppt and statistically significant responses could be detected when compared to control resting metabolic rates, regardless of salinity. These data inform the effect of salinity on ammonia toxicity and confirm the potential for using a sentinel respirometry system for detecting changes in water quality within RAS.

2.07.P-Tu-093 Freshwater Mussel Culturing: Comparison of Fatty Acid Profiles, Stable Isotopes, and Glycogen Levels between Fish-host and In Vitro Cultured Populations

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Freshwater mussels have a unique life cycle during which there are 3 stages: glochidia/ larvae, juvenile, and adult. During the larval stage of the life cycle, the mussel (glochidia) is dependent on a fish host to which they attach and have a parasitic relationship with. The larval mussels then transform into juveniles and exit the fish host. At the juvenile stage, the mussels drop to the substrate and are free living. It has been shown that a significant amount of nutrients in the glochidia is derived from the fish host via stable isotope analysis. The parasitic relationship between glochidia and fish hosts are still not well understood. Mussel propagation facilities are also moving toward the use of in vitro cultures to replace the fish host during the glochidia stage. This involves exposing the mussels to a nutrient medium in petri dishes while they transform into juveniles. This is a novel culturing technique that still has much to be learned about, including comparison to traditional culturing techniques. Bivalve polyunsaturated fatty acid profiles have been found to match their algae diet closely. They can be used as food web tracers and have use for assessing diet quality and energy storage. Glycogen has long been used to determine freshwater mussel health and stress levels. In this study, we aim to determine if there are differences in glycogen levels and fatty acid profiles, as well as carbon and nitrogen stable isotopes between mussels cultured using traditional fish host and in vitro techniques Results of this study further inform the use of in vitro propagation techniques in freshwater mussel conservation efforts and its impact on mussel quality and health.

2.07.P-Tu-095 Acute and Chronic Toxicity of Copper to a Short-Term Brooding Freshwater Mussel (*Truncilla truncata*)

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Freshwater mussels are one of the most imperiled fauna groups globally. Previous studies have demonstrated that the early life stages of unionid mussels are highly sensitive to some metals, including copper. However, most toxicity data generated from previous studies have used long-term brooding mussels because the larvae are more readily available. Long-term brooding mussels spawn in late summer and brood their parasitic larvae over winter through the following spring or early summer. In contrast, short-term brooding mussels generally spawn in early spring and release their larvae by late spring or summer. Data using short-term brooding mussels is underrepresented in toxicity databases. The objective of this study was to use a short-term brooding mussel, Deertoe (*Truncilla truncata*), to evaluate its sensitivity to copper. We used ASTM standard methods to conduct an acute 96-h test and a chronic 28-d toxicity test with juvenile mussels. Copper exposure levels were chosen based on previous work with long-term brooding unionids. The acute test exposed 1-week-old juvenile mussels to five copper concentrations (50% serial dilution) plus a control under static conditions at 20°C. The chronic test used a flow-through diluter with an auto-feeding system to expose 6-week-old mussels to five copper concentrations (50% serial dilution) plus a control at 20°C. Preliminary results indicate that acute EC50 and chronic EC20 for Deertoe fall within the range observed with other mussel species that were tested in waters with water quality characteristics similar to those used in the present study. Thus, the sensitivity of this short-term brooding mussel to copper appears not to be different from other species of mussels. However, more studies are needed with short-term brooding mussels to test additional chemicals with different modes of toxic action.

2.07.P-Tu-096 Subchronic Effects of Titanium Carbide MXenes on Zebrafish (*Danio rerio*)

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MXenes are a type of two-dimensional (2D) inorganic engineered nanomaterials, known for their unique accordion-like morphology and customizable surface functionalities. This class of nanomaterials has garnered significant attention within the scientific community and industrial sectors alike. These advanced materials hold immense promise across a spectrum of applications, particularly in domains such as water treatment, purification, and environmental remediation. Zebrafish (*Danio rerio*) were exposed to varying concentrations of Ti_3C_2 MXenes under two different light conditions (standard laboratory fluorescence and specialized Everbeam® 365nm 50W UV LED Flood Lighting designed for aquatic environments. Zebrafish, beginning as eggs and developing through larval stage in a subchronic study, were exposed to five Ti_3C_2 MXene concentrations ranging from 0.01 to 1.0 mg/L, while tracking their development through 28 days. There were statisically significant effects on zebrafish survival between all treatment groups and their respective control groups; there was only survival in the controls and the lowest exposure group (0.01 mg/L). In three out of the five exposure groups, initial sample sizes of 5 per aquarium were gradually reduced to 4, then 3, and finally 2 to ensure a sufficient number of

organisms remained alive to complete the study. The LC_{50} was calculated to be only 2.34 mg/L in contrast to an earlier acute study that calculated the LC_{50} to be 257.46 mg/L. Given that zebrafish share a significant portion of their genome with humans and were heavily affected by such low doses, the findings of this study underscore the imperative need for further research to fully understand the potential risks associated with MXene exposure.

2.07.P-Tu-098 The Effect of Non-Dioxin Like Polychlorinated Biphenyls (NDL-PCBs) (Aroclor 1254) on Calcium (Ca2+) Dependent Signaling Pathways in Zebrafish (*Danio rerio*)

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Polychlorinated biphenyls (PCBs) are a group of persistent synthetic organic pollutants that continue to pose significant threats to both aquatic organisms and humans. Non-Dioxin-like (NDL) PCBs constitute the bulk of PCB congeners and are capable of eliciting neurotoxicity. Neurotoxicity is thought to be elicited through three possible mechanisms: disruption of tissue dopamine (DA) levels, γ- aminobutyric acid (GABA) signaling, or intracellular calcium (Ca^{2+}) dynamics. Preliminary exposures to Aroclor 1254 demonstrated DA and GABA concentrations to be unaffected in zebrafish, with Ca^{2+} dynamics yet to be investigated as a cause of neurotoxicity. This study investigates the effects of Aroclor 1254 on neurological, developmental, and transcriptomic endpoints related to Ca^{2+}-dependent signaling pathways. Embryos will be exposed to Aroclor 1254 at 200, 350, and 500 µg/L for a 96hr acute non-renewal exposure. After exposures, cardiac edema, heart rate, and eye tremor will be quantified. A portion of replicates will also be utilized for transcriptional analysis. RNA extraction and cDNA synthesis will be done using the RNeasy Plus Mini Kit (Qiagen), following its provider instructions. Then RT-qPCR will be run, comparing the expressed genes TrpC1 (a transient receptor gene channel that operates as Ca^{2+} store and functions as a regulator for intracellular Ca^{2+} signaling) and RyR1 (a ryanodine receptor gene that is responsible for release of Ca^{2+} from the SR) to the reference gene Ube2a/tmem50a. The differential of the gene expression will be calculated using the ΔCt method. A downregulation of RyR1 and TrpC1 will show that Ca2+ dynamics are responsible for neurotoxicity.

2.07.P-Tu-099 Evaluations of Aqueous Toxicity and Cytotoxicity of Ziram

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Grass Carp, *Ctenopharyngodon idella*, originally from eastern Asia, are one of the four invasive carp species established in North America. Grass Carp have been recorded in 45 states in the United States, with breeding populations in the Great Lakes and several major basins, including the Missouri and Mississippi Rivers. There is a need to identify new chemicals that can be used for the selective control of invasive carp. These new chemicals should show some species-specificity, be palatable to target species, demonstrate low persistence in aquatic systems after application, and have minimal adverse effects on non-target species. One such chemical is Ziram, a dimethyl-dithiocarbamate containing zinc ($\log K_{ow}$: 1.23) is currently registered as an agricultural fungicide that may have promise as a pesticide to control undesirable fish, like the Grass Carp. Few studies have measured the toxicity of Ziram to fish, or freshwater invertebrates. The objectives of this study were to evaluate the potential acute toxicity of Ziram in an aqueous solution to a suite of non-target aquatic invertebrates and fish and to predict *in vitro* toxicity using newly developed Grass Carp gill cell lines. Six species, 3 invertebrates, and 3 fish were exposed to a series of aqueous Ziram concentrations for 96 hours. In the invertebrate bioassays, 96-h LC_{50} values (nominal concentrations) ranged from 0.14 mg/L to >2.0 mg/L, with amphipods being the most sensitive species. In the fish studies, 96-h LC50 (nominal concentrations) values ranged from 5.4 µg/L to 354 µg/L, with Lake Sturgeon being the most sensitive species. Ziram concentrations will be determined by analyzing test water for total Zn concentrations. In this study, gill cells from rainbow trout (*Onchorynkuss mykiss*; RTGill-W1) and the newly developed grass carp gill cell lines were exposed to ziram (0.01 – 1250 µg/L) for 24 hours to characterize fish cytotoxicity (EC_{50}), evaluating metabolic activity, lysosome membrane integrity, and cell membrane integrity. Cytotoxicity values were compared to toxicity values from *in vivo* exposures. Preliminary results indicate cytotoxicity is endpoint-dependent for rainbow trout and grass carp, but the ratio of *in vitro*: *in vivo* 24-hour toxicity values is less than 2-fold. Future development of a piscicide bait using Ziram could consider formulations that would increase the absorption of Ziram in the intestine by increasing its residence time in the peritoneal cavity.

2.07.P-Tu-100 Modeling Thiamethoxam Effects on Field Populations of *Chironomus riparius* Using a DEB-IBM-Based Approach

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Population models play a crucial role in predicting species population dynamics under various environmental scenarios, accounting for both natural and human-induced stressors. In ecological risk assessment, these models, often coupled with toxicokinetic-toxicodynamic (TKTD) models, have become increasingly valuable. Our study focuses on developing a model concept to simulate populations of *Chironomus riparius* with the goal of extrapolating ecotoxicological laboratory data for the neonicotinoid insecticide Thiamethoxam (TMX) to real-world field scenarios. Our model concept integrates individual-based models (IBMs) based on dynamic energy budget (DEB) modules, complemented by toxicokinetic-toxicodynamic (TKTD) module to capture both lethal and sublethal processes influenced by dynamic environmental conditions. At the core of the model is an individual DEB-TKTD model, parameterized for TMX using laboratory tests at different temperatures as well as experimental results on density dependence. In a comprehensive experimental laboratory study, we found that *Chironomus riparius* experiences a food-dependent development rate, but also a density-dependent mortality rate of more than 60 % at high larval densities, which is probably due to cannibalism. In principle, at high population densities, lethal effects from exposure to the insecticide can be partially compensated by a reduction in density-dependent mortality. In this study, we present a modular model framework for a population model of *Chironomus riparius* and apply it to assess the risk of TMX under field conditions. We analyze and discuss the effects of varying larval densities and food conditions, and we evaluate the transferability of exposure multiplication factors (EMFs) estimated at the individual level, using the moving-time-windows approach, to field populations through relevant application examples.

2.07.P-Tu-101 Assessment of Hydrogen Peroxide as a Treatment Method for the Degradation of Prochloraz in Freshwater Environments: Implications for Environmental Management

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Prochloraz is a commonly used imidazole fungicide with medical, industrial, and agricultural applications in Europe, Australia, Asia, and South America. It is often used in agriculture to treat produce prior to exportation. Prochloraz has been the focus of multiple studies assessing toxicity both in soil microbial communities as well as in mammalian exposures. When released into the environment, prochloraz is believed to influence bacterial communities in the soil because of its anti-fungal properties, as well as having potential effects on aquatic ecosystems as an endocrine disrupting chemical (EDC) and is a known cytochrome P450 (CYP) enzyme inhibitor. Because of this, the disposal and environmental fate of prochloraz is an important factor, and current and potential degradation treatments should be evaluated based on their success and improved, if necessary, to minimize environmental impact. Current treatment methods include utilizing UV exposure and the addition of an interacting chemical (ex. hydrogen peroxide) to increase the degradation rate of prochloraz prior to disposal. However, little data exists analyzing the photodegradation of prochloraz or the success rate of hydrogen peroxide as an effective treatment method to reduce residues in wash water prior to releasing into freshwater environments. The objective of the present study is to evaluate the success of hydrogen peroxide as a treatment method for the degradation of prochloraz in freshwater when exposed to UV light. To accomplish this, water samples containing 1ppm and 10ppm prochloraz will be organized into treatment groups that will each be treated with multiple concentrations of hydrogen peroxide. The water samples will then be placed in a UV chamber for a designated amount of time according to treatment group. Upon completion of the UV exposure, each sample will be removed and analyzed using HPLC. With success, the results of this study will provide useful information and improve treatment options for companies currently using prochloraz and allow for reduction in overall environmental impact.

2.07.P-Tu-102 Pesticides Mixture Damage Kidney Architecture, Induce Oxidative-Nitrative Stress, Increase Renal Expression, Instigates Cellular Apoptosis and Impair Swimming Behavior in Goldfish

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Freshwater and marine life forms are damaging in toxic conditions, on account of escalating pollutants tarnishing their natural habitats. An ever-increasing range and volume of chemical contaminants are introduced into aquatic habitats by man-made factors, particularly agriculture. These include compounds such as pesticides (more precisely biocides) that contribute to a wide variety of stressors corrupting aquatic ecosystems and deleteriously influencing the life of aquatic inhabitants. In this study, we analyzed dose-dependent and time-dependent effects of pesticide blends (metalachlor, linuron, isoproturon, tebucanazole, aclonifen, atrazine, pendimethalin, and azinphos-methyl) (exposure at 22 ^{o}C for 1 week) on the morphological and cellular changes in the gills and kidneys of goldfish. Fish exposed to pesticides showed a significant ($P<0.05$) increase in oxidative and nitrative stress biomarkers in the gill and kidney tissues. In addition, behavioral analysis showed a dose-dependent, time-dependent, decrease in

the distance swam and the prolonged time they stayed in each region of the tanks. Predominantly, these results indicate that pesticide cocktails impair cellular functions in teleost species.

2.07.P-Tu-103 Developmental Sensitivity to PFOS and PFHxSA in the Mummichog Varies Based on Early Life Stage

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Per- and polyfluoroalkyl substances (PFAS) are widely used, long-lasting environmental contaminants linked to adverse effects in fish and other species. The thousands of unique PFAS are likely to impact organisms in different ways and through different mechanisms. To address these concerns, we exposed an ecologically important estuarine minnow, the mummichog (*Fundulus heteroclitus*), to perfluorooctane sulfonic acid (PFOS) or perfluorohexanesulfonamide (PFHxSA) at concentrations from 0-100 µM during two different periods of their early life stages: embryo and larva. The early life stages of fish are important for assessing the effects of pollutants because they are often highly sensitive and toxic effects have the potential for later life impacts that can affect population persistence. Embryos were statically exposed to PFOS or PFHxSA from days 1-7 post-fertilization (dpf) and observed until 30 dpf. Larvae were statically exposed from 1-7 days post hatching (dph, 15-21 dpf) and followed until 35 dpf. After hatching, mummichogs actively feed, and therefore an additional treatment variable of feeding or fasting was investigated during larval exposures. Over the course of both embryonic and larval exposures, a multitude of biological endpoints were assessed including survival, growth, and swim bladder inflation. Fish were differentially sensitive to PFOS and PFHxSA depending on life stage of exposure. PFOS did not affect embryonic survival, however, nearly all larvae exposed to 40 µM died. In embryonic exposures to PFHxSA, survival significantly decreased at 100 µM compared to control, while larval exposures caused a decrease in survival at 40 µM. Additionally, after exposures to each PFAS, survival of larvae was influenced by feeding. Fed groups of larvae tended to experience higher mortality earlier in exposure but at lower levels overall compared to fasted groups. Further work is needed to determine if the greater sensitivity of larvae is caused by toxicokinetic or mechanistic differences between the two stages. Additionally, the role of feeding requires further investigation. Exploration of early life stage sensitivity is essential to understanding how contaminants affect overall development, health, and survival. Ongoing integration of molecular mechanistic data and apical endpoints will provide insight on potential adverse outcome pathways and help inform population modeling efforts.

2.07.P-Tu-104 Short and Long-Chain PFAS Effects on Development and Metabolism of *Danio rerio* Larvae

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Per- and polyfluoroalkyl substances (PFAS) are emerging contaminants of concern due to evidence of nondegradation within ecological systems and adverse interactions with organismal physiology. Currently, there is some limited evidence that shorter carbon chain alternative PFAS are relatively less toxic than legacy compounds in perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). We sought to investigate this current dogma by comparing a short carbon chain PFAS alternative to the longer carbon chain legacy PFAS, PFOA. We hypothesized that there will be no difference between the two regarding developmental outcomes in zebrafish (*Danio rerio)* larvae. Commonly affected endpoints regarding PFAS toxicity include embryonic development and metabolism. Therefore, we explored the development and metabolism of two different strains of zebrafish following exposure to perfluoropentanoic acid (PFPA), a shorter carbon chain PFAS, to PFOA. PFAS contamination concentrations range widely globally with no established lowest effect level (LOEL) for PFPA. Exposure concentrations ranged from 20-2000 ppb. The fish embryo acute toxicity test (FET) is an assay used for assessing zebrafish embryonic and larval development following exposure to a contaminant. Alamar Blue is a fluorescence-based assay that allows for the detection of NADH reduction. These two assays allow for monitoring of gross morphological development and acute detection of changes to metabolism during the embryonic period. Our results indicate that the longer carbon chain legacy PFOA has a greater effect on metabolism than the shorter carbon chain alternative, thus supporting the current notion regarding PFAS toxicity related to carbon chain length.

2.07.P-Tu-106 Toxicity of PFASs to Fathead Minnows Compared with Other Aquatic Species

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To better understand the risks of per- and polyfluoroalkyl substances (PFASs) in freshwater ecosystems, toxicity information is needed for a more diverse range of structures and test species. Previously, we reported that PFASs varied widely in their sublethal potency to three freshwater invertebrates: the cladoceran *Ceriodaphnia dubia*, the midge *Chironomus dilutus*, and the amphipod *Hyalella azteca.* For the perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs), most EC50s followed a trend of increasing toxicity with fluorinated chain length, without large differences in sensitivity among species. The clear exception was the much higher sensitivity of midge to PFSAs in the 7 to 9 carbon range, which showed toxicity about 3 orders of magnitude higher than was observed for the other two invertebrates or for PFCAs in general. We hypothesize the greater sensitivity represents a different mechanism of toxicity. Literature showed that fathead minnow are much more sensitive to PFOS than PFOA, which may indicate that fathead minnow follow similar trends as midge in

their sensitivity to PFSAs. In this poster, we compare these prior data with new data for fathead minnow (*Pimephales promelas*). In 7-d sublethal toxicity tests conducted with larval fathead minnows, this wide difference in toxicity was confirmed, with PFOS and PFOA EC50s of 2.2 mg/L and 212 mg/L, respectively. Although more sensitive to PFOS than PFOA, fathead minnows were notably less sensitive to PFOS than were midge, and less sensitive to PFOA than were any of the three invertebrates. Initial measurements of PFAS concentrations in tissue suggest that much greater uptake of PFOS relative to PFOA may partially explain the difference in toxicity to fathead minnow, something that was not observed with midge. Additional data on fathead minnow sensitivity to other PFSAs and PFCAs are presented, along with implications toward identifying PFASs most likely to cause toxicity at environmental concentrations. *This abstract does not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.*

2.07.P-Tu-107 Toxicity of PFOS and PFOA to Two Benthic Estuarine Organisms

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Per- and polyfluoroalkyl substances (PFAS) are a group of fabricated chemicals that includes such compounds as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). PFOS is no longer manufactured in the United States, but they are still produced internationally and can be imported into the United States in consumer goods. PFOA is used worldwide as an industrial surfactant in a variety of applications. PFOS and PFOA toxicity information on estuarine organisms is limited especially in sediment dwelling estuarine organisms. The objective of this research was to characterize the effects of PFOS and PFOA in juvenile amphipods (*Leptocheirus plumulosus*) and juvenile hard clams (*Mercenaria mercenaria*) in aqueous only acute tests and in chronic tests in the presence of sediment. *L. plumulosus* are surficial modifiers widely distributed along the East coast of USA. *M. mercenaria* are filter feeding bivalves native from Canada to Mexico. Any effects on these benthic organisms can have repercussions on their ecosystem as a whole. The parameters measured, in addition to mortality, will include growth and biomarkers of exposure. The research with these PFAS compounds will add much needed data to assess how these contaminants pose a risk to estuarine organismal health.

2.07.P-Tu-108 Mixture Effects of PFOS and Two Perfluorosulfonic Acids on Larval Sheepshead Minnows

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Per- and polyfluoroalkyl substances (PFAS) are a ubiquitous class of pollutants commonly found in aquatic ecosystems. Perfluorooctane sulfonate (PFOS) is a particularly toxic and persistent PFAS that has been shown to be toxic to fish. While PFOS toxicity has been well documented, its mechanisms of toxicity, as well as mixture effects with other PFAS are largely unknown. Previous studies in our lab have observed antagonistic effects in larval sheepshead minnows (*Cyprinodon variegatus*) co-treatmented with PFOS and several perfluorocarboxylic acids.

However, PFOS interactions with perfluorosulfonic acids are largely unknown. This study aims to characterize the mixture effects of PFOS with perfluorohexane sulfonate (PFHxS) or perfluorobutane sulfonate (PFBS) on larval sheepshead minnows. Acute toxicity of PFOS mixtures with PFHxS or PFBS will be assessed, as well as their sublethal toxicity, including oxidative stress biomarkers and differential gene expression. This research will reduce uncertainty in risk assessments of PFAS mixtures in aquatic environments.

2.07.P-Tu-109 Spatial Distribution of Polyfluoroalkyl Substances in Freshwater Fish in Maine

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Per- and polyfluoroalkyl substances (PFAS) are highly persistent and mobile chemicals known to have adverse health effects in humans. One way that humans can be exposed to PFAS is through consumption of contaminated fish. Fishing has long been an important social, economic, and cultural aspect of Maine. As a result, it is critically important we understand how the concentrations of PFAS in fish vary across the State. Created in 1993, Maine's Surface Water Ambient Toxics (SWAT) monitoring program directs Maine's Department of Environmental Protection to investigate the nature, scope, and severity of toxic pollutants in Maine's surface waters and fisheries. In recent years, PFAS chemicals have been an important focus of the SWAT program. To determine the spatial distribution of PFAS contamination in Maine's freshwater fishes, the SWAT monitoring group has been sampling brook trout, bass, black crappie, and other game fish across the state since 2014. Sample sites were selected to provide a gradient of potential exposures but often included areas that are popular among anglers to help us understand the potential contamination of fish that Mainers may be consuming. Samples were collected primarily by angling. PFAS concentrations were obtained from composites of tissues samples that were analyzed by SGS Axys. Preliminary results indicate that concentrations of PFAS in fish tend to be lowest in forested parts of the state and highest in more populated regions of the state. However, PFAS chemicals were still detected in fish in remote locations. This information has been critical for helping the State of Maine develop consumption advisories for freshwater fish throughout the state and identify areas of unexpectedly high PFAS contamination.

2.07.P-Tu-110 Toxicity of 10 Priority PFAS to Five Standard Marine Species

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Per- and poly-fluoroalkyl substances (PFAS) are coming under increasing scrutiny at Department of Defense (DoD) sites primarily due to their historical use in aqueous film-forming firefighting foams (AFFF). Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are chronically toxic to aquatic organisms at observed concentrations near AFFF sources, resulting in concerns over the ecological risk posed by these compounds. There is a lack of effects data for marine aquatic life, limiting the assessment of ecological risks and challenging the development of water quality criteria, resulting in unclear requirements for remediation at impacted sites. In the present study, the toxicity of 10 priority PFAS compounds (PFBA, PFHxA, PFOA, PFDA, PFBS, PFHxS, PFOS, PFDS, 6:2 FTS, and 8:2 FTS) were evaluated with five standard USEPA marine laboratory toxicity testing species. Species being evaluated include: 1) 7-d chronic survival and growth for topsmelt fish (*Atherinops affinis*); 2) 7-d chronic survival and growth for the mysid shrimp (*Americamysis bahia*); 3) 48-h embryo-larval normal development and normal survival for the Mediterranean mussel (*Mytilus galloprovincialis*); 4) 96-h embryo-larval normal development for the purple sea urchin (*Stronglycentrotus purpuratus*), and 5) 48-h germination and growth for giant kelp (*Macrocystis pyrifera*). All species were tested using standard USEPA methods. Observed EC50 values were typically above 1 mg/L PFAS, with the exception of PFOS and PFDA in some cases. Generally, *S. purpuratus* or *A. bahia* were the most sensitive endpoints and *M. pyrifera* was the least sensitive endpoints. In general, longer perfluorinated carbon chains were more toxic than shorter chain compounds. The perfluorosulfonic acids (PFSAs) were most toxic in the 6-8 perfluorinated carbon chain length and perfluorocarboxylic acids (PFCAs) were most toxic at the 10+ chain length. Understanding the effects of PFAS exposure on marine ecological receptors will lead to better remediation outcomes at impacted, coastal sites.

2.07.P-Tu-111 Maternal Transfer of Perfluorooctane Sulfonate (PFOS) in the Great Lakes' Rainbow Trouts and Resulting Effects on Embryo Development

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Extensive industrial and commercial use of per- and polyfluoroalkyl substances (PFAS) has led to their widespread contamination of the Great Lakes. Due to their resilient carbon-fluorine bonds, PFAS bioaccumulate, biomagnify, and cause a wide range of toxic effects in biota. Perfluorooctane sulfonate (PFOS), a legacy PFAS, has integrated itself into the life cycles of the Great Lakes' salmonid species; concerning amounts of PFOS were found to transfer from mothers to their eggs through a process known as maternal offloading. Our research objectives assess PFOS accumulation into eggs using an *in vitro* model of maternal offloading and its effect on embryonic development. This project evaluates PFOS exposure effects in rainbow trout eggs: a fish species important to the Great Lakes. Simulated maternal offloading of PFOS (0, 0.03, 0.3, 3,0 & 30 ppm) and subsequent incubation until hatching allowed for close monitoring of embryo development. Samples were taken at various timepoints and assessed for three markers of oxidative stress: protein carbonylation, lipid oxidation and antioxidant activity levels (glutathione, catalase & superoxide dismutase). PFOS compartmentalization was tracked through LC-MS/MS analysis of embryo content. The resulting data will provide key insight into the effect of PFOS on rainbow trout embryo development and hatching rates.

2.07.P-Tu-112 Effects of Metformin on Wild Fathead Minnows (*Pimephales promelas*) Using In-Lake Mesocosms in a Boreal Lake Ecosystem

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Metformin is routinely detected in surface waters globally due to its widespread use for the treatment of Type-2 diabetes. Laboratory studies have shown that environmentally relevant concentrations of metformin can adversely affect the health of adult fish, with effects often more observed in males. However, the potential risk to wild fish populations has yet to be fully elucidated and continues to be a topic of debate. To explore whether environmentally relevant metformin exposure poses a risk to wild fish populations, the present study exposed wild fathead minnows (*Pimephales promelas*) to 5 or 50 µg/L metformin via 2 m diameter in-lake mesocosms deployed in a natural boreal lake in Northern Ontario at the International Institute for Sustainable Development - Experimental Lakes Area (IISD-ELA). Environmental monitoring was performed at regular intervals for 8-weeks, metformin concentration was measured weekly, and fish length, weight (body, liver and gonad), condition factor, gonadosomatic index, liver-somatic index, body composition (water and biomolecules) and hematocrit levels were evaluated at test termination. Additionally, metabolic endpoints were evaluated using liver, brain and muscle tissue, and gonads were evaluated histologically. Results indicate that current environmental exposure scenarios may be sufficient to adversely impact the health of wild fish populations. Adult male fish exposed to metformin had significantly reduced whole body weight and condition factor and several male fish from the high-dose metformin had oocytes in their testes. Metformin exposed fish had altered moisture and lipid (decrease) content in their tissues. Further, brain (increase) and liver (decrease) glycogen were altered in fish exposed to high-dose metformin. To our knowledge, this study constitutes the first effort to understand metformin's effects on a wild small-bodied fish population under environmentally relevant field exposure conditions.

2.07.P-Tu-113 Can Sulforaphane Blunt Depleted Uranium-Induced Metabolic Injury In Vivo?

***Luke Gibbons**, Hayden L Bekkedahl and Matthew Salanga, Biological Sciences, Northern Arizona University*

Mining of depleted (DU) Uranium (U) for energy production and military use by the United States has left some areas of the country peppered with abandoned mine features and sites. This is particularly true for communities living in the four corners region of the American southwest. Many of whom are tribal entities that have faced health disparities for decades. Measurement of environmental U in these areas has unveiled sites of U concentrations that far exceed the World Health Organization and Environmental Protection Agency's maximum contamination limits of 30 ppb. The mechanism of U chemotoxicity is not fully known, as a result, the health impacts caused by elevated environmental Uranium also remain unknown. We, along with other research groups, have shown chronic low-dose depleted uranium (*i.e.,* uranium containing a significantly lower fraction of the radioisotope U234, and thus is not considered a radio-hazard.) exposure causes metabolic dysfunction and mitochondrial damage. Notably, the prestoBlue assay, a resazurin-based fluorescent assay, has been used to demonstrate DU-induced metabolic injury *in vitro*. Our investigation of mitochondrial function *in vivo* using a similar resazurin-based fluorescent assay, alamarBlue, we observe similar DU-induced metabolic injury in zebrafish larvae. Metabolic injury and subsequent oxidative damage are hallmarks of DU toxicity and are cause for concern. Strategies to blunt these effects could be therapeutically useful. Sulforaphane is an isothiocyanate that is produced by the enzyme myrosinase when cruciferous leafy green vegetables are mechanically disturbed, such as in chewing or chopping. In a cell, sulforaphane activates the transcription factor NRF2 (nuclear factor erthyroid-2 related factor) leading to trans-activation of Heme oxygenase 1 (HO-1), NADH quinone oxidoreductase 1 (NQO1), and other antioxidant-related genes. We hypothesize that NRF2 activation will blunt DU's chemo toxic effects through activation of antioxidant proteins that neutralize the DU's induction of reactive oxygen species and uranyl species. If supported, these data reinforce the need for access to fresh produce, especially in region like the four corners.

2.07.P-Tu-114 Sub-MCL Depleted Uranium Exposure Causes Reduced Mitochondrial DNA Copy Number and Increased Genomic DNA Damage in Zebrafish (*Danio rerio*) Larvae

Phillip Hua Kalaniopio *and Matthew Salanga, Biological Sciences, Northern Arizona University*

Chemical toxicity from uranium (U) is a problem for many communities. In the Southwest USA, abandoned uranium mines are exposure sources for humans and wildlife. While the effects of ionizing radiation on health are well studied, chemical toxicity from uranium is less understood. We and others have shown adverse outcomes from uranium exposure that include impaired mitochondrial function, disrupted mitochondrial ultrastructure, and increased nuclear DNA damage. We hypothesize that uranium directly interacts with DNA (mitochondrial and nuclear), causing DNA damage accumulation. To test our hypotheses, we quantify DNA damage in zebrafish by semi-long run qPCR (SLR qPCR) for three regions in the mitochondrial genome, probing 45.7% of the mitochondrial chromosome. The *ahr1* (aryl hydrocarbon receptor) gene locus is amplified to measure nuclear DNA damage as a nuclear reference. Preliminary data reveal several important outcomes, such as decreased mitochondrial DNA copy number (mtDNAcn), increased locus-specific DNA damage, and fluctuating damage accumulation in the mitochondrial genome. Furthermore, we analyzed outcomes in different wildtype and mutant strains, which revealed some differences across experimental endpoints, particularly in the patterns (i.e., more, less, equal) of mitochondrial DNA damage and effect amplitudes, but not the presence of nuclear damage or diminished mitochondrial copy number, which were reproducible across strains. These data can help explain reduced metabolism and mitochondrial dysfunction in cells and animals exposed to depleted uranium. Most notably, these effects are observed at exposure levels at or below the WHO's MCL of 30 ppb U; we identified a new lowest-observable effect level (LOEL) of 18 ppb U (UN; a common solubilized form of depleted uranium) in mtDNAcn and nuclear DNA damage responses. Furthermore, we preliminarily identified 1.8 ppb U as the potential LOEL for mitochondrial D-loop damage, over 15 times less than the WHO MCL. In future directions, we will expand mitochondrial and nuclear genome coverage, add zebrafish Comet assays, and probe for DNA damage markers by immunofluorescence. Our data provides strong evidence for increased DNA damage in both the mitochondria and nucleus, as well as alterations to mtDNAcn in response to DU at levels below the WHO's MCL. In conclusion, these data support a paradigm in which depleted uranium interacts with host DNA that negatively impacts mitochondria.

2.07.P-Tu-115 Radium-226 Toxicity to the Early Life Stages of the Great Pond Snail *Lymnaea stagnalis*

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Radium-226 (226Ra) is released into the environment during mining and milling of uranium ores, or extraction of coal, oil and gas. To date, there are very few ecotoxicity data for 226Ra with freshwater species found across North America. This gap is particularly problematic for Canada, which is one of a few countries actively involved in the full nuclear fuel cycle. Today, Canada does not have a guideline for the protection of aquatic life to guide environmental managers regarding the presence of this element in fresh waters. To generate such water quality guideline, our team and collaborators are developing new 226Ra toxicity data with Canadian freshwater species. In this study, we are characterizing the toxicity of 226Ra in the great pond snail (*Lymnaea stagnalis*), an aquatic snail that is widespread in Canadian fresh waters and that is known for its sensitivity to trace metals. It is expected that this calciphilic organism will take up large amounts of 226Ra during its developing life stages, due to the analogy between calcium and radium. We exposed the two early life stages of *L. stagnalis* (embryos and juveniles) to increasing aqueous doses of 226Ra up to 100 Bq/L, and measured 226Ra bioaccumulation in tissues, embryonic heart rate, hatching success, juvenile growth and survival. We found that 226Ra affected embryonic heart rate but not hatching, and affected juvenile survival but not growth. In ongoing experiments, we are exploring 226Ra toxicity mechanisms (e.g. oxidative damage to biomolecules) and characterizing the effects of water composition (pH, concentrations of Ca, Mg, dissolved organic matter) on 226Ra bioavailability.

2.07.P-Tu-116 Impact of Anticoagulant Rodenticides on Coho Salmon (*Oncorhynchus kisutch*): Assessing Lethal and Sublethal Effects

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Invasive species have cascading negative effects on community structure and are detrimental to many ecosystems. Rat species have been especially problematic on islands that previously served as naval bases. Eradication efforts include aerial and ground-based applications of food pellets containing anticoagulant rodenticides. This has led to a large increase in the number of islands treated to mitigate damage caused by invasive rats. Aerial application makes an attempt to be precise; however, pellets do inevitably enter the aquatic environment. There have also been documented cases of accidental spills into water during pellet transport. Two of the most commonly used anticoagulant rodenticides are brodifacoum and diphacinone. Trace amounts of these rodenticides have been detected in several captured fish species as well as fish carcasses after pellet applications on rat infested islands. Great Sitkin Island in the Aleutians Archipelago of Alaska is infested with brown rats (*Rattus norvegicus*) and under consideration for eradication by the US Fish & Wildlife Service. This island includes spawning and rearing habitat for Pacific salmon. There is a large information gap for the aquatic toxicology of brodifacoum and diphacinone. To address this gap, we are using coho salmon (*Oncorhynchus kisutch*) to generate lethal and sublethal dose-response curves for estimating median effects doses of brodifacoum and diphacinone. Our estimated 96 hour LD50 by intraperitoneal injection for brodifacoum is 83.7 µg/g (95% CI: 65.3-102.0 µg/g). The preliminary estimate for the LD50 for diphacinone is 104.2 µg/g (95% CI: 62.1-146.4 µg/g). Sublethal effects include blood clotting time which is increased by exposure to anticoagulants. This data will help inform policies regarding the future use of these chemicals for whole island eradication.

2.07.P-Tu-117 Evaluating the Potential Hazards of Anticoagulant-Containing Bait Pellets to Early Life Stage Pink (*Oncorhynchus gorbuscha*) and Coho Salmon (*O. kisutch*)

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Cereal bait pellets containing anticoagulant rodenticides (AR) are frequently employed to control rodents *(e.g.*, rats) in domestic, municipal, agricultural, and conservation settings. The USDA engages in whole-island eradication of invasive rats using aerially dropped cereal bait pellets containing AR chemicals to support the conservation efforts of native species. Currently the USDA and Alaska Fish and Wildlife are developing eradications plans to target invasive rats on the Aleutian Islands. As part of this planning, surveys are being conducted on targeted islands to evaluate the potential for non-target organism mortalities, which has identified Pacific salmon, including coho (*Oncorhynchus tshawytscha*) and pink (*O. gorbuscha*), as potentially impacted species due to aerially broadcast pellets often unintentionally entering waterways. The timing for these eradication efforts is important as they often occur in late summer to early fall and overlaps with the spawning and developmental timeline for pink and coho embryos and potentially alevin. Spawning salmon deposit their fertilized eggs in gravel redds, where the embryos develop over the winter. These redds could unintentionally receive AR-containing bait pellets during aerial broadcast resulting in embryos and possibly alevin developing and rearing in the presence of the ARs. Work in 2022-2023 began estimating the effects of pellets containing brodifacoum (BROD) and pellets containing diphacinone (DIPH) to early life stage coho embryos and alevin. Exposure to AR pellets resulted in significantly increased incidences of mortality and cranial hemorrhaging for alevin exposed to BROD. Tissue accumulation of BROD also increased over time in the developing coho, with accumulation beginning in the embryo stage. This research was expanded in 2023-2024 to assess impacts on pink embryos and alevin as well as to explore critical windows for early life stage coho exposed to BROD pellets. Again, increased rates of mortality and cranial hemorrhaging were noted for coho embryos and alevin for each of the three BROD pellet exposure scenarios . Data collected from the pink alevin also showed a sensitivity to BROD exposure, but not DIPH, which was observed as increased mortality and cranial hemorrhaging. Collectively these studies highlight a need to better understand the movement of ARs in aquatic environments and develop models on AR toxicokinetics and toxicodynamics in aquatic species.

2.07.P-Tu-118 Assessment of Trace Metals and Polyfluoroalkyl Chemicals in Sediment, Water, and Fish Tissues Collected from Mattawoman Creek (Charles County, MD)

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The Naval Support Facility Indian Head, located in the Chesapeake Bay Watershed, serves the U.S. Military as a weapons test and manufacturing facility. The manufacturing of propellants and explosives makes it a potential source of trace metals and polyfluoroalkyl (PFA) chemicals. The Waterkeeper Alliance has detected PFAs in all tested samples obtained from Maryland, Pennsylvania, Virginia, and West Virginia and have also identified Indian Head and Dahlgreen Surface Warfare Center specifically as potential sources. Mattawoman Creek and the downstream Potomac River provide significant biodiversity and ecosystem services, jobs, and drinking water to millions of residents in the DC/Maryland/Virgina region. To assess the presence and concentrations of trace metals and PFAS in Mattawoman creek, sediment, water, and fish tissue samples were collected for analysis of contaminant concentrations. For PFAS analyses, surface water samples were obtained using rinsed and methanol-cleaned 500mL nalgene bottles. A dedicated stainless-steel van veen grab sampler was utilized to collect sediment samples which were deposited into methanol cleaned 1L Nalgene bottles. Trace metal surface water samples were collected in acid washed 125mL Nalgene bottles. Sediment samples were collected using a separate van veen grab sampler and stored on ice during transport. Adult male and female Banded killifish (*Fundulus diaphanus)* were collected using minnow traps and seine nets from three locations. PFAS samples were analyzed using EPA draft method 1633 for 31 per- and polyfluoroalkyl substances using LC-MS/MS. Trace metal samples were digested and analyzed for trace metal element identification and concentrations of these ions using ICP-OES. We expect increased levels of trace metals and PFAs will be found in sediments, water, and tissue samples adjacent to the facility and that tissue contamination will be higher than water due to bioaccumulation factors. If disturbed, these sediments may release contaminants back into surface waters or transport across systems. Our research provides novel information regarding the potential impacts of anthropogenic activity on an important aquatic ecosystem and direction for future studies.

2.07.P-Tu-119 Assessment of the Source, Occurrence, and Fate of Nutrients and Legacy Contaminants in the Odaw River Basin, Accra, Ghana

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The Odaw River Basin in Ghana is a critical lifeline for both ecological systems and human populations. However, the influx of contaminants from multiple sources is posing significant environmental and public challenges. Compounding this problem, is the lack of comparative studies from tropical rivers that could guide restoration efforts. We comprehensively assessed the source, occurrence, and fate of nutrients and legacy contaminants within the Odaw River Basin by collecting surface and groundwater samples from twelve (12) strategically located sites during the dry and wet seasons. We analyzed samples for (i) nutrients (phosphorous, nitrate, nitrite, and ammonia nitrogen) and (ii) legacy contaminants (lead, copper, cadmium, iron, chromium, arsenic, and mercury). We assessed spatial and temporal variations in contaminant concentrations to identify potential sources and comprehend seasonal dynamics. Our findings indicate that the average concentrations of ammonia nitrogen (NH_3-N), phosphorus (PO_4-P), nitrate (NO_3-N), copper (Cu), iron (Fe), and lead (Pb) in the water exceed the Ghana Standard (1212:2019) for effluent discharge. We further explored factors such as hydrological dynamics, land use, and seasonal variations. Additionally, we examined the potential impacts of climate change and urbanization on the transport and transformation of contaminants within the basin, forecasting future scenarios to inform sustainable management strategies. Our findings underscore the urgent need for integrated water resource management approaches that address both point and non-point sources of pollution. By identifying contamination hotspots and assessing the effectiveness of existing regulatory measures, this research contributes to developing targeted interventions to safeguard the ecological integrity and public health of the Odaw River Basin and similar aquatic ecosystems in the tropics. Ongoing research will integrate the presence of the contaminants of emerging concern and conduct a comprehensive ecological risk assessment.

2.07.P-Tu-120 Bioaccumulation and Toxicity of Field-Collected PFAS-Impacted Sediments

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Most environmental toxicology research on per- and polyfluoroalkyl substances (PFAS) to date has focused on aqueous exposures, with less information available about effects of PFAS-impacted sediment at environmentally relevant concentrations. To better understand such effects of PFAS-contaminated sediment on aquatic organisms, our laboratory conducted a series of bioassays: estuarine sediment toxicity and bioaccumulation with the amphipod *Leptocheirus plumolosus,* freshwater sediment toxicity and bioaccumulation with the midge *Chironomus dilutus*, elutriate testing with both freshwater (the water flea *Ceriodaphnia dubia* and fathead minnow *Pimephales promelas*) and marine (the sheepshead minnow *Cyprinodon variegatus* and the Mysid shrimp *Americamysis bahia*) species. Furthermore, we conducted an additional *L. plumolosus* study to test the effectiveness of using 3D-printed structures containing a zeolite known to adsorb PFOS and PFOA in reducing PFAS bioavailability in sediment and porewater and consequently bioaccumulation in tissue. No PFAS-related toxicity was observed in any bioassay; although PFAS concentrations in most bioassays were below those known to cause mortality, *C. dilutus* were exposed to sediment concentrations up to 69 µg/kg dry weight (dw) total PFAS (57 µg/kg dw PFOS), whereas equivalent aqueous PFOS concentrations in this species have lethal effects. Though PFAS bioaccumulation varied between site and did not display a clear trend regarding chain length, median biota sediment accumulation factors (BSAFs; tissue:sediment concentrations, dw) tended to be highest in 6-8 carbon carboxylic acids, particularly PFHxA. 3D-printed zeolite structures reduced PFAS levels measured in porewater and tissue by 80-95% and 34-85%, respectively, compared to the control, offering a promising subject for future studies on scalability.

2.07.P-Tu-121 Seasonal and Spatial Sedimentation Trends in the Eleven Point River, Arkansas, USA

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Seasonal and Spatial Sedimentation Trends in the Eleven Point River, Arkansas, USA.
Land use changes in the Ozark Highland Ecoregion in Arkansas increases the potential for erosion due to deforestation and construction. This can result in not only the loss of aquatic habitat but also a sink for additional contaminants. The Eleven Point River Watershed is experiencing increased poultry production and pasture lands which is changing the surrounding landscape. The watershed has been listed on the 303d list for impaired waterways for turbidity in the years 2014, 2020, and 2022 by the Arkansas Department of Environmental Quality. Four sites of the Eleven Point River were sampled from November 2021 to September 2024 for water quality parameters including turbidity and total suspended solids (TSS). Turbidity and TSS were observed to decrease from the topmost upstream site, Dry Creek, to the downstream site, Hubble Creek. The greatest sediment value was observed in Spring 2022. Seasonal variation and trends from upstream to downstream can indicate the location and type of management practices needed to protect this scenic river known for its recreational value in Arkansas.

2.07.P-Tu-123 A Meta-Analysis: Understanding Effect Levels at the NOEC, LOEC, & MATC in Freshwater Toxicity Tests

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Environmental risk assessors have long debated the merits of using point estimates (e.g., EC50) versus the effect levels determined through hypothesis-based testing, such as No Observed Effect Concentrations (NOEC) and Lowest Observed Effect Concentrations (LOECs). Given the recent preference for point estimates and continued reliance on traditional hypothesis-based testing results in ecotoxicology, our goal was to better understand how point estimates of effects relate to NOECs, LOECs, and Maximum Acceptable Toxicant Concentrations (MATCs). The specific objective of this analysis was to (1) compile a comprehensive dataset of dose- (or concentration-) response data from high-quality aquatic toxicity tests, (2) determine the percent effect that occurs at each NOEC, LOEC, and MATC from the compiled dataset, and (3) develop factors that equate NOECs, LOECs, MATCs, EC20, and EC10 values to EC5 values. We do not intend debate the merits of regression analysis verses hypothesis-based testing. Rather, we intend to provide risk assessors with the tools to interpret the various ways chronic toxicity test data are commonly reported. Moreover, the factors developed here to equate NOECs, LOECs, MATCs, EC20, and EC10 values to EC5 values can be important tools for adjusting commonly-reported toxicity test results to low-level effect thresholds that can streamline screening-level ecological risk assessments and endangered species evaluations.

2.07.P-Tu-125 Development of Ecotoxicity Test Methods for Biodegradable Plastics ? The Decomposition Pretreatment Method to Obtain Testing Samples

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Marine pollution caused by plastics has become one of the world's major environmental problems. The prevailing use of biodegradable plastics is one of the solutions, and an effort to develop the high performance and effective biodegradable products are underway especially in Japan. The new materials are expected to have no ecotoxicity with their high molecular weight at the point of manufacturing and shipping, although, the impact of their degradated products are concerned because they are supposed to be decomposed in the environment. Therefore, the method of ecological assessment designed for biodegradable plastics and their degradated products should be available. Currently, there is a test method for the ecotoxicity of biodegradable plastics, ISO 5430, which is proposed for assuming the oceanic degradation.
The method requires 6 months to prepare test sample in a running water system, results in a low concentration of derived degradation products by a large amount of water used in the method. Those reproducibility and validity are questionable as sample preparation process.
The aim of this study is to develop a versatile ecotoxicity test method for evaluating ranging biodegradable plastics, with emphasis on simpler and more efficient way to prepare test sample, and to evaluate the environmental impacts of those plastics. In Japan, the chemicals which have large molecular weights are not required the ecotoxicity test, except when they degrade with acids or alkalis by 2% of the original weight after 14 days of treatment. The treatment is called "Stability Test." We adjusted the stability test scheme, such as the duration time from 2 weeks to 8 weeks, suitable for biodegradable plastics. If the biodegradable plastic is less decomposable from 2% of the original weight, it could be regarded as stable chemical and omit the ecotoxicity testing. But it decomposes more than 2 %, then ecotoxicity test is carried out using the decomposed sample. We tested five representative biodegradable plastics on fish, crustaceans, and luminescent bacteria using above scheme and those results will be presented.

2.07.P-Tu-126 Ecotoxicological Assessment of *Hyalella Azteca* Exposure to Titanium Carbide MXenes Under Various Environmental Conditions

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Titanium carbide MXenes, Ti_3C_2, represent a prominent category of 2D transition metal carbides, garnering considerable interest due to their exceptional characteristics and broad applicability. These MXenes exhibit versatility across various environmental industries including water treatment and remediation, solar batteries, and agriculture. In two comprehensive studies (acute - 10 d and subchronic - 42 d), *Hyalella azteca* (*H. azteca*) were exposed to Ti_3C_2 MXenes at concentrations of 10, 100, and 1000 µg/L. *H. azteca* were exposed to MXenes in three different matrices (plain culture water, culture water supplemented with sand, and culture water augmented with sand and activated charcoal) under two lighting conditions. Lighting conditions included standard lab fluorescent lighting and full-spectrum plant growth light, spanning 300 to 780 nm. Statistical analysis showed significant differences between all treatment groups, environments, settings, and the control group. Thus, emphasizing the comprehensive nature of these findings. LC_{50} values were calculated, and results ranged from 85.11 µg/L to 758.58 µg/L for the exposure of *H. azteca* to $Ti_3C_2T_x$ MXenes. Statistical analysis included three-way ANOVA followed by Tukey pairwise post-hoc analysis. The ANOVA results indicated that mortality, environment, and setting were each significantly different across all treatments when compared to control groups. This was further confirmed by Tukey's pairwise analysis. Since very low doses caused such profound effects on the survival of the *H.* azteca in all environments studied, there is a critical need to determine the extent to which these MXenes will affect other organisms in an aquatic environment.

2.07.P-Tu-127 The Effects of Cadmium Exposure in the Presence of Climate Change on Embryonic Development in the Seminole Ramshorn Snail (*Planorbella duryi*)

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Based on projected modeling, increases in surface water temperatures due to climate change will pose a host of new challenges for poikilothermic aquatic organisms. Additionally, little is known about how this added stressor will interact with the impacts of known environmental contaminants. As such, this study was designed to assess the effects of cadmium exposure on Seminole ramshorn snail (*Planorbella duryi*) embryonic and adult development under varying temperatures based on predictions from the Shared Socioeconomic Pathways (SSPs). Freshly laid (<8hr post-deposit) *P. duryi* embryonic clutches were harvested from an established adult colony and assessed for viability. Individual clutches were then placed into acid washed 60 × 15 mm glass petri dishes containing 10mL of 0, 3, 5, or 10 ug/L cadmium solution (confirmed using ICP-OES). Plates were then incubated at 20°C (standard assay conditions), 23°C (SSP 2 predictions), or 25°C (SSP5 conditions) for 11 days. During the exposure period, a micrograph of each clutch was obtained under 40x magnification using a mounted USB camera. On days 4, 6, 8, and 11, one egg from each clutch was selected and assessed for developmental stage (morula, trocophora, veliger, hippo. Individual adult snails (n = 20) were exposed to 0, 3, 5, or 10 ug/L of cadmium at 20, 23, or 25 ^{o}C in separate 200mL beakers for 15 days using a static replacement exposure method (complete exchange every 72hrs) and were fed 1hr before each water change to maintain water quality. Snails were monitored daily for mortality defined as lack of footpad response to physical stimulation. On days 1, 4, 7, and 15, individuals were placed into a custom recording chamber, acclimated for 5 minutes, and a 3 minute video obtained and analyzed for differences in locomotor behaviors using Noldus EthoVision XT 17. At the conclusion of the study snails were euthanized, whole body tissues dissected out, extracted using

nitric acid, and bioaccumulation rates assessed using ICP-OES. While this study is still ongoing, we expect to observe a correlation between increased temperature and irregular development, with a subsequent increase in cadmium toxicity at higher temperatures. The results of this study will give a deeper understanding of how sensitivity to trace metal exposure in poikilotherms may be altered by current and future shifts in climate and the potential importance of temperature modifications in current OECD testing methods.

2.07.P-Tu-128 Using Ecological Speciation and Genome Structure to Reveal Habitat Specific Chemical Risks

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Daphnia are keystone species used to understand how chemical pollution impacts our freshwater ecosystems. Typically, toxicological assessment utilizes one *Daphnia* species and clonal isolates as a one-size-fits-all model suggesting all *Daphnia* will respond similarly to chemical stressors. However, *Daphnia* have adapted to live in a multitude of habitats around the globe thus shaping unique physiological processes and preferences. In North America the predominant species are *Daphnia pulex* and *Daphnia pulicaria* which are phenotypically indistinguishable yet evolved to live in different habitats with one preferring static lakes, and the other preferring ephemeral systems (e.g. ponds and wetlands). These habitat preferences drive important physiological differences of toxicological importance including life spans, predator avoidance, reproductive and metabolic rates. A recent study from our lab has shown that only 30% of genome differs between the species, while the majority (70%) is homogenized through gene flow. These differences are primarily constrained to large, rearranged regions that persist on each chromosome that harbor genes that show the strongest signals of selection compared to genes in other regions. We hypothesize that the fixed differences between the species found within these divergent regions, when probed with a variety of chemical stressors, will reveal the mechanisms controlling the differences in physiology between the species. For instance, the commonly used triazine herbicide, Atrazine, promotes the formation of reactive oxygen species (ROS), increase of antioxidant defense enzyme superoxide dismutase (SOD), alterations in lipid peroxidation, and causing irreversible oxidative stress in *Daphnia.* The toxicity of atrazine differs between these species, and we are currently examining its effect on genes in the divergent regions compared to genes whose alleles are shared between the species. Responses across broad groups of toxicants will be discussed. This research is the first part of a dissertation project that will use chromosomal rearrangements as a tool to assess toxicity at the molecular level through exploring differences in gene expression between fixed differences and homogenized regions of the *Daphnia pulex-pulicaria* genome. This research serves as a tool to better estimate risk in lakes and ponds as well as increase our understanding of how the genome and its structure influences the sensitivity of a species to a toxicant.

2.07.P-Tu-129 Disparities in Per- and Poly Fluoroalkyl Substances (PFAS) Tolerance and Life History Traits in *Simocephalus serrulatus* Populations

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Per- and poly fluoroalkyl substances (PFAS) represent a broad class of ubiquitous and pervasive anthropogenic chemicals. While data examining the toxic effects of PFAS are accumulating in the literature, this work has largely focused on laboratory model species with little consideration given to population level differences in toxicity. In this study, we assessed the effects of three PFAS (Perfluorooctane sulfonic acid, PFOS; perfluorooctanoic acid, PFOA; and perfluorohexanesulfonic, PFHxS) on two populations of *Simocephalus serrulatus,* an abundant but unstudied species of zooplankton. We found population-level differences in acute toxicity of PFOS and PFHxS. Moreover, toxicity estimates substantially differed from commonly used toxicity reference values derived from similar species that are used to represent *S. serrulatus* in risk assessment. While PFAS had limited chronic effects on the populations, we observed population-level differences in survival, growth, and reproduction. In particular, the population from the site with a legacy of PFAS exposure showed greater mortality, smaller body size, and greater reproduction. These findings suggest that prior PFAS exposure may have selected for fecundity compensation in the population. This work highlights the need to consider both species- and population-level differences in assessing PFAS toxicity, as these insights will enhance risk assessment and give more accurate insight into effective conservation of affected habitats.

2.08.P-We One Health of Planktonic, Pelagic and Benthic Harmful Algal Blooms (HABs): The Detection, Fate, Effects, Monitoring, and Management of Blooms

2.08.P-We-063 Evaluating the Tolerance of Harmful Algal Blooms to Copper Sulfate Pentahydrate

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Harmful algal blooms can cause severe economic and ecological problems, including fish mortality and the production of toxins and off-flavor compounds. These blooms often require chemical treatments, such as copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), to mitigate damaging effects. Given that waterbodies require repeated $CuSO_4 \cdot 5H_2O$ treatments to control blooms, we hypothesized that phytoplankton communities treated with $CuSO_4 \cdot 5H_2O$ develop tolerance to treatment, which could make blooms difficult to manage over time. To test this hypothesis, the toxic effects of $CuSO_4 \cdot 5H_2O$ were evaluated at a standard dose (1.37 mg/L $CuSO_4 \cdot 5H_2O$; 0.348 mg/L Cu) and a low dose (0.69 mg/L $CuSO_4 \cdot 5H_2O$; 0.174 mg/L Cu) relative to untreated control. Treatments were applied once to 1,000 L mesocosm enclosures installed in a productive aquaculture pond at the start of the experiment and monitored for 28 days using the lab-based pollution-induced community tolerance (PICT) methodology. This method measures photosynthetic efficiency across a broad range of 0.05 to 300 mg/L Cu concentrations in acute short-term bioassays. The results of the bioassay were applied to create half-maximal effective concentration (EC_{50}) dose-response curves that can be utilized to determine tolerance to copper. Results from this experiment indicated that both doses of $CuSO_4 \cdot 5H_2O$ resulted in >99% removal of cyanobacteria in the first 7 days and reduced cyanobacteria by at least 70% throughout the experiment. In the first 3 days, communities in the standard dose and low dose treatments were 20x and 10x more tolerant, respectively. Tolerance peaked at 14 days after the treatments were applied and remained higher than the control throughout the experiment. These data suggest that a lower dose of copper sulfate is equally effective at treating harmful algal blooms dominated by cyanobacteria and that higher doses can lead to higher tolerance in the system, making blooms more difficult to treat in the future.

2.08.P-We-064 Development of Effective Herbicides Mixtures to Address Microcystis in Aquaculture Ponds

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It is well understood that water quality and eutrophication are major drivers of harmful algal blooms (HABs). Issues arising from nutrients loads, water circulation, and temperature also influence blooms of harmful algae, such as Microcystis which are the most common cyanobacteria in Alabama aquaculture ponds, which can be detrimental to cultured organisms and public food safety. Catfish producers commonly use copper sulfate, an EPA-approved algicide, to treat harmful algal blooms. While effective, repeated annual use of copper can accumulate in aquaculture ponds and potentially make the pond too hazardous for commercial production. Copper also has short-term effects on algal densities, leading to repeated doses to address HABs. Use of herbicides may be more effective in establishing long-term remediation of HABs. As herbicides have different mechanisms of action, using multiple herbicides simultaneously may be more effective in controlling Microcystis blooms. The efficacy of herbicides towards Microcystis however, is poorly characterized. The aim of the present study was to establish dose response profiles for Microcystis exposed to common herbicides and characterize effects on growth rates, chlorophyll concentrations, and chlorophyll/phycocyanin ratios (PC:CHL). Microcystis aeruginosa (UTEX 3037) was grown in house and dose response testing was conducted with 7 alternatives to copper (H2O2, Topramezone, Bispyribac Sodium, Triclopyr, Imazapyr, Diaquat Dibromide, Flumioazin, Fluridon). Effect concentrations (EC10, EC25, and EC50) were used to identify thresholds of ecotoxicity for chemicals independently. Diaquat dibromide was the most effect at inhibiting growth rates with an EC50 of 0.08 ± 0.01 mg/L and H2O2 was the second most effective method with an EC50 of 0.13 ± 0.04 mg/L. Of the endpoints, chlorophyll was more sensitive (EC50 0.06 ± 0.01 mg/L) for Diaquat dibromide and H2O2 (EC50 of 0.11 ± 0.02 mg/L) for these compounds. Herbicides decreased the PC:CHL ratio (EC50 0.15 ± 0.05 mg/L for H2O2), indicating that either the chemicals inhibited phycocyanin production more than chlorophyll or that Microcystis altered its pigment ratios to promote light capture. Follow up research is in process to identify the efficacy of the herbicides to inhibit algal growth through binary and tertiary mixtures. Additional research will investigate long term efficacy of the mixture identified to be most successful.

2.08.P-We-066 A Chemical Forensics Approach to Fingerprinting Cyanobacterial Gradients in Ross Island Lagoon, Portland, Oregon

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Anthropogenic activities are increasing the frequency, duration, and severity of cyanobacterial blooms. Cyanobacteria can produce toxins that cause serious health issues in humans, including neurological, gastrointestinal, and respiratory problems, leading to severe illnesses. Thus, it is crucial to forecast cyanobacterial blooms so municipalities can notify communities and implement strategies to protect human health. One potential strategy for predicting blooms is to develop diagnostic chemical fingerprints that can be screened for in existing water quality monitoring; however, these fingerprints are currently underdeveloped. Therefore, our goal is to use multivariate chemical forensics workflows to identify the chemical features that best predict gradients in cyanobacteria within surface bodies of water. Throughout the summer of 2020, we collected water samples from four locations at various depths in Ross Island Lagoon in Portland, Oregon. In each sample, we quantified cyanobacterial species and the non-target chemical composition. Amplicon Sequence Variant counts of individual species and mass spectrum peak area spectra were collected per sample at each site and depth, along with chemical data via liquid chromatography quadrupole time-of-flight. The analysis using Non-Metric Multidimensional Scaling and Indicator Species Analysis showed that variation in algal community composition was strongly associated with water chemistry and environmental variables such as wind speed, water temperature, and air temperature, as well as with seasonal temporal changes over the course of the year. Further analyses will include using machine learning regression to develop a predictive model of cyanobacterial gradients based on a subset of non-target chemical features. Once identified, this model could be used in conjunction with conventional mass spectrometry tools (e.g., LC-triple quadrupole) to broaden the applicability of chemical forensics workflows.

2.08.P-We-067 Evaluation of Biotechnology for the Detection of Microcystin Producing Harmful Algal Blooms in the Stones River in Central Tennessee, USA

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Harmful Algal Blooms (HABs) are becoming an increasing concern towards water quality as anthropogenic influence on the environment increases. One of the primary algal toxins of concern, microcystin, is produced by cyanobacteria and acts as a hepatotoxin. One of the primary bloom forming genera that produce microcystins is *Microcystis spp.* The ability for cyanobacteria to produce the toxin depends on the presence of the microcystin synthetase gene cluster within its genome. In this study, we used polymerase chain reaction (PCR) techniques on cultured algae to amplify genes that can inform algal community structure in field samples. DNA Primers were used that are capable of detecting cyanobacteria and *Microcystis* ribosomal RNA genes as well as detecting the microcystin synthetase E gene. Minimum concentrations for detecting a bacteria via PCR was determined before use on field samples. These primer sets were then applied to field samples from the East Fork of the Stones River to infer the variation in algal communities and toxin-gene presence through time. The Biotechnological data was compared with microcystin concentration, nutrient levels, pH, dissolved oxygen, and conductivity. This was utilized to determine correlations between environmental parameters with algal blooms and toxin gene presence.

2.08.P-We-068 Harmful Benthic Cyanobacteria in Streams and Rivers: USEPA Research to Inform Methods for Risk Assessment

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Benthic harmful cyanobacteria blooms (bHCBs) pose a risk to domestic animal, wildlife, and human health. Recent years have seen dog, cattle, bird, and bat deaths attributed to exposure to bHCBs in various states across the U.S. and internationally. U.S. states, tribes, and local agencies need consistent approaches to characterize bHCB relative risk and inform mitigation. USEPA is addressing this need with multi-year, multi-site field research and controlled experiments. In 2023, this research focused on evaluating in-stream sampling methods that both quantify spatial extent at the reach scale and assess toxin exposure risk from disturbing benthic mats. Field measurements and 2200 samples were scheduled among seven field crews who made multiple visits to seven pilot test sites across six states (CA/KS/OH/UT/VA/WA) and the work was accomplished with 98% completeness. Laboratory analyses include general water quality variables, pigments, morphospecies identification and DNA metabarcoding of periphyton samples, qPCR analyses for toxin biosynthesis genes, and quantification of several cyanotoxins. All periphyton samples were collected such that all measures could be normalized to stream bed area and biomass. Preliminary results are providing 1) a rationale for streamlining field sampling techniques so that more sites can be surveyed in 2024 and 2) insight into the variation in benthic cyanobacteria growth forms, community structures, and toxins that can help guide recommendations for future risk assessment.

2.08.T One Health of Planktonic, Pelagic and Benthic Harmful Algal Blooms (HABs): The Detection, Fate, Effects, Monitoring, and Management of Blooms

2.08.T-01 Using Unoccupied Aerial Systems to Monitor Cyanobacterial Blooms Across Seasons

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Predicting blooms of noxious cyanobacteria is critical for the protection and management of our freshwater resources. Although many tools and approaches exist for water quality monitoring, the integration of unoccupied aerial systems (i.e., drones) with geographical information systems (GIS) has opened the doors for relatively large-scale and rapid detection of cyanobacteria of small waterbodies when compared to standard lakeside sampling and satellite-based remote sensing methods. To evaluate the utility of using drones to monitor water quality in hypereutrophic ponds where phytoplankton are generally abundant and cyanobacteria dominate during the warm growing season, we conducted missions with an eBee Ag drone with a Duet M camera over 19 aquaculture ponds at one farm each month from June 2023 to March 2024 while simultaneously collecting water quality data for algal pigments (chlorophyll; all phytoplankton) and phycocyanin (cyanobacteria) and two measurements water transparency (Secchi depth and total suspended solids). Our results show strong positive correlations between the normalized

difference vegetation index (NDVI) derived from drone imagery and all studied water quality parameters despite fairly large seasonal variation in slopes between NDVI and each studied water quality parameter that is likely mediated by the presence of phytoplankton deeper than the drone cameras are capable of measuring. Our results highlight the value of using drones to monitor surface water quality in aquatic systems for algal blooms that range widely in productivity but show that seasonal variation should not be overlooked considering that phytoplankton communities change in abundance and species composition over time.

2.08.T-02 Relative Sensitivity of Cyanobacteria to Copper

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Copper (Cu) is commonly used in aquaculture ponds to control cyanobacterial blooms. In Alabama, copper is often used when blooms become evident and there is not a strong understanding of how to use dosing to mitigate bloom development. To improve management plans to address harmful algal blooms, I carried out a number of studies characterizing the effects of copper on cyanobacterial species that are commonly found in Alabama catfish ponds. Three cyanobacteria: *Microcystis*, *Anabaena*, and *Oscillatoria* were isolated from a local catfish pond and culturing methods were developed. All three species were exposed to copper using a dose-response format ranging from 0-200 µg/L to identify decreases in cell density, impacts on growth rates, chlorophyll concentrations, and the phycocyanin: chlorophyll ratio. As these algal strains are grown in aquaculture ponds which have consistent copper exposure, *Microcystis* were compared to a lab strain (UTEX 3037). The results demonstrated that Cu affected biological performance of the algal species. Using chlorophyll-a concentrations to determine Cu toxicity, the 96-h EC50 values were 15.7, 19.8, 21.4, and 12.0 µg/L Cu for UTEX 3037, *Microcystis*, *Anabaena*, and *Oscillatoria*, respectively. Based on the results of cell density measurements, the 96-h EC50 values for UTEX 3037 and *Microcystis* were 15.6 and 19.9 µg/L Cu, respectively. These EC50 values indicate that both measurement endpoints gave consistent results. Among the algal species, *Oscillatoria* appeared to be the most sensitive to Cu. These results highlight the need for species-specific knowledge of how different species react to Cu pollution in order to effectively manage aquatic environments. The development of focused mitigation methods will be greatly impacted by the resolution of dose-response connections between Cu exposure and cyanobacterial populations as well as the validation of chlorophyll concentrations as a proxy for algal density. To formulate effective management methods that mitigate detrimental impacts of Cu contamination on cyanobacterial communities, more studies are necessary to fully understand the underlying processes regulating the observed responses.

2.08.T-03 Assessing the Influence of Phosphorus Availability on the Growth and Toxicity Dynamics of *Prymnesium parvum*

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Prymnesium parvum is a harmful algal bloom species that has caused great economic and ecological impacts. This study aims to explore the growth and toxicity of *P. parvum* across varying phosphate concentrations, a crucial yet underexplored aspect. *P. parvum* test cultures were inoculated under four sodium phosphate levels maintained constant throughout the study (5 µg mL^{-1}, 2.5 µg mL^{-1}, 0 µg mL^{-1}, and control (starting at 5 µg mL^{-1} but not maintained)). *Daphnia pulex* was used for assessing *P. parvum* toxicity (10,000 cells mL^{-1}) following EPA's guidelines for acute daphnia toxicity tests. *P. parvum's* growth significantly suffered under phosphate limitation (0 µg mL^{-1}). Despite this, the growth rate of cultures at 5 µg mL^{-1} and 2.5 µg mL^{-1} with constant phosphate addition surpassed that of the control. Nevertheless, they reached the stationary phase with significantly lower density. By day 6, 2.5 µg mL^{-1} and 0 µg mL^{-1} phosphate cultures exhibited the highest toxicity. By day 9, 0 µg mL^{-1} phosphate cultures reached the stationary phase and became significantly more toxic than all treatments. On days 12 and 15, all treatments were significantly less toxic than the control, suggesting that the addition of phosphate may alleviate nutrient stress. These results confirm the complex interactions between phosphorus availability and *P. parvum's* growth and toxicity, emphasizing the importance of exploring alternative approaches for mitigating *P. parvum's* harmful blooms.

2.08.T-04 Using Multiple Linear Regression (MLR) to Optimize Copper Sulfate Pentahydrate Dosages for Controlling Harmful Algal Blooms

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Copper sulfate pentahydrate has been used extensively over the last century to control harmful algal blooms (HABs) in aquatic systems; however, its application can cause negative effects on community structure and function due to its non-selective nature. Traditional copper dosing methods are based on the total alkalinity of a waterbody; however, this method was based on observational studies, not empirical data. This study aimed to develop a novel, predictive multiple linear regression (MLR) model that can be used to determine an optimal algicidal dose that minimizes non-target effects on the overall aquatic ecosystem. This model was developed from a series of comprehensive controlled laboratory bioassays relating key water quality parameters such as pH, hardness, alkalinity and dissolved organic carbon (DOC) to algal toxicity. Rigorous testing found that DOC and pH were the only significant predictors of algal toxicity (R^2=0.816, P <0.0001). A field-based validation of the model was conducted using a replicated, 28-day experiment in a functioning aquaculture pond located at the E.W. Shell Fisheries Station at Auburn University. Results from this experiment show that the MLR derived dose, which contained 60% less copper than the standard dose, resulted in identical control of harmful algae (95% reduction). In addition, the MLR dose caused less harm to the overall plankton community than the alkalinity-based dose. These results hold promise in the development of more sustainable water management practices that allow for harmful algal control while also preserving natural ecosystem function.

2.08.T-05 Effects of Glyphosate on the Growth and Toxicity of *Prymnesium parvum*

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Nutrient-rich runoff causes eutrophication, increasing the incidence of harmful algal blooms (HABs), such as those caused by *Prymnesium parvum*. Glyphosate, one of the most used herbicides worldwide, contributes phosphorus to the environment as it degrades. To investigate whether the phosphorous input from glyphosate alters the growth of *P. parvum,* we exposed cultures to four glyphosate concentrations (0 (control), 0.1, 0.25, and 0.5 $\mu g\ L^{-1}$) in each of two media types, P-sufficient (f/2) and P-limited. *Daphnia pulex* were used to assess *P. parvum* toxicity (10,000 cells mL^{-1}) following EPA's guidelines for acute daphnia toxicity tests. In P-limited media, cultures at 0.5 $\mu g\ L^{-1}$ showed consistently higher growth compared to control on days 3 and 6. However, contrary to P-sufficiency, at the stationary phase (day 14), cultures at 0.1 $\mu g\ L^{-1}$ had the highest density. Growth rate did not vary significantly in P-sufficient media, but under P-limitation, 0.1 $\mu g\ L^{-1}$ exhibited the highest growth rate. The lowest maximum density achieved was in P-sufficient cultures at 0.1 $\mu g\ L^{-1}$ glyphosate, but this treatment achieved the highest density in P-limited media. Toxicity was consistently lower for cultures at 0.1 $\mu g\ L^{-1}$ in both media types. Our results underscore glyphosate's impact on *P. parvum's* growth and toxicity, particularly in nutrient-imbalanced environments. Understanding these interactions is key for targeted mitigation against HABs in aquatic ecosystems.

2.08.T-06 Comparative Lung Toxicity of Size-Fractionated Cyanobacterial Particulate Matter

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The global expansion of harmful algal blooms (HABs) poses an increasing threat to public health. While the aerosolization of toxins from marine HABs (red algae) have been well documented, little information is available on the inhalation of aerosolized cyanotoxins in association with particulate matter (PM) in freshwater systems and their subsequent health outcomes. Thus, it is important to determine whether aquatic cyanobacteria can be aerosolized and how inhaled cyanobacterial PM affects the lungs. In this study, we collected size-fractionated PM (fine and coarse) from three different freshwater lake/rivers during periods of rapid HAB growth: Chowan River, North Carolina (NC Coarse and NC Fine), Lake Erie, Ohio (OH Coarse and OH Fine), and Discovery Bay and Stockton, California (DB Coarse and ST Coarse). PM samples were chemically analyzed and assessed for lung toxicity and lung function in CD-1 mice via oropharyngeal aspiration (100 μg of PM). Results showed that coarse PM had relatively higher organic carbon and inorganic element contents than fine PM. On an equal mass basis, DB Coarse and OH Coarse had the highest microcystin content and elicited the greatest pro-inflammatory responses in the lungs (neutrophil influx, increased protein and cytokine levels) and significantly reduced lung function (increase in airflow obstruction) at 4 and 24 h post-exposure. NC Coarse and OH Fine significantly increased lung inflammation at 4 h post-exposure but other biological and physiological endpoints remained unchanged. NC Fine and ST Coarse had the lowest microcystin content and increased (but not significantly) cytokine levels and did not change lung function at either 4 or 24 h time point. These results show that HAB aerosols from freshwater systems can generate contrasting chemical and cyanotoxin spectra depending on their size fraction and inhalation of airborne cyanobacteria and PM mixtures can cause acute respiratory problems. Our findings can be used to fill a gap in current HAB knowledge and public safety education. *This abstract does not represent U.S. Environmental Protection Agency policy.*

2.09.P-We-070 Introducing the Paint Library Of Plastic Particles (PLOPP) for FTIR: a Tool for Improving the Identification of Paint Microplastics

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The degradation of paint is thought to be one of, if not the largest, source of microplastics in the environment. However, researchers lack paint-specific spectral libraries that represent a wide variety of paint sources (e.g., roads, boats, buildings) to characterize paint microplastics in the environment and differentiate paint microplastics from non-paint microplastics. Here, we help fill this gap by creating a Fourier transform infrared spectroscopy (FTIR) library of 263 spectra from 90 paints using attenuated total reflection (µATR-FTIR) – called here a Paint Library of Plastic Products (PLOPP). The paints in the library were sampled directly from paint cans, tins, and jars and are primarily used in seven sectors (architectural, automobile, general industrial, marine, other, road marking, and wood). Paints are in 15 colors and five appearances (glitter, gloss, matte, pearl, and semi-gloss). With the library, we are also working on a spectral analysis pipeline using machine learning to help with the accurate identification of paint vs. non-paint microplastics. To aid in microplastic characterization as paints or non-paints, we will also create a visual key for paint microplastic identification. Using environmental microplastics collected in Plymouth, United Kingdom, we will verify PLOPP's utility to characterize paint microplastics. This research creates a spectral library, data pipeline, and visual tools to further our understanding of paint microplastics.

2.09.P-We-071 The Environmental Fate of Marine Paints: A Study in Metal and Polymeric Particle Release

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Marine antifouling paints used on shipping vessels can release potentially toxic metallic and polymeric particles to the aquatic environment when exposed to environmental stressors such as UV irradiation and freeze-thaw cycles. The release of the smallest fractions of metal and polymeric particles is not well understood due mainly to analytical limitations. By using a variety of novel techniques, we can characterize and quantify the particles being released from weathered painted surfaces. In our study, steel coupons painted with marine antifouling paint were submerged in water and exposed to laboratory-controlled weathering conditions (UV irradiation, freeze-thaw and a combination). The resulting paint leachates were sampled biweekly for six weeks. The paint leachates were then analyzed and quantified using Optical Photothermal Infrared (O-PTIR) spectroscopy, Nanoparticle Tracking Analysis (NTA), and Single Particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS). Results showed that large polymeric particles were present in conditions exposed to freeze-thaw, which could be identified positively using O-PTIR. We expect to find low concentrations of metallic nanoparticles released from the paints; metals were most probably released in their ionic form at high concentrations. Our findings will give us a better understanding of the types and concentrations of particles that can be released into natural waters when painted surfaces are exposed to natural weathering conditions. This new knowledge on the potentially toxic materials released to the natural environment from antifouling paints will provide insights into the consequences of the extensive use of marine vessel paints.

2.09.P-We-072 Visualization of Barnacle Plate Morphology in Response to Petroleum- and Non-Petroleum-Based Materials using Microcomputed Tomography

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Biofouling, the unwanted attachment of organisms on submerged surfaces, costs industries billions of dollars annually and threatens coastal infrastructure, food security, human and environmental health. Barnacles are problematic biofouling organisms. Barnacles routinely settle on surfaces from floating plastic debris to the hulls of ships. Ships are often coated with petroleum-based materials like epoxy resins and plastics to protect underlying metals from corrosion and to manage biofouling; a primary constituent of epoxy coatings is bisphenol A (BPA), an endocrine disrupting compound (EDC) with known impacts in dysregulating calcium homeostasis. Other additives in plastics with endocrine disrupting effects, like phthalates and antimony, have also been shown to disrupt calcium homeostasis. It is not fully understood how these and other materials impact the calcification, morphology, and adhesion of barnacles. Utilizing microcomputed tomography (μCT), we analyzed barnacles attached to different surfaces to visualize and better understand interactions at the barnacle-substrate interface. Given that EDCs are common additives in both petroleum- and non-petroleum-based materials, we hypothesized that barnacles growing on these surfaces would result in altered plate morphology and degree of plate calcification. μCT results show that petroleum-based materials are bioactive and elicit responses in barnacle morphology including plate thickness, convexity, height, and angle of formation. Our results also demonstrate that a single barnacle reared on multiple bioactive surfaces can respond regionally to those surfaces, suggesting that barnacle physiology is impacted by the local diffusion of compounds from the material's surface. Overall, these findings suggest that barnacles exhibit phenotypic plasticity in response to substate type, and these responses may be localized to physical points of attachment. Understanding how barnacles respond to surfaces will fill knowledge gaps regarding basic barnacle biology and may help to provide insight necessary to advance technologies for biofouling management.

2.09.P-We-073 Microplastics from Paints Taste Like Food

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In 2017 we were surprised to discover that animals like corals and anemone that could only sense tactically and chemically treated plastic like food. More surprising was microplastics exposed to biofouling were less tasty than unexposed microplastic. We discovered that anemones ingested NIST standard preproduction pellets and egested different composition pellets at different times. Pellets lost their flavor with repeated re-feeding of the same pellet and anemones could extract metal additives like lead and tin. Aqueous extracts of plastics dried on combusted glass fiber filters were retained 4 times as long as filters with vehicle alone. Preliminary LC MSMS revealed that water extracts of everyday plastics contained 50 to over 150 compounds. Any wine or spirits drinker knows metals and organic compounds like hydrocarbons have flavor and that complex mixtures are tasty. In addition to taste, many extracts of plastics are toxic at surprisingly low concentrations. Here, we use anemones and test their responses to microplastics from common paints. Our bottom line is microplastics are polymer platforms for the delivery to and removal of chemicals from consumers' bodies.

2.09.P-We-075 Painting by the Numbers: Quantifying Paint Particles and Other Microplastics in Roadway Adjacent Salt Marshes Subject to Nuisance Flooding

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In Charleston, South Carolina, nuisance flooding associated with extreme high tides has become increasingly more frequent over the past 50 years. In addition to public health concerns, increased frequencies of flooding can put unexpected strain on city infrastructure and increase human exposure to various microplastic particles. Studies in Charleston Harbor have shown that stormwater and floodwater may be important pathways by which microplastics are transported to estuaries. The overarching goal of this project is to identify and quantify the microplastics present in local roadways adjacent to salt marshes subject to frequent nuisance flooding to characterize these pathways and their exposure to salt marsh biota. Salt marshes adjacent to three street locations which frequently experience nuisance flooding were selected for sampling: Hagood Avenue and Johnson Street on the Charleston peninsula were used as test sites based on high daily traffic, and Rosemead Road in Mount Pleasant was used as a control site based on low daily traffic. Sediment samples were collected using a transect design in triplicate. Microplastics were extracted using a two-step NaCl/Na-polytungstate density separation procedure to remove particles with densities ≤1.9 g/mL. Particles were enumerated under a microscope and color/type of particle were recorded. Suspected microplastics were confirmed using a hot needle test, and a subset of particles were tested for polymer type using Fourier Transform Infrared Microscopy (FTIR). It was found that tire and road wear particles (TRWP) made up between 20 and 40 percent of all microplastics found in all three of the sample locations. An unexpected number of bright yellow microplastic fragments (between 7 and 23 percent of total particles) were also found at the Hagood Avenue testing site. When a subset was tested using FTIR, it was found that the spectra of these yellow particles matched that of the yellow road marking paint present on Hagood Avenue, suggesting that the road paint was degrading and transported into the marsh sediments. Road paint was also found to account for between 4 to 25 percent of microplastic particles found at the Johnson Street sampling site. Assessing transport and fate are critical first steps toward a comprehensive understanding of the environmental risks associated with the interactions between climate change and microplastic pollution.

2.09.V Paint Microplastics: Sources, Fate, and Ecotoxicological Effects in Aquatic Ecosystems

2.09.P-We-076 Construction Projects: A Substantial Point Source of Microplastic Pollution of Land and Water

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Construction workers consume water for trade related tasks. This water, when used for tasks such as tool washing, becomes contaminated with various construction materials including applied finishes like paint, plaster, render and adhesives. Some of these construction materials contain microplastics and some will become plastic films when partially or totally dry on tools during application. When these products and washed from tools, the wash water released contains microplastics. The session will delve into how the construction industry handles wash water from trades activities, including exploring the volumes generated and the potential environmental impact. We will analyze available data on the type and level of microplastic contamination in the waste material, shedding light on the industry's role as a potential significant point source of microplastics to the environment. Drawing on verifiable industry data, testing results from wastewater samples, and expert opinions, we will assess current practices in construction liquid waste management and evaluate the effectiveness of various mitigation methods. Additionally, we will examine innovative approaches and best practices for reducing wash water generation and promoting responsible waste management practices. The session will also address the limitations of wastewater treatment plants in removing microplastics from construction wash water, as well as how microplastics emerge in the wastewater treatment plant outputs and their environmental impact. Preliminary findings suggest that conventional liquid waste management practices for trades activities significantly contribute to microplastic pollution of water bodies and landmasses. This underscores the urgency of addressing this issue as the construction industry plays a crucial role in global microplastic pollution. Given the imperative to transition to net zero and nature-positive practices in construction to address climate and urbanization challenges, this topic is of paramount importance. The session aims to raise awareness, facilitate knowledge exchange, and identify actionable strategies to minimize the industry's environmental footprint and promote sustainability.

2.10.P-We Stormwater Runoff Impacts, Solutions, and Innovative Research

2.10.P-We-077 Particle Size-Based Assessment of Stormwater Control Measures to Limit Sediment Recontamination of Hydrophobic Organic Contaminants

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Stormwater runoff is a major non-point source of water and sediment pollution. Stormwater Control Measures (SMCs) can help reduce runoff and contaminant loads to mitigate this issue. Historically, stormwater assessment has focused on total contaminant loads to receiving waters, with little attention paid to the particle-size distribution of contaminants—critical information necessary to assess their fate in receiving water bodies. The current study focuses on better understanding different SMCs' performance in reducing particle-size-based loads of Hydrophobic Organic Contaminants (HOCs), specifically Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs) and their implications for receiving water systems. Several SMCs were sampled, including Biofilters, Media Filters, a Hybrid Biofiltration

+ Media Filter system, Hydrocyclones, and a Retention Pond. Contaminant concentrations were then measured in the selected particle-size fractions and the filtered water for each system's inlet and outlet. Passive sampling was also used to determine the freely dissolved contaminant concentrations. Stormwater intensity, volume, and rain characteristics were measured or estimated to calculate the contaminant loads pre-and post-treatment. Collected pollutant data was combined to statistically determine the removal efficiency of similar technologies as a function of the particle association of the contaminants. The analysis revealed that coarse sediments (>20µm) have a significantly higher fraction of organic carbon (f_{oc}), contrary to common belief. These particulates also contain higher concentrations of HOCs. Most SMCs effectively remove these contaminated coarse particles that would normally settle near receiving water bodies. However, dissolved contaminants and those associated with fine solids were generally not effectively removed in most systems, except for the hybrid system. The performance of the various stormwater management systems was summarized and contextualized with recontamination risks in the receiving water systems, providing valuable insights for future research and practical applications.

2.10.P-We-078 Characterizing Stormwater Runoff from Various Land Uses in Heavily Urbanized South Florida Watersheds

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The Biscayne Bay watershed is a heavily managed system of canals and rivers that serve as flood protection for Miami Dade County that discharges stormwater into Biscayne Bay through several important tributaries. For the last several decades there has been a decline in the water quality of Biscayne Bay regarding contaminants and excess nutrients which has worsened as urbanization continues to increase. Multiple pollution sources contribute to this decline such as stormwater runoff, groundwater intrusion, and sewage and septic influence. Of these, stormwater inputs are one of the least studied. Due to its episodic behavior and overall lack of regulatory framework, stormwater is hard to characterize systematically and long-term. It is critical to understand the overall anthropogenic chemicals emitted from stormwater outfalls and to assess how they are contributing to the overall pollution of the main tributaries and the Bay itself. In this study, we analyzed stormwater among several rivers and canals to determine differences in contaminants between stormwater and canal water and between stormwater sites based on various land uses. Nontargeted analysis was conducted by online solid phase extraction high pressure liquid chromatography mass spectrometry on a Q-Exactive Orbitrap (SPE-HPLC-HRMS). Compound Discoverer 3.3 was used to process HRMS data, broadening our understanding of the difference in chemical makeup from one stormwater outfall to the next. Kendrick Mass Defect (KMD) plots were used to visualize the chemical space among water sources. Principial Component Analysis (PCA) plots were used to statistically compare stormwater sites and compare stormwater to canal water using compound's intensity and features. Stormwater runoff had 618 features while surface water had 409. KMD plots showed unique features among surface water and stormwater sites and PCA plots revealed compound and intensity distinctions between surface water and stormwater. Pharmaceuticals, corrosion inhibitors, rubber products, pesticides, detergents, and personal care product compounds have been tentatively identified as having the highest intensity among most stormwater samples from January 2024.

2.10.P-We-079 Developing Analytical Protocols for 6PPD-Q in Natural Seawater

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6PPD-quinone (6PPD-Q), as an oxidized product of 6PPD, is highly toxic, and was found to be responsible for the acute mortality of coho salmon (*Oncorhynchus kisutch*) in the Pacific Northwest. However, analyzing 6PPD-Q in natural waters can be challenging due to its amphiphilic properties and the interference with natural particles and dissolved organic matter. In this study, we sought to develop analytical protocols aimed at enhancing the extraction efficiency and analysis of 6PPD-Q in natural seawater. Several variables were tested to observe their effects on solid phase extraction (SPE) and LC-MS analysis in seawater samples, including the type of SPE cartridge, filter size, and salinity. Also tested were the effects of solvent (ACN vs DMSO), freezing and thawing of DMSO, sonication, and bottle type (plastic vs glass) on the measured concentration of 6PPD-Q. For seawater samples, it was observed that Bond Elut C18, Bond Elut PPL, and Oasis Prime HLB delivered better recovery rates of 6PPD-Q than Bond Elut C8 and Oasis Prime MCX, and that pre-filtration through 0.2µm filters helped the extraction speed without much loss of 6PPD-Q to particles. Salinity did not significantly affect the extraction efficiency of 6PPD-Q. We recommend sonicating as well as freezing and thawing samples in DMSO, as these techniques improved recovered 6PPD-Q concentrations as compared to samples with no sonication or those that were not frozen and thawed. Additionally, the measured concentration of 6PPD-Q was higher when using glass bottles, and when using DMSO instead of ACN at initial stock solution concentrations of 500 ppb, although the opposite was true for both variables at 50 ppb. Furthermore, recommended instrumental conditions are suggested, such as mobile phase, column, m/z ratio, and linear range. Overall, these evaluations offer multiple angles to more effectively quantify 6PPD-Q in natural seawater samples.

2.10.P-We-080 Best Management Practices for 6PPD-Quinone Stormwater Mitigation: Systematic Review

Sara Hutton*, Casey R. Remmer, Emily E. Smith and Kenia Whitehead, GSI Environmental*

Over the past 20 years, Coho salmon in the Pacific Northwest (PNW) of the United States have experienced an increase in pre-spawn mortality; a phenomenon in which adult Coho salmon, returning to freshwater rivers to spawn would develop sporadic swimming behaviors, gapping, and eventual mortality. Pre-spawn mortality was eventually correlated with rain events and the release of unfiltered stormwater into receiving waters and was subsequently termed Urban Runoff Mortality Syndrome (URMS). In 2021, a degradation product of the rubber anti-ozonate N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) was identified as the primary pollutant responsible for URMS. Since the discovery of the link between this oxidation product, 6PPD-quinone (6PPDq), and URMS, there has been a surge in research focusing on the toxicity, chemistry, fate & behavior, sources, and mitigation strategies of 6PPDq. Alternatives to 6PPD for use in tires and other rubber products are being urgently studied, but the replacement of 6PPD in tires and rubber products will take time. Stormwater best management practices (BMP), designed to reduce contaminants from stormwater before they reach receiving waters, are currently the most effective mitigation strategy to reduce environmental exposure to 6PPDq. As a reflection of the importance of management practices in 6PPDq mitigation, this has been an intense area of study. Here we present a comprehensive review and meta-analysis of existing BMPs that effectively treat 6PPDq in stormwater. Both field and lab-based treatments of 6PPDq in stormwater are discussed, including biofiltration and porous pavements. Abiotic factors relevant to BMP selection, maintenance, and efficacy will also be considered. This review provides information necessary for stormwater managers, aquatic conservationists, and researchers to act quickly to protect Coho salmon populations and aquatic ecosystems from the detrimental effects of 6PPDq contamination in stormwater runoff.

2.10.P-We-082 Chitosan Has the Potential to Improve Water Quality Without Negative Effects on the Stony Coral, Porites lobata

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Coral reefs off the coast of West Maui in Hawai‘i are frequently subjected to highly turbid water, caused in part by terrigenous sediment inputs from stream gulches after rain events. Sediment, and its associated contaminants, have numerous deleterious consequences on scleractinian corals, including impacts on growth and survival. The West Maui Watershed Management Plan includes recommendations to reduce sources and conveyance of land-based pollution to increase resiliency of coral reefs. This study investigated the use of the flocculant, chitosan, for potential use near sensitive coral reef ecosystems. In a laboratory-based experiment, exposure to chitosan did not impact growth of *Porites lobata* during a 30-day experiment. Additionally, the presence of chitosan significantly reduced turbidity in the test chambers within one hour of its addition in both seawater and freshwater. These data suggest that chitosan may not negatively impact one of Hawai‘i’s commonly found coral species. This study provides the foundation for further research investigating the ecosystem effects of chitosan when used as a potential mitigation action to reduce sedimentation in West Maui’s coral reefs.

2.10.P-We-083 Wipes Versus Pipes: The Trouble with Wet Wipes When Stormwater Runoff Causes Overflow in Combined Sewer Systems

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Wet wipes are one of the most common types of plastic pollution in urban waterways. When wipes are inappropriately flushed, they can create a direct pathway for plastic to enter local waterways, leaking from combined sewage overflows (CSOs) during storm events. Our overarching research objective is to inform prevention of plastic wet wipes in the environment by: (1) increasing our understanding of wet wipe packaging and proper disposal, (2) determining current emissions into the environment, and (3) assessing the degradation of wet wipes into microfibers. To understand the wet wipe market, and how and whether customers receive information about proper disposal, we conducted store surveys across six supermarkets. Wet wipe packages were recorded by brand, intended use, composition, and disposal information. Using Toronto, Canada, as a case study, wet wipe emissions were calculated using empirical data from field surveys in the Don River, one of the most urbanized rivers in Canada. The shedding of wet wipes into microfibers was measured in an experiment where we exposed wet wipes (made of polypropylene, polyester, and cellulose) to different environmental conditions. This presentation will share the results of our study. Briefly, store surveys showed wet wipe packages lack information on disposal and composition, potentially leading to improper disposal. In the field, 25.7% of all observed plastic waste was wet wipes, with hotspots concentrating around CSOs. Almost all wet wipes collected were plastic (i.e., polypropylene or polyester). Microfiber release tests demonstrated shedding from wet wipes likely occurs in nature and thus, wet wipes are a source of microfibers to the environment. Shedding was generally most influenced by the presence of water and mixing, conditions similar to those found within rivers. These findings can be used to advance policy targeting wet wipe pollution, including whether to mandate “Do not flush” labelling, regulating companies to prove International Water Services Flushability Group (IWSFG) flushability certifications, and/or substituting the materials in wet wipes to those that are not plastic. Mobilized through flood events, plastic is an important stormwater contaminant and should be monitored to inform prevention and mitigation.

2.10.P-We-084 Leveraging Multi-Omics Analyses to Explore the Toxicity of Urban Road Runoff Contaminants in Juvenile Salmonid Species

Miranda Elizabeth Jackson[1], *Chloe Fender*[2], *Stacey L Harper*[2] *and Manuel Garcia-Jaramillo*[2], *(1)Environmental and Molecular Toxicology, Oregon State University, (2)Oregon State University*

Urban roadway runoff is correlated to escalating pre-spawn mortality (PSM) events in Pacific Northwest salmon populations. Recently, a chemical derived from tires, N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-q), was isolated from roadway runoff and observed to induce acute mortality in salmonids, however sensitivity among salmonid species is varied. It is predicted that exposure to 6PPD-q will enhance salmonid sensitivity to other cooccurring contaminants commonly detected in surface waters, such as the polycyclic aromatic hydrocarbon 9,10-Anthraquinone (AQ). To determine sublethal concentrations, juvenile salmonid species (chinook, coho, and rainbow trout) were exposed to a range of concentrations of 0 – 10 µg/L 6PPD-q for 24-hours. A targeted multiple reaction monitoring (MRM) method was developed in a triple quadrupole mass spectrometer, coupled to an ultra-high-performance liquid chromatography system to quantify 6PPD-q and AQ in water samples. To better understand the mechanisms of toxicity of 6PPD-q, fish were exposed to established sublethal concentrations of 6PPD-q and AQ separately and in combination over a five-day period. AQ and 6PPD-q quantification in water tanks were validated and measured within a 15% margin of error. Coho and rainbow trout exhibited PSM symptoms leading to mortality at 6PPD-q concentrations after 24-hour exposure to 0.01 µg/L and 10 µg/L, respectively. PSM symptoms and mortalities were observed in coho and rainbow trout, but not chinook. Non-targeted mass spectrometry-based metabolomics analyses were performed on brain and liver samples from exposed and non-exposed fish. Using in-house and open-source spectral libraries we annotated 290 and 260 metabolites in the brain and liver tissues, respectively, with high level of confidence. A larger number of significant changes were observed in the liver tissues within each specie when compared to the brain tissues (Wilcoxon rank sum test, $p < 0.05$). Fatty acid biosynthesis was the pathway most significantly affected by 6PPD-q exposure in coho salmon, as revealed by metabolic pathway enrichment analysis. QuantSeq 3’ mRNA-sequencing transcriptomics analysis will be integrated with the metabolomics data. This study aims to clarify the impact of prioritized urban road runoff contaminants on salmonids health, contributing to a deeper understanding of the observed variations in toxicity among different salmonid species and mechanisms of action.

2.10.P-We-086 Metal Speciation in Stormwater Control Measures to Limit Sediment Recontamination of Heavy Metals

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Stormwater runoff causes significant sediment recontamination as a non-point source, especially in historically contaminated urban areas. Stormwater Control Measures (SCMs), such as retention ponds, bio-filters, media filters, and hydro cyclones help reduce the risks of sediment recontamination in the surrounding area. In this work we focus on the fate and speciation of heavy metals in SCMs based upon measurements in the field. Previous studies have shown that heavy metal contamination is related to particle sizes in stormwater runoff. However, different metals exhibit varying behavior in terms of association with solid and subsequent removal in SCMs. To gain a better understanding of the transport and fate of heavy metals in stormwater runoff, metal speciation was used to analyze the form of metal in the runoff and its ability to partition to particulates. The results were then compared with the removal of metals based on size-based removal efficiency analyses. The results from metal speciation analysis can be further applied to the Bio-Ligand model to evaluate the ecological risks of stormwater to the environment, suggesting a new way of evaluating the connection between ecological risks and particle sizes in SCMs.

2.10.T Stormwater Runoff Impacts, Solutions, and Innovative Research

2.10.T-01 Time-Course and Latency of Toxicity of 6PPD-Quinone to Three Salmonids and a Centrarchid

David J. Soucek[1], *Rebecca Dorman*[1], *James Candrl*[2], *Olivia Watt*[2], *Jeffery Steevens*[1], *Rachael Lane*[1], *Justin Greer*[1] *and John Hansen*[3], *(1)U.S. Geological Survey, (2)U.S. Geological Survey, CERC, (3), Western Fisheries Research Center, U.S. Geological Survey*

The tire rubber antioxidant, N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine, or 6PPD and its transformation products (TP), including 6PPD-quinone (6PPD-q), are frequent contaminants found in storm water runoff, the latter having been identified as the causative factor in "Urban Runoff Mortality Syndrome" in Coho Salmon (*Oncorhynchus kisutch*). Subsequent studies with commercial standards of 6PPD-q confirmed the extreme sensitivity of *O. kisutch* to this compound, with 24-h LC50s ranging from 41 to 95 ng/L. Some other salmonids, including Rainbow Trout (*Oncorhynchus mykiss*), Brook Trout (*Salvelinus fontinalis*), and White-spotted Char (*Salvelinus leucomaenis pluvius*) have been shown to be highly sensitive to 6PPD-q as well. Thus far, no species outside of the family Salmonidae has been shown to be sensitive to 6PPD-q at concentrations below the solubility limit of the compound. While several studies demonstrate the sensitivity of *O. kisutch, O. mykiss*, and *S. fontinalis* to 6PPD-q, the data are not all ideal for the development of Ambient Water Quality Criteria. Some data quality issues identified include inappropriate test durations, excessive fish loading rate, and insufficient analytical chemistry/variable 6PPD-q concentrations in static solutions. Our objective was to conduct 24- and 96-hour acute 6PPD-q toxicity tests with *O. kisutch*, *O. mykiss,* and *S. fontinalis*, and the previously untested Smallmouth Bass (*Micropterus dolomieu*) under pulsed flow-through conditions with fish that were three weeks post-swim up. This allowed us to meet fish mass loading requirements while maintaining stable toxicant concentrations, meeting data quality objectives outlined by USEPA, and comparing similar aged fish of different species. We recorded mortality hourly until 12 hours, then at 24 h intervals thereafter. At 96-h, we removed

the 6PPD-q and held fish in clean water (with feed) for 7 days to document potential latent mortality. We confirmed the sensitivity of *O. kisutch* and *S. fontinalis*, and observed that for these species, approximately 90 percent of mortalities occurred within the first 24 hours of the test. No latent toxicity was observed in the 7-day clean water depuration period. These data illustrate the ephemeral nature of the 6PPD-q effects and will assist with the development of protective criteria for sensitive species.

2.10.T-02 Habitat and Life Stage Impact Acute Lethality of 6PPD-Quinone to Coho Salmon

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Habitat and life stage can impact toxicokinetics and toxicodynamics of environmental pollutants for aquatic animals. The novel tire-derived chemical 6PPD-quinone is acutely lethal to juvenile coho salmon at environmentally relevant concentrations and is implicated in annual acute mortality events for adult coho salmon returning from the ocean to spawn in streams of the Pacific Northwest of North America. Regulations to protect coho and other sensitive fishes from 6PPD-quinone in receiving waters are being developed around toxicity tests conducted under standard laboratory conditions that do not capture the variability in habitat and life stage experienced by coho in the wild across their native range. To address this data gap, we conducted acute toxicity tests under varied water quality for four parameters that can alter contaminant bioavailability: temperature, pH, conductivity, and dissolved organic matter. We also tested whether fish activity altered toxicity. Finally, we tested acute toxicity under standard laboratory conditions for four life stages: embryo, alevin, fry, adult. All results were compared to survival for juveniles under standard laboratory conditions. Temperature was the only habitat parameter that affected lethality; coho were significantly less sensitive at the warmest temperature tested (20 °C). Embryos were invulnerable to 500 ng/L 6PPD-quinone pulsed twice weekly during development, whereas all other life stages were sensitive to that concentration. Dechorionated embryos were also sensitive, although mortality was delayed and was coincident with the timing of hatch for intact embryos. Life stage sensitivity was in the order of embryos < alevin < fry < parr = adults. These results suggest that regulations to protect coho salmon from 6PPD-quinone across most of their range can rely on tests conducted with juveniles under standard laboratory conditions.

2.10.T-03 Tracking 6PPD-Q Concentration Dynamics in Coho Salmon-Bearing Streams Following Rain Events

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Urban runoff mortality syndrome in coho salmon (*Oncorhynchus kisutch*) is attributed to 6PPD-Quinone (6PPD-Q) and is associated with watersheds containing high densities of roadways. Our research is revealing that, during rain events, average 6PPD-Q concentrations in salmon-bearing streams within the intensively urbanized metropolitan Vancouver area typically exceed established acute lethality concentration thresholds, although the duration over which levels remain elevated is currently unknown. We have conducted continual rain event sampling and hydrological measurements in coho salmon-bearing streams throughout the metropolitan Vancouver area. Concentrations of 6PPD-Q were measured in stream water samples collected continuously from baseflow under dry conditions (≥3 days negligible rain) though the first rainfall and ensuing stormflow for up to three days. Results indicate that, following an initial 5% increase in stream depth from baseflow, time-weighted average 6PPD-Q concentrations in water generally remain above pre-rainfall concentrations (95% CI) for three days. Concentration time series of 6PPD-Q correspond to hydrograph dynamics, with maximum first-flush concentrations analyzed to date ranging from 74.6 to 99.7 ng/L. Time-weighted average concentrations exceeding published LC50 values for juvenile coho are observed for up to 24 hours (43.4 ng/L) during rainfall events. This ongoing research will provide more detailed insights regarding risks to coho and other salmonid species in coastal British Columbia.

2.10.T-04 Transport and Fate of 6-PPD Quinone in a Full-Scale Stormwater Bioretention Pond

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Tire wear particles have associated pollutants such as the antiozonant N-phenyl-N'(1-3-dimethylbutyl)-p-phenylenediamine (6-PPD) whose transformation product 6-PPD-quinone (6-PPD-Q) was discovered to have adverse effects towards some salmonids critical to tribal subsistence and culture. It is currently unknown whether stormwater management features such as hydrodynamic filter systems and retention ponds are conduits of these pollutants or if they can be employed as effective mechanisms to manage the risks of tire-related pollutants. This study examines the fate and transport of 6-PPD-Q in an urban stormwater retention pond that is equipped with an up-flow hydrodynamic filter system and discharges into an estuarine ecosystem (Bayou Chico, Pensacola, Florida). We hypothesized that if the transport of 6PPD-Q is highly associated with sediment and roadway particles (SPM), such management systems would be a viable option to reduce and/or prevent the impact of 6-PPD-Q on our water systems. Influent and effluent water samples collected from a total of 10 storm events were analyzed for 6-PPD-Q concentrations (ng/L) using the EPA Draft Method 1634. Average concentrations of all influent samples were 127 (±55.4) ng/L, 3 times higher than the lowest reported LD_{50} values (40 ng/L) for Coho Salmon. Filter effluent average concentrations of were 8.5 ng/L (±7.2), with a reduction of overall 6PPD-Q concentrations into the receiving estuary by over 90%. Water samples were also analyzed in the pond at surface and bottom 4-10 hours before and after a single storm event to evaluate the possible photodegradation of 6PPD-Q in the pond. The results showed little difference in 6PPD-Q concentrations between storm effluent (6.3 ng/L), surface water (5.8 ng/L), and bottom water (6.3 ng/L; approx. 3m depth). Given that total suspended sediment in influent water from all storm events (avg. 26.9 (±20.1) mg/L) is much higher than the respective effluent (approximately 6.3 (±6.5) mg/L), our results suggest that the up-flow hydrodynamic filter system functioning to retain the majority (>80%) of the particulates in stormwater also plays a key role in retaining 6PPD-Q, indicating this is a viable option for efficient mitigation and source control strategies to reduce 6PPD-Q impacts on aquatic ecosystems. These results are the first report on the effectiveness of a hydrodynamic filter system-assisted stormwater bioretention pond in reducing 6-PPD-Q in stormwater runoff.

2.10.T-05 The Influence of Saltwater Intrusion on PFAS Release from AFFF-Contaminated Aquifer Solids

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Understanding the impact of stormwater runoff on per- and polyfluoroalkyl substances (PFAS) release from AFFF-contaminated aquifer solids is vital for effective management of contaminated sites. Stormwater runoff presents significant challenges in environmental management due to its diverse pollutant mix, including PFAS. This study employs meticulously designed saturated column experiments to simulate real-world conditions and understand PFAS behavior in the presence of stormwater. By constructing columns with varying mixtures of sand, clay, and organic carbon, we replicated natural vadose zone soils. We introduced diluted AFFF solutions to mimic infiltration scenarios and eluted the columns with stormwater-simulating solutions. Additionally, we investigated electrostatic interactions with clay minerals, including iron, and assessed the impact of electrochemical fluorination (ECF) and fluorotelomer-based AFFF on PFAS release. Our findings reveal that perfluoroalkyl carboxylate anions at environmentally relevant pH values experience faster release regardless of water matrix salinity. We observe that while saltwater slows down the release of zwitterionic PFAS with terminal negative charges, it accelerates the release of those with terminal positive charges and causes a preferential release of branched PFAS. This research sheds light on how stormwater influences PFAS release dynamics based on their chemical properties, offering insights crucial for devising management strategies to address PFAS contamination in AFFF-impacted sites, particularly in the context of stormwater runoff. The findings of this study underscore the importance of interdisciplinary collaboration in tackling the multifaceted challenges posed by stormwater pollutants and their impacts on contaminated sites.

2.10.T-06 Stormwater Quality and Quantitative Microbial Risk Assessment in Southern California

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Southern California is known for its stunning shorelines and warm weather, even in winter, where beaches attract 250 million beach-goers annually. However, every time it rains, Public Health Officers warn swimmers to stay out of the water due to high bacteria levels despite sanitary sewers and storm drains being completely separate (i.e., no combined sewer overflows). The goal of this research is to measure indicator bacteria, human fecal source markers and human pathogens across the entire southern California region (approximately 5,900 km^2 and 28 million people). A quantitative microbial risk assessment (QMRA) was used to assess health risk. More than 30 sites were sampled during at least two storm events between 2021-2023. Indicator bacteria nearly always exceeded standards, the human specific fecal marker HF183 was quantified in 90% of the samples and at least one human pathogen such as Norovirus, Adenovirus, Campylobacter, and Salmonella was detected in up to 90% of the samples. There was a statistically significant but variable concentration relationship between the indicators and the pathogens. The QMRA identified that there was a median probable risk of about 140

additional gastrointestinal illnesses per 1,000 exposures based on the concentrations of pathogens, pathogen-specific dose-response functions, and volume of water ingested by swimmers. Campylobacter and Norovirus were the primary pathogens responsible for this increased risk. Approximately 10-fold stormwater dilutions with pathogen-free receiving waters would provide a risk level comparable to the US EPA's acceptable median risk level of 32 illnesses per 1,000 exposures. A risk-based threshold was proposed for the human-specific fecal marker HF183.

Track 3: Toxicology, Ecology and Stress Response

3.01.P-Mo Beyond the Deepwater Horizon: Recent Wildlife Petroleum Ecotoxicology Research and the Path Ahead

3.01.P-Mo-039 Effects of In Ovo Chrysene and Phenanthrene Exposure on Chicken Embryo Development and Cardiac Function: Is There Evidence for Synergism?

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Polycyclic aromatic hydrocarbons (PAHs) are naturally occurring toxic chemicals found in crude oil and are known to transfer from the external eggshell surface to egg contents. Previously, we conducted an egg-injection study with White Leghorn chicken (*Gallus gallus*) eggs and identified two PAHs, chrysene (Chr) and phenanthrene (Phe), that increased embryonic heart mass and decreased embryonic heart rate. In this study, we investigated whether co-exposure to Chr and Phe resulted in additive or synergistic effects on chick embryo development. Chicken embryos were exposed to Chr (800 ng / g of egg mass), Phe (800 ng / g egg mass), and Chr and Phe in combination (∑ PAH 1600 ng /g ng egg mass) via egg-injection, and we collected embryonic organ mass, heart rate, metabolic rate, and cardiac and hepatic mRNA expression of detoxification enzymes on embryonic day (ED)18. We observed a decrease in ED 18 heart rate across all treatments. We also saw an increase in ED 18 liver mass in eggs exposed to Chr and Phe simultaneously, and shifts in metabolic rate and mRNA expression of cardiac detoxification enzymes. However, embryonic growth or morphology did not vary among treatments. Collectively, these data suggest *in ovo* exposure to PAHs may lead to congenital heart defects, which may have long-term implications for hatching success and hatchling survival.

3.01.P-Mo-040 Exposure to Individual Polycyclic Aromatic Compounds Impairs the Cardiac Performance of American Lobster (*Homarus americanus*) Larvae

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The periodic occurrence of oil spills poses potential threats to marine organisms. The toxicity of oil has been primarily attributed to polycyclic aromatic compounds (PACs) which are widely known to cause cardiotoxicities including bradycardia and arrythmia in vertebrates. However, this is not well characterized in crustaceans. Predictive tools such as the target lipid model (TLM), a quantitative structure-activity relationship that can predict the acute toxicity (LC50) of a PAC based on the logK_{OW}, have been developed to assess these risks, but few studies have applied the TLM framework to cardiac endpoints. As a step towards better understanding the toxicity of PACs in crustaceans the cardiotoxicity of 10 structurally diverse PACs was assessed in American lobster (*Homarus americanus*) following a 48-hour exposure using a passive dosing system. Larvae were exposed to 5 concentrations of each PAC, a clean O-ring control, and a seawater control. Exposure to carbazole, dibenzofuran, fluoranthene, fluorene, 9-methylanthracene, 1-methylnaphthalene, naphthalene, and phenanthrene caused bradycardia and arrythmia in a concentration-dependent manner, similar to effects observed in vertebrates. Exposure to acridine and dibenzothiophene did not affect either endpoint. The TLM framework was then used to calculate a critical target lipid body burden (CTLBB) for bradycardia in lobster larvae which is among the most sensitive endpoints included in the CTLBB database at 12.5 ± 27.8 μmol/$g_{octanol}$. This is among the first applications of the TLM to a cardiac endpoint and contributes to improving predictive models for assessing sublethal impacts of oil spills on early life stages of American lobster.

3.01.P-Mo-041 Ecological Disturbance in the Anthropocene: Legacy Effects of Orphaned Wells on Vegetative Community and Metabolic Phenotype of Free-Living Rodents

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Persistent organic pollutants have been studied intently for the last thirty years, and many are known to be mutagenic, some carcinogenic. Among the most commonly studied environmental organic contaminants are polycyclic aromatic hydrocarbons (PAHs), 16 of which are listed by the United States Environmental Protection Agency (US EPA) as priority contaminants of concern. Many PAHs are found in crude oil and can remain in the environment long after crude oil spills have occurred. While catastrophic large marine oil spills receive considerable media attention, smaller-scale inland spills occur much more frequently, resulting in legacy PAH contamination. Oklahoma currently has 15,965 documented orphaned oil rigs that were operated under less regulatory oversight. Oklahoma Energy Resources Board (OERB) is working diligently to plug orphaned wells however, the toxic legacy effects on surrounding ecosystems are not well understood. Using two separate field sites in Cushing, Oklahoma, we targeted areas surrounding unplugged oil wells and will compare soil PAH concentrations to EPA soil screening levels (SSLs) and a reference site with no crude oil extraction. To assess site-specific disturbance, we will conduct vegetative surveys to generate Floristic Quality Assessments (FQAs). In accordance with data showing PAH exposure causes hematological damage, altered immune function, and shifts in organismal metabolic rates, we hypothesize legacy PAH contamination will lead to physiological differences in free-living Peromyscus populations inhabiting the sites. We will measure white blood cell differentials, packed cell volume, hemoglobin concentration, and organismal resting metabolic rate. Collectively, this data will allow us to evaluate legacy effects of unplugged oil wells on multiple ecosystem components.

3.01.P-Mo-042 Lingering Oil and Lasting Impacts: Prince William Sound 35 Years After Exxon Valdez

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The 1989 *Exxon Valdez* oil spill (EVOS) introduced 11 million gallons of crude oil to Prince William Sound (PWS), Alaska, and a multitude of studies have been conducted in the past three and a half decades to better understand the long-term fate and transport of the oil, as well its impacts on surrounding ecosystems and communities. The goal of this study was to review

relevant literature pertaining to lingering oil from EVOS within PWS to understand the nature, extent, and effects of lingering oil and evaluate the current state of marine species injured by EVOS. Socio-economic impacts related to EVOS were also investigated, with an emphasis on subsistence use and harvest of aquatic species. Evidence for long-term persistence of oil from EVOS on beaches in PWS was outlined by several studies; oil sequestration within sediment has contributed to a lack of degradation due to reduced bioavailability, resulting in beaches classified as “oiled” generally not changing in status in the last 20 years. Therefore, lingering oil is likely to persist within sediment unless disturbed and made bioavailable. Species recovery rates have varied, and environmental drivers independent of EVOS have added a layer of complexity to understanding the role EVOS has played in the certain species inability to recover, such as pigeon guillemots, marbled murrelets, and killer whales. Spill-affected communities have reported declines in subsistence use and harvest since EVOS, which has primarily been attributed to changes in local economies introduced by EVOS, as well as broader socioeconomic changes unrelated to the spill. Overall, the presence, location, and bioavailability of lingering oil within PWS should be considered in future monitoring efforts, and the extensive network of information on EVOS allows for invaluable insight into the dynamics of large-scale oil spills as well as understanding what factors can influence the long-term recovery of injured populations.

3.01.P-Mo-043 Characterizing Exposure Risks to Resident and Migratory Waterbirds at Oil Sands Liquid Impoundment Facilities in Northern Alberta

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The Athabasca oil sands region of northern Alberta is under a convergence of four major migratory flyways where frequent bird landings occur on holding ponds containing oil sands process-affected water (OSPW) also known as liquid impoundment facilities (LIFs). OSPW contains high levels of potentially toxic naphthenic acids (NA), polycyclic aromatic hydrocarbons (PAH) and heavy metals in complex mixtures. These sites may be an attractive migratory stopover or breeding site for resident and migratory waterbirds as they may be vegetated and the water is seasonally open longer due to the constant efflux of warm OSPW. The extent of toxicant exposure to transient and resident waterbirds through ingestion of contaminated water, sediment, plants, and aquatic invertebrates is largely unknown. We characterized 12 LIFs to determine if invertebrate and macrophytic plants occur as habitat and food resources. Passive samplers were also deployed for 14 days during spring to characterize contaminant levels in each LIF. Current and historical avian species abundance surveys have been completed daily from April – October from 2014-2022 and focal behavioral observation surveys were conducted from May to July (2023 and 2024) weekly on each of the LIFs and nearby reference freshwater ponds. Historically, California Gulls (*Larus californicus*), Canada Goose (*Branta canadensis*) and Eared Grebes (*Podiceps nigricollis*) occur on the ponds among 84 other recorded waterbird species. Higher numbers of historical bird landings were statistically correlated with greater open water area and decreasing proximity to the Athabasca River. Behavioural observations revealed birds were primarily using the ponds for resting and feeding while search efforts have confirmed the presence of nesting waterfowl on and around the LIFs. Stable isotope of dD, $d^{18}O$ and $d^{34}S$ in water and tissues is being explored as a tracer for LIF exposure but preliminary data was unable to differentiate isotope profiles between LIFs and nearby natural waterbodies. Previous work has focussed on deterrent strategies, but this comprehensive assessment will help quantify pond use and exposure risk to waterbirds that differentially use liquid impoundment facilities to inform mitigation strategies for the energy sector.

3.02.P-Mo General: Terrestrial Toxicology, Ecology, and Stress Response

3.02.P-Mo-044 Oviposition by Monarch Butterflies onto Clothianidin Contaminated Milkweed Plants

***Tim Bargar**[1] and Michelle L. Hladik, U.S. Geological Survey*

Contact chemoreceptors on various appendages of Lepidopterans detect chemicals in plants that enable ovipositing Lepidopterans to detect larval host plants. The possibility that pesticides could interfere with chemoreception and host plant selection has led to laboratory studies confining monarch butterflies within chambers with contaminated and uncontaminated milkweed plants to evaluate pesticide effects upon oviposition. In addition, we are conducting field studies in which wild ovipositing monarch butterflies are presented with a choice among potted contaminated and uncontaminated milkweed plants. Soil of potted milkweed plants was treated with Arena 0.25G (16.4 and 640 mg/pot approximating 5 and 200 g ai/acre) to achieve target clothianidin concentrations in leaves of approximately 12 and 300 ng/g. The higher of the two exposure concentrations is reported to affect monarch larvae growth and survival while the lower concentration has translated to prepupal mortality. Each month beginning in May 2024, sets of 6 plants (treated and untreated) will be deployed at three to four locations in the field for up to two weeks. Plants will be checked every other day for eggs and larvae. Leaves from all plants will be analyzed to determine clothianidin concentrations. To date in the first experiment, eggs were laid on plants at one location. The number of eggs on plants ranged from zero to eight, and the total number on plants in the treatments was 11 (untreated), 10 (16 mg/pot), and 8 (640 mg/pot). Results for the May through September experiments will be summarized and presented.

3.02.P-Mo-045 Validation of a Quantitative Polymerase Chain Reaction and Immunoassay to Assess Parasite Abundance and Quantify Stress in Passerines at the Interface of Agrochemical Exposure

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The eyeworm *Oxyspirura petrowi* has been measured in great prevalence and abundance in the Northern bobwhite quail (*Colinus virginianus*). The Northern bobwhite is a non-migratory, economically important, North American game species, and has been historically abundant in arid and semi-arid regions of Texas and Oklahoma. They are also commonly present in agricultural landscapes. Epidemics of this parasite have been linked with population crashes and pathological consequences in the host's eye tissue. Migratory songbirds have been reported to also have high *O. petrowi* abundances. It is hypothesized that migratory songbirds may contribute to spikes in *O. petrowi* infections for non-migratory Aves such as bobwhite when they arrive in the ecoregion. Additionally, the use of agrochemicals may increase susceptibility of bobwhite to infection as they have been linked to suppressed immune function and increased stress hormone levels in other birds. This impairment of immune function likely creates a potentially lethal scenario for those birds. This is of particular concern as migration is already a strenuous life history event. The purpose of this research is to validate a non-lethal method to quantify *O. petrowi* infection and a minimally invasive method to measure stress in some songbirds so that epidemiological comparisons can be made. First, a nonlethal quantitative polymerase chain reaction will be validated to determine *O. petrowi* infection intensity from a fecal sample collected from the passerines. Next, an immunoassay will be validated using passerine feces that will quantify the amount of corticosterone metabolites present, equating to the amount of corticosterone used. These tools can be used to quantify stress to make epidemiological comparisons in response to both parasite infections and agrochemical exposure.

3.02.P-Mo-046 The Effects of Different Sample Storage Conditions on Enzyme Immunoassay Results for Measuring Corticosterone in Northern bobwhite (*Colinus virginianus*)

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The Northern bobwhite (*Colinus virginianus*) is an economically and ecologically vital species in North America, and recent population declines have prompted extensive research into reversing this trend. With the recent validation of an enzyme immunoassay (EIA) to detect the stress hormone corticosterone (cort) metabolites in feces, there are many opportunities for its scientific application. Cort has many beneficial functions that help bobwhite fight infection. However, chronic levels of elevated cort can be detrimental as well. Therefore, determining what causes increases in cort is vital for bobwhite conservation efforts. Furthermore, bobwhite are also standard test subjects for many of the Environmental Protection Agency's contaminant studies, so there is potential for the application of the cort EIA in broader toxicological research. Proposed research includes investigating the effects of pesticides, climate, disease, management strategies, and more on bobwhite stress levels. However, the differing methodologies exploring these relationships may result in different ways the feces are stored and processed. Studies on wild bobwhite would likely have varied sample storage conditions and times before cort extraction. Additionally, feces not immediately collected from a bird (e.g. collected from a nest for a reproduction study) may allow for the bacterial and UV degradation of cort metabolites. Therefore, this study aims to determine if EIA results from fecal samples that were immediately frozen or left exposed in the environment before cort extraction differ from samples where cort is immediately extracted. Feces will be collected from pen-reared bobwhite. On 1d, cort will be extracted immediately from Group A's feces. The feces from Group B will be frozen for 30d before extraction. The feces from the final group will be placed outside for 24h before being processed. On 2d and 3d, the feces from each group will receive a different treatment. Treatments are rotated to account for individual variation in cort levels. Multiple groups are proposed to account for possible daily variations in cort levels. If storage conditions do affect the levels of cort metabolites detected by the EIA, researchers will know that values obtained using different storage methods or times will not be comparable. If no difference was detected between storage techniques, this would broaden the applicability of the EIA and allow for a wider variety of comparable sampling techniques.

3.02.P-Mo-047 Measuring Stress in Northern Bobwhite (*Colinus virginianus*) Parasitized by the Eyeworm *Oxyspirura petrowi* by Quantifying Heat Shock Proteins 60, 70, and 90

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Populations of Northern bobwhite quail (*Colinus virginianus*), an economically and ecologically important gamebird, have been declining across North America for the last few decades. While there are many causes for this decline, including climate change and habitat loss, bobwhite numbers have continued to fall even in well-managed areas under favorable climate conditions. This infers that there are additional factors of which we are not aware. One explanation is that bobwhite are experiencing chronic elevated stress levels due to compounding elements. However, there is a significant lack of understanding of how bobwhite respond to stress. Habitat, chemicals, and disease can all elicit a stress response in wildlife. One such response is the production of heat shock proteins (HSPs), a highly conserved family of proteins that play a vital role in maintaining cell homeostasis. Originally found to be induced by heat stress, we now know that many other types of stress including loud noise, isolation, disease, and parasitism can induce the production of HSPs as well. While the induction of these proteins is generally beneficial, chronically high levels of HSPs have been linked to numerous health issues including increased insulin resistance, cardiomyocyte inflammation, and increased tumorigenic potential in cancer cells. Sustaining high levels of HSP production also limits the energy and resources available for other biological functions. The well-understood mechanism of cause and effect between HSP production and cellular stress makes HSP concentrations a reliable way to measure chronic stress caused by a specific irritant. The current study aims to quantify the concentrations of HSPs 60, 70, and 90 in bobwhite parasitized by the eyeworm *Oxyspirura petrowi*. This parasite lives and feeds within the harderian gland in the back of the bobwhite's eye and has been linked with bobwhite population declines in the Rolling Plains ecoregion of Texas. The HSPs chosen for quantification are frequently found in vertebrates and have all been shown to respond to a variety of stressors in multiple bird species including other types of quail. This study will not only provide insight into the physiological effects of parasitism in bobwhite, it also serves as an example of a method for measuring stress in an EPA model avian species, the Northern bobwhite.

3.02.P-Mo-048 Validation of an Immunoassay to Quantify Immunoglobulin Response in Northern Bobwhite Quail (*Colinus virginianus*) and Scaled Quail (*Callipepla squamata*) to Parasites on the Microplastic Frontier

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Starting in the 1960s the Northern bobwhite (*Colinus virginianus*) and scaled quail (*Callipepla squamata*), ecologically and economically important gamebirds, experienced the largest declines of grassland birds in the United States. Both species have extensive geographical ranges including agricultural settings that overlap in the arid and semi-arid regions of Texas and Oklahoma. Many factors are suspected to contribute to the decline including pesticides, habitat loss and degradation, climate change, and diseases. Strongly supported evidence has indicated

two helminth parasites infecting both quail species, the cecal worm (*Aulonocephalus pennula)* and eyeworm (*Oxyspirura petrowi),* linked to quail population crashes. These helminths are notorious for their ability to suppress host immune systems leading to severe pathological consequences. While there is increasing research into the impact helminths have on wild quail populations, there is a lack of data on the adaptive immune response, especially at the intersection of microplastic exposure coupled with parasitic infection. The presence of microplastics in agricultural soils has raised concerns for species such as quail that inhabit these environments. Microplastics have demonstrated the ability to suppress immune function, resulting in immunotoxic effects in avian species, an area yet to be explored in quail. This leads to the hypothesis that microplastic exposure may increase susceptibility to parasitic infection. Immunoglobulins are critical for the adaptive immune system providing defense against pathogens. These may be useful as indicators of parasitic infection and host immune function. However, there are no studies documenting quail immune responses with immunoglobulins respective to parasitic infection, let alone microplastics. This research aims to validate an enzyme immunoassay utilizing blood serum to quantify immunoglobulin concentration. These concentrations will be compared to DNA extracted fecal samples through quantitative polymerase chain reaction to determine parasitic levels. By validating this tool, we can advance our understanding of the quail's immune system and conduct comparisons between sympatric species such as Scaled quail. This will also create a bridge to investigate combined effects of emerging contaminants, such as microplastics, that may alter immune function within quail.

3.02.P-Mo-049 Using Anthelmintics to Increase Abundance of Northern Bobwhite (*Colinus virginianus*) a Socially and Economically Important North American Game Bird: Safety, Efficacy, and Population Response

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The Northern bobwhite quail (*Colinus virginianus*) is a valuable North American gamebird that is estimated to generate over $2 billion (US) annually, much of which goes to fund conservation. However, *C. virginianus* populations have been declining across most of their range for several decades. There are several possible reasons for the decline including agrochemicals, urbanization, climate change, and disease. In the American Southwest, wild *C. virginianus* are plagued by two parasitic nematodes, with epidemics of those parasites corresponding with region-wide population crashes. An anthelmintic medicated feed was developed in response to those observations in an attempt to reduce the impacts of those parasites on wild *C. virginianus* populations. The anthelmintic medicated feed underwent stringent experimentation under U.S. Food and Drug Administration oversight to determine safety for *C. virginianus* and humans and efficacy against the nematodes. Blood chemistry health metrics of *C. virginianus* given the medicated feed were not different between treatment groups or controls. All pathology was reported as unremarkable. Human food safety was assessed using LC-MS/MS to assess the tissue dissipation rate from *C. virginianus* livers. Treated individuals were euthanized at time point intervals and concentrations of the conjugated metabolite were estimated. At no time point was the concentration greater than the maximum allowable concentration established by regulations and was below the limit of detection after 36 hours. Field efficacy trials demonstrated significant decreases in parasite burden of treated *C. virginianus* from pretreatment and placebo-treated individuals. Finally, the anthelmintic feed was used for 5 years to estimate the impact of parasites on wild *C. virginianus* populations. A private ranch in western Oklahoma was used as a treatment site and a neighboring public property was used as a reference site. By year three, and despite a prolonged drought, the treated site had increased by 500% and had over twice the *C. virginianus* abundance as the reference site. The treated site also saw a 50% reduction in one nematode and about a 30% reduction in the other. Annual survivorship on the treated site was also over twice that of previous reports and over 3-fold greater than the reference site. Thus, we have demonstrated a safe and effective means to improve conservation outcomes in the face of increasing agrochemical use, habitat loss, and climate change.

3.02.P-Mo-050 Emerging Contaminants in Neglected Australian Vertebrate Species

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For Australian terrestrial ecosystems, there are significant gaps in our understanding of chemical exposure to emerging contaminants, as well as information on bioavailability of these chemicals to sensitive receptors like birds of prey and reptiles. Despite their worldwide use and ongoing environmental contamination issues, the presence of per- and polyfluoroalkyl substances (PFAS), polybrominated diphenyl ethers (PBDEs) as well as both legacy and emerging pesticides remain largely unknown with Australian species. For this reason, ecological risk assessment (ERA) is hugely limited by the availability of environmental contamination studies focused on species relevant to Australian conditions. In this study we addressed the presence and concentrations of PFAS, pesticides and PBDEs in native Australian birds of prey (n = 45) as well as a reptile species, the lowland copperhead *Austrelaps superbus* (n = 12). Raptor species included wedge-tailed eagles *Aquila audax* (n = 29), white-bellied sea eagles *Haliaeetus leucogaster* (n = 7), brown goshawks *Accipiter fasciatus* (n = 3), brown falcons *Falco berigora* (n = 4), and powerful owls *Ninox strenua* (n = 2). For PFAS, there were 13 chemicals detected in all species and like international studies, PFOS was the most common (Total PFOS ranged between not detected (nd) to 110 ng/g wet weight). Levels of PFAS in white-bellied sea eagles showed the highest contamination, likely due to their reliance on marine food sources. In addition, metabolic intermediate fluorotelomer acid (7:3 FTCA) was detected for the first time in Australian species, these were copperheads, brown falcons and brown goshawks (range nd-16 ng/g ww). Levels of PBDEs and pesticides found in all species are likely lower than those reported in international literature. However, the presence of these chemicals highlights the need for further studies relevant to Australian species. As global regulators such as the European Union shift away from traditional ecotoxicological endpoints towards effects-based monitoring, Australian studies on the presence of emerging contaminants and chemical mixtures present in sensitive, terrestrial species will be key to setting relevant, ecological guidelines.

3.02.P-Mo-051 Investigating Snakes as Sentinel Species for Per- and Polyfluoroalkyl Substances (PFAS) in two Different Continents

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Per- and polyfluoroalkyl substances (PFAS) represent a class of synthetic chemicals known for their persistence in the environment, propensity for bioaccumulation, and adverse health effects. While extensive research has been devoted to understanding PFAS contamination in various ecological niches, studies on their accumulation in snakes remain notably sparse, leaving a critical gap in our understanding of PFAS dynamics within terrestrial ecosystems. This study bridged this gap by examining PFAS in the liver tissues of four snake species sampled from diverse habitats in South Africa and Florida, USA. The selected species included*,* Burmese pythons (*Python bivittatus*), Black mamba (*Dendroaspis polylepis*), Mozambique spitting cobra (*Naja mossambica*), and Eastern green mamba (*Dendroaspis angusticeps), which* serve as sentinel species offering invaluable insights into PFAS exposure and bioaccumulation within their respective ecosystems. By integrating ecological, toxicological, and conservation perspectives, this study will not only enhance our understanding of PFAS levels in snake populations but also will underscore the urgent need for proactive measures to mitigate PFAS contamination and preserve biodiversity in vulnerable ecosystems.

3.02.P-Mo-052 Comparative Reproductive and Developmental Effects in Mice Exposed to a PFAS-Containing AFFF and a PFAS-Free Firefighting Foam

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Although the replacement constituents for per- and polyfluoroalkyl substances (PFAS)-containing aqueous film-forming foams (AFFFs) should have reduced persistence and bioaccumulation by design, there are limited data on the ecotoxicity of the firefighting foam products, themselves. Combined repeated-dose toxicity studies and reproductive and developmental toxicity screening tests with CD-1 mice were conducted with two newly developed candidate products and one legacy PFAS-containing AFFF. Developmental data from mice exposed gestationally, via lactation, and post-weaning are selectively presented herein. Prior to weaning at postnatal day 21 (PND21), endpoints such as litter weight, pinna unfolding, eye opening, and anogenital distance (AGD) were observed as indicators of early pup development. Starting at PND22, selected first filial animals were dosed via oral gavage, and body weight gain and attainment of puberty via vaginal opening (VO) and balanopreputial separation (PPS) were assessed as indicators of effects as pups approach maturity. We observed reduced body weight gain at high doses in pups, which aligns with short-term exposures in adult mice, indicating that high oral exposure to full products may be a bioenergetic stressor. Considering reproduction and development are impacted in some species exposed to PFAS, these data are especially valuable for the comparison of replacement products and for informing decision-makers through the transition away from use of PFAS-containing AFFFs.

3.02.P-Mo-053 Assessment of Ecotoxicological Effects to Earthworms (*Eisenia fetida*) Exposed to Titanium Carbide Mxenes

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Titanium carbide MXenes, Ti_3C_2, are being explored for several innovative applications in agriculture that leverage the unique properties of MXenes, such as their high surface area, excellent electrical conductivity, and tunable surface chemistry. Some key uses for Ti_3C_2 MXenes in agriculture include fertilizer delivery systems, soil remediation, pesticide delivery, plant growth enhancement, and water treatment for irrigation. Adult *Eisenia fetida* earthworms were exposed to Ti_3C_2 MXenes at concentrations of 0.01, 0.1, 0.5, 1.0, 5.0 and 10.0 mg/L for 28 days. At the conclusion of the initial exposure period, the five replicate earthworms in each MXene exposure group were combined into one container with identical soil composition for a 28-day reproductive toxicity study. After 28 days, adult earthworms were retrieved, and cocoons were counted. Cocoons were then reintroduced into their respective containers and monitored for an additional 14 days to assess hatching success. Statistical analyses conducted revealed noteworthy distinctions across all treatment groups relative to the control. Specifically, the Exposure to the lowest dose, 0.01 mg/L, caused 46% of the cocoons to fail to hatch, compared to only 6% in the control group that failed to hatch. The 0.5 mg/L exposure group caused the highest hatch failure rate (94%).

3.02.P-Th Late Breaking Science: Terrestrial Toxicology, Ecology, and Stress Response

3.02.P-Th-142 Microplastic in Northern Gulf of Mexico Waterfowl

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Some of the highest concentrations of microplastics, globally, have been reported in the Gulf of Mexico, which is home to the majority of plastic manufacturers in the United States. Microplastic ingestion not only poses a physical threat to humans and wildlife, but also an ecotoxicological threat from chemicals associated with plastic production and those readily adsorbed from the environment onto the plastic particles. A comprehensive understanding of the risk microplastic contamination poses to wildlife is critical to the development of scientifically sound mitigation and policy initiatives. However, very little knowledge exists regarding patterns of microplastic ingestion in Gulf of Mexico birds or waterfowl in general, and specifically, no knowledge in Gulf of Mexico waterfowl. In this study, we assess microplastic quantities, probability of ingestion, and microplastic ingestion risk factors for hunter-donated waterfowl from Texas and Louisiana. We collected carcasses of dabbling and diving ducks from hunters, dissected out the intestinal tracts, digested organics, filtered out microplastics, and counted them under a dissecting microscope. The probability of ingestion for wintering ducks from both states was approximately 60% with microplastic quantities ranging from zero to 80 microplastic pieces per individual. Microplastic fibers were overwhelmingly prevalent and 100% of ducks with detectable microplastic ingestion had ingested fibers. Fragments, films, foams, foils, and nurdles were also all detectable at much lower percentages. The most prevalent microplastic colors were blue (~90% of ducks), and black, clear, and green (~85%, each), followed by red (~60%), white (~50%), and brown, yellow, and gray (<30%, each). We are currently evaluating potential risk factors for microplastic ingestion, including foraging mode (*i.e.,* diving/dabbling), and proximity to known microplastic hotspots. Overall, our results suggest widespread ingestion of microplastics, and especially microplastic fibers, by wintering waterfowl in the northern Gulf of Mexico. Future research is needed to understand the implications of this contamination for waterfowl health, productivity, and potential trophic transfer to humans.

3.02.P-Th-143 Honey Bee Cell Culture as a Non-Intrusive Tool for Understanding Non-Target Effects of Fungicides

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Outbreaks of plant fungal pathogens are predicted to increase with global climate change and the expansion of crop production in new areas. This increased infection risk threatens global food security, as plant fungal pathogens erode gains in crop yield and produce quality. Fungicides are an important tool for the control of plant fungal pathogens; however, their use, especially on pollinator-visited crops, is at odds with honey bees that utilize these crops for food resources. Although once considered safe, there is increasing evidence that chronic, sublethal exposure of honey bees to fungicides can result in negative effects on their development, behavior, and response to infectious diseases. Our understanding of how fungicides detract from honey bee health is achieved mainly through field and caged-bee studies. While these approaches work well for understanding organismal and social responses to exposure, high resolution, less intrusive, and highly controllable approaches are needed to explore mechanisms of cellular toxicity and host-pathogen interactions in the presence of fungicides. Cell lines are routinely used in many areas of human, animal, and plant toxicological research, but are surprisingly lacking for such a prominent model organism as the honey bee. This deficiency prompted us to use a continuous cell line derived from honey bee embryonic tissues, AmE-711, as a platform for characterizing the cellular response of select fungicides commonly applied to flowering crops that honey bees are likely to visit. We demonstrate that exposure of AmE-711 to a range of concentrations of the active ingredients found in Pristine, boscalid and pyraclostrobin, and chlorothalonil negatively affected cell viability and mitochondrial performance. Use of AmE-711 as a platform for toxicological research can aid assessment of the risks that fungicides, as well as other pesticides, pose for honey bee health. Moreover, AmE-711 as a screening tool could lead to the development of safer, highly specific alternatives that ultimately benefit beekeepers, growers, and the environment.

3.03.P-Th Integration of 21st Century Approaches to Wildlife Risk Assessment for Pesticides in North America

3.03.P-Th-029 Accounting for Uncertainty in Pesticide Risk Assessments for Wildlife: Approaches at Different Tiers

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In the United States and elsewhere, wildlife risk assessments for pesticides should follow a tiered approach. The initial screening-level assessment is based on simple, highly conservative models (e.g., T-REX, T-HERPS for birds, mammals, herptiles in the United States). Such models assume, for example, that wildlife forage exclusively on treated fields immediately after application and are exposed to 95th percentile concentrations on dietary items. Although useful for determining which receptor group and use pattern combinations are a potential risk concern and those that are not, relying solely on these models without a corresponding uncertainty analysis presents a distorted risk picture. This distorted risk picture is problematic because the

U.S. Environmental Protection Agency only infrequently conducts higher tier, probabilistic assessments even when potential risk concerns are identified for wildlife. As a result, the Agency is often sued when pesticides are registered or re-registered because it's own analyses indicated risk quotients that exceeded levels of concern for wildlife. The obvious solution would be to conduct more realistic and probabilistic assessments for scenarios identified as being potential risk concerns in the screening-level assessment. However, the U.S. Environmental Protection Agency has resource limitations, thus limiting their ability to routinely conduct higher tier assessments for wildlife. In this presentation, I will discuss simple steps that may be taken to account for uncertainty in screening-level assessments that require few additional resources but result in a more accurate risk picture for wildlife. Examples include: (1) conducting best-case and most likely analyses alongside the typical worst-case analyses, (2) creating generic probabilistic models for a suite of wildlife receptor groups that have standardized distributions for key parameters such as food ingestion rate, dietary residue concentrations, and rates of degradation and metabolism, and (3) conducting what if analyses for parameters that are uncertain (e.g., proportion time foraging on and off treated fields). When higher tier assessments are required, other refinements such as the use of species-specific, spatially explicit exposure models and dose-response curves can be incorporated in wildlife assessments for pesticides. The latter are resource intensive and thus unlikely to be conducted on a frequent basis.

3.03.P-Th-030 The History and Future of MCnest for Pesticide Risk Assessment

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The Markov Chain Nest Productivity Model (MCnest) was written to allow risk assessors at U.S. Environmental Protection Agency to evaluate the effect of pesticides on reproductive success of birds exposed to pesticides in agricultural settings. The model incorporates test endpoints from three standard avian toxicity tests submitted to U.S. Environmental Protection Agency by chemical registrants. The Avian Acute Oral Toxicity test [850.2100], the Avian Dietary Toxicity Test [850.2200] and the Avian Reproduction Test [850.2300]. The first version of MCnest used EPA's Terrestrial Residue Exposure Model (T-REX) for exposure estimates. The second version also incorporated U.S. Environmental Protection Agency's Terrestrial Investigation Model (TIM), allowing users to choose between T-REX and TIM for exposure modeling. Throughout its lifetime, MCnest has been coded in Matlab®, and has modeled only the potential effects of pesticides during the avian breeding season. Recently, MCnest has been reprogrammed in R and expanded to the full annual cycle with the intent of improving versatility and utility for researchers to adapt its algorithms and to include their own exposure and effects subroutines while still leveraging the powerful lifecycle modeling capabilities within the MCnest model. Although the new version has not yet been released, I will preview some of its design and analytical features, review the expanded functionality, and discuss future directions for MCnest. In particular, I will focus on the integration of the new MCnest model with adverse outcome pathway models and with the recently proposed framework of endogenous lifecycle models. *The views expressed in this abstract do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.*

3.03.P-Th-031 Exploring Avian Toxicity Data for Pesticides in the ECOTOX Database

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Currently, registration of pesticides in regions including the US, Canada, and Europe requires several avian toxicity studies, generally including the acute oral (OCSPP 850.2100/OECD TG

223), chronic reproduction (OCSPP 850.2300/OECD 206), and in some cases, sub-chronic dietary (OCSPP 850.2200/OECD TG 205) study designs. Collectively, the results of these studies are used to assess the potential acute and chronic risk of pesticides to birds. In recent years, interest has grown in possible approaches for reducing vertebrate animal use for toxicity testing, including in the area of avian toxicity. A key aspect of designing, validating, and implementing new approach methods (NAMs) is gaining an in-depth understanding of the underlying data that is intended to be approximated or predicted. One available resource for avian toxicity data is the U.S. Environmental Protection Agency's ECOTOX database, which includes data both from studies available in the scientific literature as well as from guideline toxicity studies submitted to support pesticide registrations. In this exercise, several aspects of the available data were explored: (1) species sensitivity by study type, (2) comparison of acute and chronic endpoints, and (3) comparison of avian data to mammalian endpoints extracted from ToxValDB (an *in vivo* mammalian database). The influence of chemical class/mode-of-action on the analyses was also explored.

3.03.P-Th-032 Estimating the Exposure of Pesticide Residues in Nectar and Pollen to Bee Pollinators

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A pesticide registration requires a risk assessment that considers both toxicity and exposure to pollinators. Toxicity is defined as the dose that causes an adverse effect, while exposure – we will focus on dietary – considers the pesticide concentration in the diet (i.e., nectar and pollen) and the amount of diet consumed. The magnitude and duration of pesticide exposure vary widely according to plant type and bee attractiveness, physio-chemical properties, application rate, method and timing, and soil type. Regulatory authorities rely upon model-generated estimates to characterize exposure when measured pesticide residue data are unavailable. These theoretical pesticide residue estimates in the United States for pollen and nectar are based on T-REX model tall grass values (foliar applications), and the Briggs plant-soil uptake model (soil applications). The Pollinator Research Task Force analyzed systemic and non-systemic pesticide residue data from nectar and pollen samples collected across multiple studies that were submitted to U.S. Environmental Protection Agency and the European Food Safety Authority. Our goal was to provide a statistically refined pesticide residue estimation in the form of an interactive calculator. These results will strengthen the exposure component of pesticide risk assessment for bee pollinators by utilizing a robust pesticide residue data set of pollen and nectar samples covering various use patterns in a diversity of crops.

3.03.P-Th-034 Aerial eDNA—A New Approach to Identify and Monitor Species in Farmland

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All species leave DNA traces in the environment. So-called Environmental (eDNA) is released by any organism into the environment, via breathing, faeces, hair, urine, skin, gametes, etc. This eDNA can be extracted from non-invasively collected environmental samples such as soil, water or faeces without capturing potential target organisms in focus. More recently, studies have revealed that air also serves as a medium for transporting DNA. Approaches focussing on eDNA to monitor species are now widely recognized as a valuable tool for Biodiversity assessments. In European environmental risk assessment of agrochemicals, species of birds, mammals and other vertebrates that utilize cropped fields, need to be known and monitored. Therefore, in our study conducted in a farmland landscape in southwestern Germany, we investigated the potential of aerial eDNA sampling for identifying species or specific taxonomic groups within the contexts of agrochemical registration procedures with a particular emphasis on vertebrates. We compared the effectiveness of active and passive air sampling devices. Both types of air samplers were placed in two different sites for 8h or 2 weeks, respectively. After the sampling period in spring 2024, DNA was extracted from the filters using a commercial DNA extraction kit. Afterwards, two different genetic markers (12S and 16S) targeting vertebrates, were amplified via polymerase chain reaction PCR. Following PCR-based amplification, amplicons were sequenced using the real-time portable nanopore sequencing. The sequencing data was analyzed using the National Center for Biotechnology Information (NCBI) public genetic reference for the identification of present species available in the database. Our initial results are promising, showcasing potential for widespread application of eDNA sampling and analysis for species detection, monitoring and finally complete biodiversity surveys, and the use of these non-invasively and time-saving method in the context of species, crop or farmland community based environmental agrochemical assessments.

3.03.P-Th-035 Availability and Attractiveness of Treated Seed to Wildlife after Corn and Soybean Planting Events

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Although treatment of seed with pesticides prior to planting has been used for decades, recent advances in insecticide and fungicide chemistries have resulted in an increase in seed treatment and now most of corn and soybean seeds are treated with at least one pesticide prior to planting. Ingestion of treated seeds by vertebrates becomes possible due to events such as spillage, dropped seed during planter refills, and misplant occurrences. To evaluate the risk posed by treated seeds, more information is needed on both availability and the attractiveness of the seeds. We conducted a field experiment in north central Missouri where 10 fields, 5 corn and 5 soybean, were monitored for available seeds. Uncovered seeds were characterized as either misplants or spills. In addition, each field received two artificial spills, 1000 seeds that were treated with colorant without any active ingredients. One spill was located at a field edge near potential wildlife habitat, and one was placed in the middle of the field. Spills were monitored for 7 days with cameras to evaluate the species that visited the spill and seed loss was counted at the end of the observation period. For corn, 0.02% (SD = 0.04) of seeds were available due to misplanting and 0.004% (SD = 0.009) were available due to spillage, resulting in 0.03% (SD = 0.01) of total planted seeds available. For soybean, 0.48% (SD = 0.31) of seeds were available due to misplanting and 0.02% (SD = 0.03) due to spillage resulting in 0.5% (SD = 0.3) of total planted seeds available. These values are near or below the 1% unincorporated threshold frequently used for exposure to precision drilled seeds within risk assessments. Greater than 90% of consumed seed from artificial seed spills was attributed to mammals including cottontail rabbits, white tailed deer, racoon, opossum, and field mice. The primary bird species that ingested seeds were northern cardinals and red-winged blackbirds which ingested 1-3 seeds per incident. For corn, 62% of corn in artificial seed spills at the field edge was consumed while less than 10% was consumed in the middle of the field. Similarly for soybean, 21% of the corn at the field edge was consumed, while <10% was consumed in the middle of the field. This data can be used to develop environmentally relevant exposure estimates of treated corn and soybean seeds to birds and mammals and ultimately be used to refine wildlife ecological risk assessments.

3.03.P-Th-036 Neonicotinoid Exposure in Migrating and Resident Birds: Testing Hypotheses at a High-Volume Spring Stopover Site in Coastal Texas

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Neonicotinoids are the most widely used insecticides in the world and are an important threat to migrating birds. Birds can be exposed to them by ingesting contaminated insects, seeds, water, nectar, or soil and through dermal and inhalation routes; migrating birds may be predisposed to neonicotinoid exposure through consuming large quantities of food at unfamiliar stopover sites during migration. Neonicotinoids have been experimentally shown to affect migrating birds through severe mass loss, impaired orientation, and delayed departure timing. These effects could carry-over as lower condition birds arrive later to their breeding grounds, resulting in reduced reproductive output. To investigate how phenological and ecological variables relate to neonicotinoid exposure in migrating birds, we collected 496 blood samples and 135 fecal samples at a high-volume stopover site in the Gulf of Mexico throughout 2022 and 2023 spring migrations, with focal representation of Gray Catbirds (*Dumetella carolinensis*; a migrant) and Northern Cardinals (*Cardinalis cardinalis;* a resident). While Gray Catbirds and Northern Cardinals are one focus of this work, samples from 40 species were included in this analysis in order to look more broadly at exposure in common species and migrating groups overall. Plasma samples were analyzed for a number of neonicotinoids including acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam along with metabolites by high performance liquid chromatography mass spectrometry. Detection of parent compounds in plasma indicates acute exposure whereas detection in feces can indicate weeks-old exposure, allowing us to build novel “exposure timelines” for each bird with paired samples. We will determine if migratory strategy, phenology, and foraging guild predict neonicotinoid quantities detected. We expect later-season and insectivorous migrants to have the highest overall neonicotinoid exposures and expect migrating birds to have overall higher exposure incidence and detected quantities than resident birds. This field study augments our understanding of neonicotinoid exposure during a physiologically critical period of the avian annual cycle and is

foundational to future mechanistic studies to understand how pesticide exposure impacts migratory bird health.

3.04.P-Th Let's Talk About Snakes, Baby! (And Frogs, Lizards, Salamanders, and Turtles, Too)

3.04.P-Th-037 Ammonium: Anuran Nemesis or Environmental Niche? Investigating the Impacts of Aqueous Ammonium on Frog Biodiversity and Distribution in New Jersey and New York Wetlands

***Laina Lockett**, Rutgers University*

Ammonium compounds, such as those found in ammonium sulfate fertilizers, have been shown to cause negative impacts on amphibian species during acute and chronic studies. However, it is unknown if ammonium alone impacts habitat selection or amphibian species biodiversity. This analysis aimed to determine whether or not aqueous ammonium impacted the occurrence and species richness of anuran species in wetlands in New Jersey and New York. Frog calls and visual observations of frogs were recorded during the spring and summer of 2019. Additionally, water samples were collected during the same spring and summer and analyzed for aqueous ammonium concentrations using a colorimetric analysis. Mann-Whitney tests were run to compare sites with and without three species of frogs (Atlantic Coast leopard frogs (*Lithobates kauffeldi*), spring peepers (*Pseudacris crucifer*), and green frogs (*Rana clamitans*)) and Kruskal-Wallis tests were run to determine whether or not those same species had similar ammonium preferences in habitat selection. A generalized linear model was run to determine if there was a difference in the number of species present based on ammonium levels across time. Results suggest that there are no statistically significant differences in ammonium levels between sites with and without the selected species. The Kruskal-Wallis test further indicated that the three species exhibited similar preferences in habitat regarding ammonium concentrations. While the linear model did not detect a significant overall effect of ammonium on species richness, it found a potential impact during the third sampling period. These findings suggest that ammonium alone may not largely impact anuran biodiversity and species distribution at levels that are found in the environment, but there could be impacts at certain time points. Further investigations are needed on potential confounding factors and long-term population level impacts.

3.04.P-Th-039 Using Omics-Based Ecosurvellience Approaches to Assess Pollution Impact in Toads

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The absence of comprehensive knowledge regarding the risks posed by the majority of environmental chemicals hinders informed decision-making by regulators and stakeholders, impeding efforts to safeguard ecosystems. Integrating novel omics-based techniques in wildlife studies, in collaboration with state regulatory monitoring programs, offers a unique avenue for establishing omics-based effects monitoring within regulatory frameworks. Current risk-based monitoring methods often fail to provide an accurate assessment of chemical contaminants in the environment or consider the cumulative impact of multiple sublethal stressors. These approaches typically focus on a limited set of contaminants and short-lived model organisms, neglecting the broader spectrum of chemicals and their potential long-term effects on larger wildlife. Consequently, policy decisions tend to overlook the overall chemical profile and accumulating health impacts on wildlife, hindering effective mitigation and restoration efforts. To address these challenges, omics-based ecosurveillance tools, encompassing transcriptomics, proteomics, lipidomics, and metabolomics, offer a promising solution. By quantifying sublethal biological responses in wildlife exposed to chemical contaminants, these tools can fill critical gaps in risk assessment and provide more accurate ecosystem health metrics. Combining omics data with chemical profiling enables the identification of key genes, proteins, and metabolic activities perturbated by complex chemical mixtures. This information facilitates the establishment of omics-based adverse outcome pathways and multi-omics points-of-departure, informing future monitoring activities and guiding the development of new chemical mixture thresholds. Furthermore, it paves the way for data-driven models, leveraging machine learning and artificial intelligence, to predict ecosystem-wide adverse health outcomes. In conclusion, the integration of omics-based approaches holds promise for revolutionizing environmental risk assessment and management, ultimately contributing to the preservation of ecosystems and societal well-being. Here we provide a case study example—sample collection, preparation, and analytical tools—that highlights the utility of omics-based ecosurveillance applied to an invasive toad species within an urban catchment impacted by multiple chemical stressors.

3.04.T Let's Talk About Snakes, Baby! (And Frogs, Lizards, Salamanders, and Turtles, Too)

3.04.T-01 Exploring Options for Pesticide Risk Assessment for Aquatic and Terrestrial Life Stages of Amphibians

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This presentation will explore the possibilities of performing a risk assessment for plant protection products (pesticides) and aquatic and terrestrial life stages of amphibians. Considerations of exposure through water, soil, food items, as well as direct overspray are briefly reviewed. The dominant exposure routes, i.e. oral and dermal, are considered not only in terms of importance, but also when looking for surrogate taxa for which toxicity data and/or risk assessment approaches are already available. Typically, birds (and/or mammals) are used as surrogates for amphibian terrestrial life stages, while fish are used as surrogates for the aquatic life stages. While the whole-body exposure and ectothermy of fish fits to the aquatic life stages of the amphibians, the focus on dietary exposure for the endothermic birds and mammals does not match the more important role of dermal exposure in ectothermic terrestrial amphibian life stages. For instance, factors like energy demand and metabolism can be quite different between endo- and ecothermic organisms. In the search for suitable surrogates, several data reviews were undertaken and the (cor)relation between acute and chronic toxicity values between vertebrate taxa was investigated. While acute toxicity values for aquatic life stages (i.e. larvae and tadpoles) are quite abundant in the literature, chronic aquatic values and toxicity values for terrestrial life stages in general are quite scarce. Nevertheless, on the basis of the available data, some conclusions can be drawn and some methods are considered useful in screening risk assessment. The importance of identifying surrogates is underlined by the fact that amphibians are vertebrates and testing should be avoided or minimized as much as possible. Hence, non-testing methods such as QSARs and species' extrapolation techniques, such as those embedded in the US EPA software WebICE, can provide toxicity estimates that may be used as input for the risk assessment. Also, *in vitro* techniques using amphibian cell lines or *in vivo* studies using non-protected life stages such as eleutheroembryos can provide useful additional information. As a final step in the risk assessment process, potential mitigation measures are discussed. These measures serve to reduce exposure and may be of temporal nature (e.g., avoid spraying during amphibian spring migration to breeding ponds) or of spatial nature (e.g., consideration of buffer zones around water bodies).

3.04.T-03 In Vitro Toxicity of PFOA, Crude Oil, and Other Organic Contaminants in Sea Turtles

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Sea turtles face a number of anthropogenic and natural threats. *In vitro* toxicology provides a crucial means to investigate the impact of pollutants in these species for which *in vivo* experiments are prohibited. Primary and immortalized fibroblast cultures were successfully established from skin biopsies and internal organs of healthy loggerhead (*Caretta caretta*) and hawksbill (*Eretmochelys imbricata*) sea turtles. This project examined the *in vitro* toxicity of perfluorooctanoic acid (PFOA, 0.05 µM, 0.5 µM, 5 µM, 50 µM and 500 µM), crude oil, Corexit™9500A, polychlorinated biphenyl PCB77 (0.01µM, 0.1µM, 1.0µM, or 10µM), and benzo[a]pyrene (B[a]P, 0.01µM, 0.1µM, 1.0µM, or 10µM) after 24-96h exposure. These concentration ranges encompass concentrations found in the water column, prey items, and sea turtle tissues as well as higher concentrations to mediate the short exposure period. Toxicity was investigated using two in vitro assays commonly used in toxicity testing: The MTT (methylthiazolyldiphenyl-tetrazolium bromide) assay which reports on cell death measured as mitochondrial dysfunction and the LDH (Lactate Dehydrogenase) assay which reports on cell death measured as loss of cell membrane integrity. In general, MTT assays were superior to LDH assays in detecting toxicity, inferring mitochondrial damage generally occurred more rapidly than cell membrane damage. In the crude oil and oil dispersant study, hawksbill skin cells were dosed with either media accommodated fractions of crude oil and/or Corexit™9500A. Results revealed significant toxicity compared to the control after exposure to Corexit. Exposure to Corexit for 96h resulted in only 1% cell viability while 24h exposure resulted in 3% cell viability. In both loggerhead liver and ovary cells, B[a]P and PCB77 exerted comparable ranges of cytotoxicity and were significantly more potent than PFOA. However, PFOA had greater efficacy than B[a]P and PCB77 in both ovary and liver, reaching 100% and 96% cell death, respectively. A similar efficacy pattern among all three contaminants was detected in Hawksbill skin cells. The doses of a toxicant expected to cause death in 50% of the cell population after 24h and 96h, $24hLC_{50}$ and $96hLC_{50}$, were estimated for MTT data using general linear model (GLM) with logistic link function. These findings provide a critically needed understanding of the relative toxicity of known contaminants in these endangered species.

3.04.T-04 Bioaccumulation and Metabolic Impact of Environmental PFAS Residue on Wild-Caught Urban Wetland Tiger Snakes (*Notechis scutatus*)

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PFAS contamination of urban waters is widespread and has significant implications for human health and the environment, but understanding the biological impact of its accumulation is limited to humans and common ecotoxicological model organisms. Here, we combine PFAS exposure and bioaccumulation patterns with whole organism responses and 'omics-based ecosurveillance methods to investigate the potential impacts of PFAS on a top predator of wetlands, the tiger snake (*Notechis scutatus*). Tiger snakes (18 male and 17 female) were collected from four wetlands with varying PFAS chemical profiles and concentrations in Perth, Western Australia. Tiger snake livers were tested for 28 known PFAS compounds, and Σ28PFAS in liver tissues ranged between 322 ± 193 µg/kg at the most contaminated site to 1.31 ± 0.86 µg/kg at the least contaminated site. The dominant PFAS compound detected in liver tissues was PFOS. Lower body condition was associated with higher liver PFAS, and male snakes showed signs of high bioaccumulation, whereas females showed signs of maternal offloading. Biochemical profiles of snake muscle, fat (adipose tissue), and gonads were analyzed using a combination of liquid chromatography triple quadrupole (QqQ) and quadrupole time-of-flight (QToF) mass spectrometry methodologies. Elevated PFAS was associated with enriched energy production and maintenance pathways in the muscle; had weak associations with energy-related lipids in the fat tissue, and lipids associated with cellular genesis and spermatogenesis in the gonads. These findings demonstrate the bioavailability of urban wetland PFAS in higher-order reptilian predators and suggest these compounds have a negative impact on snake health and metabolic processes. This research expands on omics-based ecosurveillance tools for informing mechanistic toxicology and contributes to our understanding of the impact of PFAS residue on wildlife health to improve risk management and regulation.

3.04.T-05 Predicting the Sensitivity of Reptiles to Dioxin-Like Chemicals: A Quantitative Adverse Outcome Pathway Approach

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Reptiles represent the least-studied group of vertebrates in ecotoxicology. This lack of toxicity data represents a significant uncertainty in ecological risk assessments of this taxon. However, the process for assessing the toxicity of chemicals is undergoing a fundamental shift from an emphasis on whole-animal testing of apical-level toxicities to a greater focus on conserved mechanistic end points. A prior study developed a cross-species quantitative adverse outcome pathway (qAOP) model capable of predicting full dose-response curves for early life stage mortality following exposure to dioxin-like chemicals (DLCs) in any species of fish or bird using an 50% effective concentration (EC50) as input. Calculated EC50s were derived from a standardized in-vitro aryl hydrocarbon receptor (AHR) transactivation assay of COS-7 cells transfected with an AHR cloned from the species of interest. The objective of the present study was to see if this same qAOP could accurately predict early life stage mortality for reptiles. Specifically, the AHR1 and AHR2 were cloned from the common snapping turtle (*Chelydra serpintina*) as a model reptile. The sensitivity to activation of each isoform was determined for four DLCs, namely 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 2,3,7,8-tetrachlorodibenzofuran (TCDF), and 3,3',4,4',5-pentachlorodiphenyl (PCB 126). The 50% effect concentration (EC50) for activation of each isoform to each DLC was determined. There were no significant differences in sensitivity to activation between the two AHR isoforms of common snapping turtle to any of the DLCs. Thus, it is unclear which isoform drives toxicity in reptiles, but prior studies in birds suggest that the AHR1 is most likely the driving isoform. Then, the accuracy of the qAOP for predicting early life stage mortality for the 4 DLCs was assessed using the EC50s for each isoform and 50% lethal doses (LD50) from a prior study. Using the qAOP, the EC50s for AHR1 and AHR2 of common snapping turtle could predict LD50s to within 10-fold on average. This accuracy is within the range of accuracy for fish and birds suggesting that reptiles fall within the domain of taxonomic applicability for this qAOP. Overall, this study demonstrates the potential of predictive tools, such as the qAOP, and how they could play an essential role in enabling objective ecological risk assessments for taxon that are difficult to study in toxicity testing, such as reptiles.

3.04.T-06 Mystery in the Marsh: Uncovering Hidden Herps with eDNA

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Amphibians and reptiles are indicator species. Their presence or absence in an environment provides researchers with critical information about a habitat quality or changes that may be occurring within it. Unfortunately, these species are often cryptic—burrowing underground, hiding in murky ponds, or emerging only during certain times of year or specific weather conditions—and thus can extremely difficult to monitor. Researchers have begun to pilot the use of environmental DNA (eDNA) to increase their chances of detecting these cryptic species compared to traditional methods. Despite the numerous studies documenting eDNAs effectiveness at detecting elusive species, questions remain about the protocols for deploying eDNA as part of a regular land monitoring process. How many samples should be taken? What is the frequency across the year? Should you use qPCR or metabarcoding? Which primers work best? What organisms can you expect to find? How do you analyze the data? How does it relate to other data types? In this collaborative talk, we bring together case studies from a range of organizations and agencies who have been following advanced monitoring practices using eDNA to study a variety of species in California, Texas, and beyond. We will present the methods used, the lessons learned, and the best practices we have developed to monitor herps using eDNA. Let's explore how eDNA can move beyond the pilot stage to be operationalized as part of a larger herpetology monitoring program.

3.05.A.T Wildlife Toxicity: Innovative Approaches for Evaluating Exposure and Effects of Contaminants in Free-Ranging Wildlife and Laboratory Animal Models

3.05.A.T-01 Do UV Absorbents Activate Avian Aryl Hydrocarbon Receptor 1 in a Species Dependent Manner?

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Ultraviolet absorbents (UVAs) are organic chemicals used in industrial and consumer products for their photoprotective properties. They are ubiquitous in the environment and are taken up by aquatic and terrestrial organisms. UVAs are known to activate the aryl hydrocarbon receptor (AHR) in fish and mammals, but data for birds is currently lacking. The AHR mediates effects of various classes of chemicals, and the primary structure of its ligand-binding domain is conserved across terrestrial and aquatic species. Previous work in our lab suggested that UVAs are associated with few morphological effects, and they can be weak inducers of Cytochrome P450 1A4 (CYP1A4) expression from Japanese quail liver tissues. Here, our objective was to determine whether UVAs activate AHR in different avian species. We measured AHR activation in four avian species following exposure to six UVAs with a luciferase gene reporter assay using full-length AHR1 receptors cloned from each tested species. We used species with differing AHR subtypes associated with differential sensitivity to some classes of AHR ligands. The species were chicken (*Gallus gallus*; subtype 1), ring-necked pheasant (*Phasianus colchicus*; subtype 2), Japanese quail (*Coturnix japonica*; subtype 3) and double-crested cormorant (*Nannopterum auritum*; subtype 3). Preliminary data show UV-P (2-2H-Benzotriazol-2-yl)-p-cresol) and UV-9 (2-(2H-benzotriazol-2-yl)-4-methyl-6-(2-propenyl)phenol) activated AHR1 for all bird species, and this agrees with data on fishes where these were the only two active UVAs. In contrast to fishes, where the range in species-specific potency of these UVAs exceeded 100-fold, there were no species-specific differences among tested birds. However, the potency of UV-P and UV-9 for activation of the avian AHR1s was similar to the most sensitive fish species. Ongoing work will determine the chemical potency for the remaining chemical and species combinations. Results of this study improve our understanding of the risks associated with UVAs to birds.

3.05.A.T-02 The Role of Animal Energetics on Contaminant Body Burdens and Biomarkers of Health

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Assessments of wildlife health often use correlations between body burdens of contaminants with biochemical or physiological responses to infer toxicity. The scientific literature is replete with many examples of negative correlations between body condition (a proxy for energy reserves) and contaminants such as PCBs and are often cited as evidence of toxicity of these chemicals. However, as the adage goes, “correlation is not causation”; further, for correlations, the direction of the cause-effect relationship is not necessarily obvious. A common consequence of changes in body condition is the redistribution of lipophilic contaminants among tissues following the loss or gain of lipid stores, which can also lead to negative correlations between burdens and body condition. In addition, many measures of animal health used to assess the effects of contaminants, such as immune function, stress response, and thyroid function, are

driven by availability of energy or energy reserves. If body condition affects both contaminant burdens and biochemical measures of animal health, significant relationships may be found between contaminant levels and health measures without any toxicity. To illustrate this phenomenon, first we demonstrate the mechanics of how changes in body condition can alter body burdens, based on both laboratory and field experiments. Second, we show how thyroid hormones are regulated in vertebrates in relation to energy reserves, from published literature as well as the putative mechanistic pathway driving this relationship. Hence, the simplistic assumption that negative correlations between body burdens and health measures are due to toxicity may lead to many "false positives" for inferring toxicity.

3.05.A.T-03 Using Museum Specimens to Document Historical Contamination

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Natural history museums are an important wealth of data and opportunities for hands-on research. Specimens from museums can offer scientific information and data over snapshots in time while providing the means for scientists to minimize animal collecting; thus, reducing animal use. Oklahoma State University has a small natural history museum of vertebrate specimens with holdings of mammal specimens that have been collected from regions of notable environmental contamination, including the Tar Creek Superfund Site. Tar Creek and surrounding Ottawa County are both a designated NPL (National Priority List) Site in Northeastern Oklahoma. This mining area was responsible for most of the lead and zinc ore mined for both World Wars. The destruction of habitat and landscape from years of mining practices caused the displacement of generations of people from the region, and it also created environmental contamination that is still being remediated today. While studies in the area tend to focus on aquatic wildlife and surrounding landscape toxicity, fewer studies have examined metal distribution amongst mammal populations across broader taxa. This study focuses on select metal distribution in bone tissues in a comparison of both ground dwelling and flying mammals from the Superfund and surrounding locations. Findings indicate disproportionate levels of iron, cadmium, zinc, and lead in bone tissue of mammals in the Superfund Site as compared to reference specimens with the latter three metals showing significant accumulation in the specimens from Ottawa County resulting in possible displacement of iron. This type of study is important to further the knowledge of how large-scale environmental contamination can affect non-static populations without the necessity of additional on-site collecting.

3.05.A.T-04 Field Measurement of Cesium-137 in Live Passerine Birds Inhabiting Radiological Contamination Areas

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Cesium-137 (Cs-137) is a radioactive byproduct of nuclear fission that occurs in the environment due to global fallout, accidental releases of nuclear waste, and reactor malfunctions. Efforts to contain Cs-137 contamination rely on its strong affinity for sediment, which limits its mobility and bioavailability. However, the disruption of sediment by wildlife can remobilize adsorbed Cs-137 and increase exposure risk for humans. Thus, biomonitoring of Cs-137 remains a priority but is limited by a reliance on lethal sampling and a lack of portable, field-based detection systems with adequate sensitivity. The Savannah River Site (SRS) is a U.S. Department of Energy property in South Carolina where Cold War-era production led to the release of thousands of GBqs of Cs-137 to the environment. Passerine birds are suspected biotic vectors of Cs-137 from legacy contamination zones to highly trafficked areas on the SRS, but the extent to which passerines uptake Cs-137 remains unknown. The goal of this study was to evaluate the transfer of Cs-137 to passerines inhabiting SRS radiological contamination areas. We captured 143 birds of 31 species at two sites impacted by Cs-137 influx from nuclear reactor effluent and two unimpacted sites. We conducted in-situ measurements of Cs-137 activity in live birds using a portable counting system with an average detection limit of 1.75 Bq. Cesium-137 was detected in 27 birds (mean = 48 Bq/kg, SD = 41 Bq/kg). Hurdle models indicated that the likelihood of detection and the magnitude of Cs-137 activity were significantly higher in birds inhabiting contaminated sites compared to birds at unimpacted sites. Additionally, Cs-137 was more likely to occur in birds that forage and/or nest on the ground. This study reveals that passerines can accumulate Cs-137 from contaminated soil, though the radioactivity levels in all birds were below the SRS threshold of concern. We demonstrate an in-situ detection method that is suitable for non-lethal, field-based measurements of small animals emitting low levels of radiation from Cs-137.

3.05.A.T-05 Wildlife Toxicity: Innovative Approaches for Evaluating Exposure and Effects of Contaminants in Free-Ranging Wildlife and Laboratory Animals Models

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Following U.S. Environmental Protection Agency guidance, wildlife risk assessment at contaminated sites intends to evaluate effects on populations, rather than individuals; however, the number of individuals that comprises a population is not defined. One concept that has been used to define a population for risk assessment is the minimum viable population size (MVP). A number of publications have estimated the MVP for many species using population modeling, where the definition of an MVP is often defined as the size at which there is a 99% probability the population will remain extant for 40 generations. However, the MVP for any particular species can vary depending on the assumptions and methods used in the population model. We evaluated how the MVP for one example species, the American robin, changes when using different population modeling software (RAMAS, Vortex, Poptools, R), model assumptions, and model inputs (e.g., inclusion of density-dependence, ceiling carrying capacity, catastrophes, inbreeding). These different model approaches result in wide variation in the MVP and its confidence interval for the American robin. We present recommendations on methods and assumptions to use to develop MVPs and, for non-modelers, discuss selection of the best MVP available in the literature for risk assessment purposes. The recommendations depend on the site-specific conditions, species evaluated, and objectives of the wildlife risk assessment.

3.05.B.T Wildlife Toxicity: Innovative Approaches for Evaluating Exposure and Effects of Contaminants in Free-Ranging Wildlife and Laboratory Animal Models

3.05.B.T-01 Associations of Environmental Chemical Mixtures on Growth of Nestling Tree Swallows

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Wild birds are exposed to a mixture of environmental pollutants that include perfluoroalkyl acids (PFAAs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs). Our objectives were to determine possible influences of these pollutants, both individually and in combination as a chemical mixture, on growth of nestling tree swallows (*Tachycineta bicolor*) raised by the effluent outflow of a large urban wastewater treatment plant and at a reference site (conservation area). Ten-day old nestlings were analyzed for 16 major PFAAs (12 perfluorocarboxylic acids (PFCAs), four perfluorosulfonic acids), 35 PCBs, 21 PBDEs, and 18 OCPs. PFAAs were highly prevalent in nestling carcasses, with 14 of 16 measured isomers detected in > 50 % of samples. Detection rates were more variable for the remaining compounds, being measured in > 50 % of samples for 9 of the 35 measured PCB congeners, 7 of 21 PBDE congeners, and 5 of 18 OCPs. Individually, legacy perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and BDE-28 concentrations in the chicks were positively correlated with their weight, suggesting that larger chicks had higher body burdens of PFOS, PFOA and BDE-28. In contrast, PCB-105, -110, -118, -138, and -153 were negatively correlated with nestling weight, and hexachlorobenzene (HCB) and perfluoroundecanoic acid (PFUdA) were negatively associated with wing chord length. Generalized linear models identified which chemicals in this mixture of PFOS, PFOA, BDE-28, PFUdA, HCB and the five PCB congeners significantly explained chick growth. Preliminary results suggest that, in this environmental mixture, only BDE-28 significantly explained nestling weight, whereas BDE-28 and PFUdA explained wing chord length. That is, higher BDE-28 concentrations may lead to heavier chicks with longer wings, the latter also influenced by chicks accumulating PFUdA. As nestling growth and size is optimized to coincide with maximal food availability to ensure fledging success and fledgling survival, heavier chicks with longer wings may have increased energetic demands that may or may not exceed available food resources. Results of additional modelling investigating the influences of environmental contaminant mixtures on fledging success may be presented.

3.05.B.T-02 Modeling PFAS Bioaccumulation in Terrestrial Food Webs

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Food web models allow ecological risk assessors to use concentrations of per- and polyfluoroalkyl substances (PFAS) in abiotic media (soil, sediment, water) to predict concentrations of PFAS in wildlife diet items, enabling estimation of site-specific dietary doses for wildlife. This provides a critical tool to determine potential risks to wildlife receptors in the ecological risk assessments (ERAs) at PFAS-impacted sites. Validation of food web models for PFAS is critical to ensure confidence around food web modeling for PFAS and use of this tool at PFAS-impacted sites. The abundance of abiotic and tissue data at PFAS field sites that have emerged in the last few years allowed our team to complete validation of aquatic food web models using abiotic data for sediment and surface water and paired tissue data from ten high quality field studies of PFAS in aquatic environments. Model-predicted concentrations of PFAS

in biota (invertebrates, plants, and fish) were generally within a factor of 5 to 10 of the measured values for these studies. Model agreement was robust for forage fish, especially for higher PFAS concentrations that would be expected at Aqueous Film-Forming Foam (AFFF) sites. While terrestrial field data at PFAS-impacted sites are more limited, we have validated terrestrial food web models for PFAS using soil and biota concentrations from available field studies. As part of the model validation effort, Monte Carlo functionality was incorporated into the model so that validation can demonstrate the variability from soil PFAS concentrations and terrestrial bioaccumulation factors as part of the model results. This research will allow us to better understand the current performance of the terrestrial bioaccumulation model and determine whether any improvements or refinements are warranted. The validation effort will also lend further confidence to the terrestrial PFAS food web model and support its use in determining potential risks to wildlife at hazardous waste sites impacted by PFAS.

3.05.B.T-03 Do Highly Organohalogen-exposed Ring-billed Gulls Nesting in an Urbanized Environment Have Perturbed Hormones and Energy Metabolism?

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Previous studies on ring-billed gulls (*Larus delawarensis*) breeding in the greater Montreal area have reported high plasma levels of a range of legacy and emerging halogenated flame retardants (HFR) and per- and polyfluoroalkyl substances (PFAS). The exposure of wild birds to these organohalogen contaminants has been suspected to disrupt hormone regulation and energy metabolism, suggesting potential adverse effects on reproduction and health. While the sources and pathways of exposure to these organohalogens in Montreal-breeding ring-billed gulls have been documented, little information is available on their endocrine and metabolism related effects. This study aimed to explore the linkages between PFAS and HFR exposure and major hormones involved in energy metabolism and other markers of energy metabolism in ring-billed gulls. A total of 60 breeding gulls, males and females, were equipped with miniature GPS dataloggers coupled to accelerometers to characterize their foraging habitat use strategy and energy expenditure based on the measurement of overall dynamic body acceleration (ODBA) for 48h. Plasma levels of thyroid hormones and their metabolites (T_3, T_4, rT_3, $3,3T_2$, and $3,5T_2$), major glucocorticoids (e.g., corticosterone, cortisol, cortisone), energy metabolites (carbohydrates e.g. glucose, lactate or lipids e.g. β-hydroxybutyrate (BHB)) as well as PFAS and HFR were analyzed in plasma, and the relationships between these variables and the gull's foraging habitat use and energy expenditure were investigated. Preliminary results showed that both male and female gulls preferentially foraged in agricultural (25%) and residential areas (31%) during the last 48h prior to recapture. Significantly greater energy expenditure was calculated for males (0.57 ± 0.02 *g*) compared to females (0.50 ± 0.01 *g*) during the same period. ΣPFAS concentrations in plasma were in general greater in males (118 ± 13.6 ng/g ww) than females (47.9 ± 11.5 ng/g ww). Plasma $3,3T_2$, $3,5T_2$ and rT_3 levels were negatively correlated (r =-0.34 to -0.50; $p < 0.001$) with ΣPFAS concentrations, particularly in females. Additionally, correlations were observed in females between ΣPFAS concentrations and those of the energy metabolites BHB ($r = 0.30$; $p = 0.022$) and lactate ($r = -0.41$; p =2 0.0016). These preliminary findings suggest that highly organohalogen-exposed ring-billed gulls nesting in an urbanized environment may exhibit perturbed hormone regulation leading to altered energy metabolism.

3.05.B.T-04 Gastric Lavage is an Imprecise Method for Sampling Ingested Microplastics in a Wild Passerine Bird

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Microplastic pollution has become a global concern and understanding its impact on wildlife requires effective sampling techniques that quantify exposure. In particular, non-lethal sampling techniques are needed for passerines for which microplastic exposure is poorly understood. In this study, we evaluated the efficacy of gastric lavage as a non-lethal method to assess microplastic ingestion in passerine birds. We sampled Brown-headed Cowbirds (*Molothrus ater*) (*n* = 105) from Government Canyon State Natural Area in San Antonio, Texas, United States (US). We performed gastric lavage to recover microplastics from each bird, before euthanizing them and dissecting gastrointestinal tracts. We recovered microplastics from 99% of birds. Gastric lavage recovered an average of 50.4% of ingested microplastics although recovery rate was highly variable (range: 0-100%, coefficient of variation: 59.52%), indicating much uncertainty in estimating individual total microplastic loads from gastric lavage. Sampling date influenced microplastic loads and recovery rates, which may be due to untested microplastic-environment interactions or may be an artifact of sampling conditions. Recovery rate was unaffected by time of day, bird age, sex, or body condition, or microplastic shape. Overall, our findings suggest that gastric lavage may not be a suitable non-lethal microplastic sampling technique for passerine birds when precise data are needed. Instead, it may be useful for studies where coarse measures of exposure are wanted, or when large differences in microplastic loads are expected.

3.05.B.T-05 Isomer Specific Formulations of Anticoagulant Rodenticides May Reduce Risk to Non-target Wildlife

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Anticoagulant rodenticides (ARs) have a long history of use for control of vertebrate pest species. Despite regulatory efforts to mitigate risk, non-target wildlife can be unintentionally exposed to ARs through various pathways, and depending on dose, adverse effects and mortality may occur. Second-generation ARs (SGARs) are mixtures of *cis*- and *trans*-stereoisomers that are equipotent when tested *in vitro* (microsomal vitamin K epoxide reductase activity from susceptible rats) and *in vivo* (no-choice bromadiolone and difethialone feeding trials in rodents). However, some stereoisomers are preferentially metabolized *in vivo*, resulting in residue patterns in exposed target rodents that differ from the bait formulations. Use of less persistent but equally potent SGAR isomers in baits result in lower tissue residues in target rodents and constitute lower risk when consumed by non-target wildlife. We examined the toxicity of diets with varying brodifacoum stereoisomer composition in captive American kestrels (*Falco sparverius*), an important model species used to assess AR hazard in non-target hawks and other raptors. In a

7-day dietary trial, kestrels were fed brodifacoum formulations with stereoisomers having markedly different elimination half-lives in rats (Formulation A with the 2 least persistent stereoisomers in rats; Formulation B containing only the most persistent stereoisomer). Based on previous studies, brodifacoum formulations A and B were each provided at 3 dietary concentrations (0.05, 0.1 and 0.5 ug/g diet, 4 kestrels/dose level) predicted to cause a range of toxicity. Compared to unexposed controls, all kestrels ingesting 0.5 ug/g diet of the longer-lived Formulation B exhibited extreme coagulopathy (prolonged prothrombin time and Russell's viper venom time), while 0.5 ug/g diet of the shorter-lived Formulation A yielded only a modest lengthening of clotting time in just 1 of the 4 birds. There was evidence of altered immune function (increased plasma haptoglobin-like activity and β-globulin fraction, decreased albumin to γ-globulin ratio) in kestrels exposed to 0.5 ug/g of Formulation B. At these dose levels, microscopic evidence of hemorrhage was not related to dietary concentration of either brodifacoum formulation. These findings support the notion that SGAR baits enriched with less persistent stereoisomers pose lower hazard and risk to non-target wildlife and are likely more eco-friendly than currently used SGAR formulations.

3.05.B.T-06 Phthalate Metabolite Detection in Blubber of Common Bottlenose Dolphins (*Tursiops truncatus*) Stranded Near Sarasota Bay, Florida, USA

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Phthalates are endocrine-disrupting chemicals found in many consumer and industrial products, leaching into the environment and potentially impacting wildlife, including common bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida, USA. Evaluation of phthalate exposure in this community has previously used urine samples from free-ranging dolphins collected during health assessments, which demonstrated measurable exposure across sexes and age classes. This study aimed to examine possible phthalate exposure in stranded dolphins targeting a suite of phthalate metabolites (mPAEs) in blubber since urine sampling from stranded animals can be challenging. Blubber samples were collected from 44 deceased bottlenose dolphins (codes 2 and 3) during 2010-2023 (adults: n = 21, subadults, n = 11; calves: n = 12; females: n = 23, males: n = 21) by the Mote Marine Laboratory Stranding Investigations Program. This study utilized an updated and modified method to isolate mPAEs from blubber samples (~200 mg) using sonication in ammonium acetate and solid-phase extraction. Twelve mPAE compounds were quantified via liquid chromatography tandem mass-spectrometry. Overall, 43% of samples (19 of 44) had at least one mPAE concentration above the limit of detection. Detectable concentrations of seven mPAEs were observed, including monobutyl phthalate (MBP; n = 1), monobenzyl phthalate (MBzP; n = 2), mono-2-ethylhexyl phthalate (MEHP; n = 16), monoisobutyl phthalate (MiBP; n = 1), monomethyl phthalate (MMP; n = 3), mono-n-octyl phthalate (MnOP; n = 15), and monoisodecyl phthalate (MIDP; n = 1). MEHP, one of the most frequently detected mPAEs in this study and others, ranged from 2.3 ng/g wet weight to 29.9 ng/g wet weight, consistent with free-ranging dolphin blubber concentrations. Among the 19 dolphins with detectable mPAE levels, 12 were known to the Sarasota Dolphin Research Program as Sarasota Bay or Gulf of

Mexico residents, providing an opportunity to explore potential relationships between phthalate exposure, habitat use, and indicators of morbidity and mortality.

3.05.P-Mo-055 Navigating the Complexity of Bird Life History Traits to Better Evaluate Exposure to Environmental Chemicals

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As part of a constant effort to protect wildlife, risks from exposures to environmental chemicals should be evaluated as accurately as possible. This is a formidable challenge, considering the number of species involved, and their complex interactions with each other, and with their environment. Consequently, exposure evaluations must often be simplified, using a series of approximations. Often, some assumptions are necessary to compensate for species-specific data gaps, but also to simplify calculations used in a screening approach. Consequently, exposure estimates rarely represent actual field conditions. More specifically, characterizing birds' exposure to environmental chemicals is particularly challenging given their species diversity, ecological and physiological plasticity, complex seasonal life histories and seasonal niche partitioning, and physiological variations. Part of this complexity is reflected in EFSA's recent regulatory guidance, which accounts for parameters such as foraging guild or habitat preference to refine exposure evaluations in higher tiers of the risk assessment (EFSA 2023). Furthermore, efforts using models that include habitat preferences in spatial environments have been shown to result in more realistic exposure estimates. However, in both cases, species-specific data such as food and water ingestion rates, food choices, and habitat preference, although they are important, are rarely available. To advance knowledge in this space, a sub-group of HESI's Next Generation Ecological Risk Assessment Committee is working on establishing a state of science on the influence of birds' life history traits (LHT) on chemical exposure. We explore the value of integrating species-specific traits and behaviors (e.g., habitat preferences, migratory and dispersal capacity, foraging guild, seasonal changes in food choices), that can modulate chemical exposure and biological responses. We also developed a database of LHT to identify potential impactful data gaps and establish trait-dependent commonalities among birds to help more accurately characterize their exposure to various types of contaminants. Based on species vulnerability, we propose a prioritization strategy for these traits to help determine those having the greatest influence on chemical exposure predictions. We provide a qualitative analysis of behavioral and LHT most useful in increasing the precision of exposure estimates and highlight areas in need of additional research.

3.05.P-Mo-056 Evaluation of Minimally Invasive Metabolomic Methods for Assessing the Sex and Health of Sturgeons

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Almost all species of sturgeon are at risk of extinction throughout their natural range due to a variety of human activities. Unfortunately, efforts to conserve and restore stocks of wild sturgeon are hindered by their typically slow rate of maturity and infrequent spawning. Additionally, the difficulty of determining sex without highly invasive methods has confounded efforts to effectively monitor sex ratios in populations and assess sex-specific responses to environmental stressors. Here we have evaluated the efficacy of metabolomics for differentiating sexes among captive populations of Russian sturgeon (*Acipenser gueldenstaedtii*) and Siberian sturgeon (*Acipenser baerii*), using minimally invasive sampling techniques that are well suited for vulnerable species. Specifically, we report the serum and epidermal mucous metabolomes, with extensive endogenous metabolite assignments, using high resolution NMR spectroscopy. Blood serum samples were further characterized for higher molecular weight components (e.g., lipoproteins and acute phase proteins) that are closely associated, for example, with growth, development, and stress responses. Multi- and univariate statistical analyses of these datasets suggest that the Russian sturgeon blood serum metabolome and the Siberian sturgeon epidermal mucous metabolome are highly sex specific. Apart from sex-specificity, these datasets also provided rich biochemical information that should improve assessments of health and wellbeing of sturgeons in the natural environment. These findings support the use of non-lethal metabolomics for monitoring sex-specific and general health effects from contaminant exposures in wild sturgeons. *The contents of this abstract neither constitute nor reflect U.S. Environmental Protection Agency policy.*

3.05.P-Mo-057 Investigation of Fecal Microplastic Accumulation, and Associated Changes in Gut Microbiome in Florida Manatees (*Trichechus manatus latirostris*)

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Microplastics, defined as plastic debris measuring 5 millimeters or less in size, are a pollutant of emerging concern that are known to be in nearly all aspects of marine environments including the air, sediment, waterbodies, plants, and animals. Due to the highly variable size, shape, and density of microplastics, they are widely dispersed across marine ecosystems. Specific to Florida, the peninsula is surrounded by the Gulf of Mexico and the Atlantic Ocean, both of which are known to be polluted with microplastics. This leads to a high availability for ingestion across different trophic levels, including marine megafauna. When introduced to the body, microplastics can cause mechanical damage to the digestive tract by causing perforations or abrasions to the gut mucosal lining, the first line of defense against pathogens and bacterial translocation. Microplastics can also influence the type or amount of food being eaten which leads to the gut symbionts being deprived of crucial nutrients and substrates which could change the overall gut community. This study proposes an innovative, noninvasive method that pairs the investigation of microplastic presence and microbiome changes in Florida manatees (*Trichechus manatus latirostris*) by examining their fecal matter. Studies on other animals have been conducted using fecal samples as a proxy for gut microbiome but this approach introduces microplastic accumulation as a possible explanation for microbiome changes in a novel species. Floating fecal samples were collected from the water and analyzed for microplastic presence and sequenced to analyze microbiome conditions. Archived samples from other Florida areas and time points over the last decade were used as a comparison to assess microbiome differences. Our data suggests that there were changes in the manatee gut microbiome potentially related to microplastics. Studies are ongoing to evaluate these trends over varying Florida locations and time periods using this combined method in conjunction with archived manatee fecal samples from U.S. Geological Survey. This information will be used to compare fecal collection and storage methods and give insight into how microplastics could influence the microbiome given changes in time and location. This study will provide a deeper understanding of how the health of manatees, and other marine mammals, including changes in gut microbiome, might be affected by the ingestion of microplastics.

3.05.P-Mo-058 Per- and Polyfluoroalkyl Substances (PFAS) in Small Cetaceans Used for Human Consumption in St. Vincent and the Grenadines, Eastern Caribbean

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At least 54 species of small cetaceans are consumed by humans in 86 countries worldwide, and there is evidence that these species are increasingly exploited around the world, especially in countries of the Global South. Cetaceans are high trophic level marine predators with long life spans, which makes them ideal sentinels of emerging contaminants. Per- and polyfluoroalkyl substances (PFAS) are persistent organic pollutants found ubiquitously in the environment, including marine ecosystems. With concerns regarding their potential adverse effects on human health, understanding their presence in marine species consumed by humans is crucial. PFAS have been found to accumulate in cetaceans, but there is no information on their presence in cetaceans used for human consumption. Every year, several hundred small cetaceans, primarily short-finned pilot whales (*Globicephala macrorhynchus*), are harvested for local consumption in St. Vincent and the Grenadines (SVG), in the eastern Caribbean. In this study, we investigated the occurrence and concentration of 30 PFAS in the muscle tissue of 30 short-finned pilot whales harvested in SVG from 2022-2023. Specimens were measured, sexed, and muscle samples were collected, freeze dried, homogenized, mixed with internal standard and a 0.01 mol/L methanol solution containing potassium hydroxide for 30 minutes ultrasonic extraction. The resulting extracts underwent clean-up, evaporation, and filtering process prior to liquid chromatography-tandem mass spectrometry analyses. The findings of this research will address ecological and human concerns, since these compounds are endocrine disruptors and can lead to adverse immunological, reproductive, and even carcinogenic effects. Considering that small cetacean food products are consumed by nearly 70% of Vincentians, continuous PFAS exposure monitoring in these sentinel species is paramount to aid government agencies in formulating informed mitigation and conservation strategies to reduce impact of PFAS for both human and cetacean health.

3.05.P-Mo-059 Colonial Waterbirds as Sentinel Species for Long-Term Monitoring of Population, Reproductive, and Immune Effects at Contaminated Great Lakes Sites in Michigan

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Colonial waterbirds are excellent sentinel species for assessing and monitoring effects of persistent organic pollutants (POPs), including population trends, reproductive problems, and health effects such as immunotoxicity. Ongoing effects in these species are relevant to Great Lakes issues managed by federal and state agencies, including considerations about legacy contaminants and chemicals of emerging concern (CECs) at specially designated Areas of

Concern (AOCs), in contaminated areas not designated as AOCs, and in lake-wide regions. This program has monitored effects in fish-eating birds in the Saginaw River and Bay and River Raisin AOCs and in Grand Traverse Bay from 2010-24 (for most sites). Although not an AOC, Grand Traverse Bay was studied because of its high levels and unique mixture of contaminants. Herring gull eggs in the two AOCs had high dioxin equivalents (TEQs) driven by planar polychlorinated biphenyls (PCBs), while eggs from Bellow Island in Grand Traverse Bay had elevated TEQs from dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs). Reference sites were in the lower St. Mary's River (herring gulls and Caspian terns), eastern Lake Superior (terns), and eastern Lake Huron (black-crowned night herons). Relative risk ratios for embryonic nonviability (infertility and mortality) in gull embryos were 2-3-fold higher than reference in both AOCs and Grand Traverse Bay. Deformities (e.g., crossed bills, gastroschisis, edema) were found in embryos and chicks at contaminated sites. Productivity of 4-week-old tern chicks in Saginaw Bay was 38% lower than reference. In the River Raisin AOC, productivity of 4-week gull chicks was poor in 7of 12 years. Breeding Caspian terns, a state-threatened species, declined 42% in the Saginaw River and Bay AOC, and nesting herring gulls decreased >95% in the River Raisin AOC. The mean phytohemagglutinin skin response (T cell-mediated immunity) was suppressed 51-57% in gull chicks in both AOCs and Grand Traverse Bay, and 50% in terns and 33% in herons in Saginaw Bay. Antibody responses in gull chicks in the River Raisin AOC and Grand Traverse Bay were 1.4-1.7-fold lower than reference. Effects did not improve with time. Tissue residues demonstrated ongoing elevated exposure to PCBs in the River Raisin and Saginaw Bay AOCs. PFAS and brominated CECs were elevated in tissues at most sites. Overall, this biomonitoring program demonstrated ongoing immunological, reproductive, and population effects at contaminated sites.

3.05.P-Mo-060 Evaluation of Per- and Polyfluoroalkyl Substances (PFAS) in Eggs of Higher Trophic Level Birds

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The study aims to evaluate the exposure of populations of water-associated bird species to per- and polyfluoroalkyl substances (PFAS). We evaluated if modeling risk from PFAS in bird eggs and fish, combined with existing laboratory-derived toxicity reference values (TRVs), reflects ongoing field monitoring. We focused on bird species at a high trophic level, taking advantage of stores of bird eggs collected from past and ongoing monitoring programs. Repurposing previously collected samples extended their use to provide a non-invasive means of studying exposure to and effects in free-ranging wildlife and provided significant cost savings. PFAS analyses was performed on archived egg samples from four piscivorous species: hooded merganser (*Lophodytes cucullatus),* common loon (*Gavia immer*), double-crested cormorant (*Nannopterum auritum*), and osprey (*Pandion haliaetus*). Homogenized whole egg PFAS concentrations were compared to laboratory-determined chemical-specific bird egg TRVs for two PFAS compounds (perfluorooctane sulfonate [PFOS] and perfluorohexanoic acid [PFHxA]) to determine hazard quotients (HQs) as a means of predicting adverse effects. Health assessments from development data were qualitatively compared with HQs. Next steps will include assessing spatiotemporal trends, evaluating PFAS exposure relative to known source areas, and determining anthropogenic background concentrations for PFAS in eggs of these piscivorous bird species. We will use findings to evaluate the application of laboratory-based

TRVs to wild bird populations. Results will also aid in understanding PFAS contamination, PFAS risk assessments, and provide insights for contaminated site investigations.

3.05.P-Mo-061 The Ecological Protective Concentration Level (PCL) Database—An Online Tool for Streamlining Ecological Risk Assessments in Texas, USA

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Ecological Risk Assessment (ERA) is an integrated part of contaminated site management and often drives cleanup decisions. Performance of an accurate, integrated, and well-researched ecological risk assessment is paramount to site remediation and closure. However, this process is often time and resource consumptive, requiring long periods of literature review on contaminant toxicity, fate and transport, and exposure profiles of native species. There is a major need for a tool which facilitates the process of Tier 2 or 3 ERAs by generating accurate and defensible protective concentration levels (PCLs). Over the past 24 years, West Texas A&M University (WTAMU) and the Texas Commission on Environmental Quality (TCEQ) have developed the PCL Database, and the TCEQ continues to support improvements and maintenance of the Database through a contract with WTAMU. This database calculates accurate, defensible PCLs for 126 chemicals of concern (COCs) and 113 species of wildlife native to Texas. These chemicals and wildlife were chosen based on discussions within the Database work group and are representative of commonly found contaminants at Texas Risk Reduction Program sites. Each COC contains its own toxicological profile which allows the user to review relevant information on the COC's fate, transport, bioaccumulation, and toxicity. Species were chosen based on food webs presented in TCEQ ERA guidance and are divided amounts the seven major habitats in Texas, with an additional two "minor" habitats also represented. Each species has a file containing its own exposure factors, which contains input data on the species' ingestion rates, home range, and other life history information. PCLs were developed based on the protection of growth, reproduction, and mortality of the indicator organisms, leading to potentially tens of thousands of PCL calculations. The WTAMU/TCEQ has revisited the methodology for predicting bioaccumulation of COCs using empirically derived models (e.g., ORNL, U.S. Environmental Protection Agency). Integration of recent bioaccumulation models for inorganic compounds, polycyclic aromatic hydrocarbons, halogenated organics, and other COC classes including perfluorinated compounds will facilitate the calculation of defensible PCLs for receptors located throughout the state's diverse habitats. The PCL database is a useful tool in facilitating rapid and accurate ecological risk assessments and ensuring more cost-effective and scientifically defensible cleanup decisions.

3.05.P-Mo-062 Consumption of Thiamethoxam Coated Seeds Causes Multilevel Effects to the Passerine Agelaioides Badius

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Thiamethoxam (TMX), one of the main neonicotinoids used for seed coating, has previously been detected in wild birds. TMX has medium to low acute toxicity to birds, but its sublethal effects have been little studied in passerines. This study aimed to characterize the effects at multiple levels of consumption of TMX treated seeds in the grayish baywing (Agelaioides badius). Four groups of adults (N = 8) were fed ad libitum for 21 days with seeds containing 0 (control), 0.027 (low), 0.33 (medium), and 4.3 (high) g TMX/kg seed, these are approved concentrations for seed coating in the Pampa Region of Argentina. Seed consumption and survival were estimated daily. Behavioral parameters (i.e., response to predator call, feeding events, and activity), and weight were assessed weekly. At the end of exposure were measured: the hematocrit, enzymatic activity in multiples tissues (i.e., catalase [CAT], cholinesterase [ChE], and glutathione-S-transferase [GST]), and genotoxicity with comet assay. Average daily doses were 2.94 (low), 38.0 (medium), and 619.7 (high) mg TMX/kg body weight. Mortality occurred only in high treatment by 50% between 3 to 15 days. Birds from high treatment experienced an early loss of body weight that remained throughout the experiment ($p < 0.001$), in spite an increase in the number of feeding events ($p = 0.041$), and an increase in seed consumption ($p = 0.0019$), which indicates a lack of aversion to treated seeds. No treatment effect was observed on the activity of birds, measured as the number of jumps ($p = 0.499$), but there was an effect of time, being the birds less active as the experiment progressed ($p < 0.001$). Response to predator stimuli was decreased in high treatment, and a similar tendency was observed in medium treatment ($p = 0.046$, $p < 0.1$, respectively). At the end of exposure, the hematocrit was reduced in medium and high groups in a dose-response way. Compared to control, high treatment individuals increased the activities of GST (i.e., muscle and kidney), ChE (i.e., muscle), and CAT (i.e., kidney); while low treatment increased the activities of ChE and GST in the liver ($p < 0.05$). Medium and high treatments showed an enhancement of the genetic damage ($p < 0.01$ and $p < 0.001$, respectively). This study shows that consumption of seeds treated with approved concentrations of TMX can trigger multiples effects in a passerine bird, which may impair survival in the wild.

3.05.P-Mo-063 What Can('t) PCBs Teach Us About PFAS Bioaccumulation?

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Halogenated compounds that became globally distributed, accumulating in people and wildlife everywhere before they were accidentally discovered. A class of chemicals so persistent they represented "perhaps the most stable organic substances in existence." When Sören Jensen wrote these words in 1972's "The PCB Story", they were not about PFAS but about PCBs. Fast forward more than half a century, and now PFAS are considered the most ubiquitous and persistent organic contaminants. The focus on PFAS has launched countless new scientific studies, models and approaches, because they seemingly broke the mold about much of our understanding of bioaccumulative organic contaminants. For example, many PFAS are charged, making the definition, let alone the measurement, of traditional partition coefficients intractable. They bind to proteins rather than accumulate in adipose tissue. Yet much in the same way as PCBs are still found in top marine predators today, so too are PFAS. How different are the drivers of their bioaccumulation, really, and how much can we still learn about patterns observed (and perhaps ignored) about PCBs? This talk will discuss the current state of the science on PFAS bioaccumulation, encompassing trophic magnification, phospholipid partitioning, and protein interactions, based on research from the author and the broader PFAS community, and will draw comparisons with long-term observations about PCBs. The focus will be largely on wildlife, though some implications for human exposure and public health will also be touched on.

3.05.P-Mo-064 How Could Earlier Phase-out of PCB Production Have Reduced Diabetes Cases in the U.S.?

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Although the U.S. entirely ceased PCB production in 1979, the history of PCB regulation in this country is, unfortunately, characterized by a series of missed opportunities for an earlier phase-down. There were multiple critical events that could have prompted an earlier reduction in PCB production, underscoring the contrast between potential proactive measures and the actual lack of action. In this presentation, we estimate the number of cases of diabetes that could be avoided if prompt measures had been implemented to phase out PCBs during certain pivotal moments, decades ahead of the actual timeline. Specifically, we designed three alternative scenarios for the phase-out of PCB production: Scenario 1 starts in the early 1970s, aligning with the peak of public pushback; Scenario 2 starts in the mid-1950s, aligning with the wide recognition of PCBs' hazards by downstream users; and Scenario 3 starts in the early 1940s, aligning with academic discovery of PCBs' hazards. We modeled the prevalence of diabetes attributable to PCB exposure in the U.S. Our quantitative analysis shows the greatest public health benefits would be achieved if PCBs were phased down at early stages where concerns about PCBs' toxicity were concentrated in occupational settings and were limited to the academic sphere (Scenario 3). This scenario helps avoid to the largest extent PCB levels observed in blood across the cross-sections of 1940 (>3%), 1980 (>96%), 2020 (>85%) and prevent most cases of diabetes (1940: 170; 1980: 55000; 2020: 40700). Unfortunately, early warning signs of PCBs' toxicity were constricted to Monsanto employees and a few academics, leading to insufficient public knowledge or public health evidence to realistically halt production. By contrast, phasing down PCBs would become far less effective for public health if actions were taken only after widespread contamination and public impact (Scenario 1). This scenario slightly reduces PCB levels observed in blood in the cross-sections of 1980 (>3%) and 2020 (>5%) and prevents smaller numbers of diabetes cases (1980: 4200; 2020: 3000). This means waiting until public outrage to halt the production of a toxic chemical is ultimately waiting for public and environmental harm to justify the cessation. It can be most cost-effective to halt the production of a toxic chemical when the harm of a chemical becomes a consensus among downstream users in the industry.

3.05.P-Mo-065 In-Depth Analysis of Heavy Metal and Pesticide Presence in Fecal Samples from African Savanna Elephants of Lower Zambezi National Park, Zambia

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Heavy metal and pesticide exposure is a global issue with severe ecological impacts. Large herbivores like elephants, with diverse diets and migration patterns, are particularly vulnerable to these toxins. The Lower Zambezi National Park in Zambia, home to wild African savanna elephants and various native species, faces significant environmental challenges due to copper mine construction, despite the government's recent license cancellation. The construction already damaged a large area in 2023, and future activities remain uncertain. Increased human activities and poaching also pose high risks, leading to exposure to toxic chemicals. In this study, we focused on heavy metals and pesticides linked to human activities. We used elephant fecal samples as a non-invasive method to assess the accumulation of these chemicals in both the animals and the park's environment. In November 2022, we collected samples from the east side of the park, including 30 plant, 59 soil, and 33 elephant fecal samples. In September 2023, samples were collected from the west side, covering 150 soil, 120 plant, and 75 elephant fecal samples. We quantified metals like Cr, Cd, Pb, As, Cu, Co, Ni, Se, Zn, and Hg using Inductively coupled plasma-mass spectrometry and analyzed 368 pesticides using two type of liquid chromatography(LC-MS/MS and Q-TOF/MS) and gas chromatography mass spectrometry (GC-MS). Our results showed concentration differences based on the sampling area and season, with significant variations in Se, Co, and Ni concentrations between 2022 and 2023. Ni exhibited higher levels in 2023, while Co showed the opposite trend. High concentrations of metals such as Cd, Pb, As, Cr, Co, Cu, and Se were found in one elephant fecal sample from 2023, indicating potential higher exposure in the nearby environment. The 2023 sample site received more tourists, possibly contributing to this difference along with seasonal variations. Hierarchical clustering analysis revealed a group of elephants with high metal concentrations, predominantly females (70%) compared to males (23%). Trace amounts of pesticides were detected in some samples but were low. The accumulation of heavy metals was observed even without extensive mining. These findings provide valuable insights for evaluating the environmental impact of chemical substances and implementing measures for environmental protection within the park.

Track 4: Chemistry and Exposure Assessment

4.01.P-Mo 21st-Century Challenges in Developing Countries

4.01.P-Mo-066 Challenges in Water Reuse: Pharmaceutical Removal Efficiency in Durban's Wastewater Treatment Plants

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As global water scarcity increases, treated wastewater reuse has emerged as a critical component of sustainable water management. Sewage wastewater contains pharmaceutical micropollutants, making wastewater treatment essential to prevent serious environmental adverse effects. Pharmaceuticals and other micro-pollutants are found in sewage in high amounts and can harm the ecosystem. Pharmaceuticals have been detected in aquatic systems worldwide due to widespread use, inadequate wastewater treatment plant (WWTP) removal efficiency, or no WWTPs. The increasing presence of pharmaceutical contaminants in wastewater presents a significant challenge for WWTPs, especially in regions with high consumption rates of pharmaceuticals, such as Durban, South Africa. Over four days, wastewater samples were taken from the Modern Advanced Membrane Aerated Biofilm Reactor (MABR) and Durban Water Recycling Plant (DWR), as well as conventional Northern, KwaMashu, Amanzitoti, and Umbilo WWTPs. These samples were then analyzed using advanced SPE-based LC-MS/MS techniques to screen 50 pharmaceuticals. The results indicated variable removal efficiencies among the different WWTPS, with some pharmaceuticals showing high persistence through conventional WWTPs. The overall removal efficiency of the six WWTPs tested ranged from 0% to 98 %, with an average of 30%. Modern high-tech MABR (42%), and DWR (39%) WWTPs demonstrated the best average removal effectiveness, while conventional Northern (18%) and Amanzitoti (19%) WWTPs had the lowest. In our study, several compounds like Famciclovir, Clonazepam, Mirtazapine, Trihexyphenidyl, Acyclovir, Acetazolamide, Flecainide, Promethazine, Clotrimazole, Loperamide, Cimetidine, and Clozapine had lower average removal rates (≤10%), while only Trimethoprim, Paracetamol, Caffeine, and Nitrofurantoin had higher average removal rates (≥70%) by six WWTPs. The study highlights the limitations of current WWTPs in Durban in effectively eliminating these contaminants from sewage wastewater. The findings underscore the challenges and potential strategies for enhancing the removal of pharmaceuticals in wastewater treatment, contributing to safer water reuse practices. This study provides critical insights for policymakers and engineers looking to improve WWTP performance and protect public health and the environment in water-stressed regions.

4.01.P-Mo-067 Determination of Glyphosate, Aminomethylphosponic Acid (AMPA), and Glufosinate in Drinking Water Using Direct Analysis by LC-MS/MS

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Accurate and reliable methods are required to monitor water samples for compliance with regulatory limits and gain a better understanding of the presence of these pesticides in water. Typical methods of analysis have involved derivatization following by LC-MS/MS analysis. However this can be laborious with the excess reagents required to be removed before analysis. A direct injection method on LC-MS/MS removes these steps and is significantly quicker to perform. This approach requires a stable chromatographic method that will give good performance coupled to a high sensitivity tandem quadrupole mass spectrometer. The use of stable isotope analogue internal standards helps mitigate matrix effects. Three different drinking waters (hard, soft and mineral) were used for single laboratory validation work, spiked at 20, 60 and 100 ng/L, with 11 replicates at each level, repeated on three occasions per water type. The interlaboratory validation (9 laboratories) was conducted on mineral water with seven replicates at 20 and 60 ng/L, analyzed once. Labelled internal standards and EDTA were added before analysis. Analysis was by LC-MS/MS on a mixed mode liquid chromatography column connected to a high performance tandem quadrupole mass spectrometer. A 50 µL injection was used and data was acquired for the following analytes: glyphosate, AMPA and glufosinate (plus internal standards). Calibration standards were in the range of 10 to 200 ng/L prepared in deionized water. Results from the single laboratory validation demonstrated good accuracy (trueness and precision) for the quantification of all three compounds in drinking water. The accuracy was determined from the measured values for concentration in the spikes, in all three drinking waters, at 20, 60, and 100 ng/L, after adjustment using the appropriate internal standard. Pooling the results from all three water samples, the trueness, determined as measured recovery, was shown to be between 96 and 102%, repeatability within the laboratory (pooled RSD_r) between 7.9 and 11% and values for reproducibility within the laboratory over 3 days (RSD_{WLR}) were between 7.6 and 15%. These values are within the acceptance criteria set out in the ISO 21253-1:2019 standard (part 2), when using internal standards: “yield” (measured recovery) between 70 and 120% and intermediate precision (RSD_r) ≤20%.

4.01.P-Mo-068 Effortless Alkalinity Analysis Enabled by AI and Smartphone Technology, Without the Need for Equipment

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Alkalinity, defined by its four components (bicarbonate, carbonate, hydroxide, and hydrogen ions), is a crucial water quality parameter with significant environmental and engineered system applications. In natural water systems, it regulates pH, supports primary production, and mitigates ocean acidification, linking to the carbon cycle through biogeochemical processes. In engineered systems, alkalinity influences drinking water treatment, wastewater treatment, biogas production, and boiler water management. Various analysis methods exist, from traditional titrations to advanced spectrophotometric and electrochemical techniques, each with specific benefits and limitations. Developing simple, affordable techniques for alkalinity analysis is essential to facilitate extensive and reliable water quality monitoring, empowering citizen scientists and overcoming financial barriers in traditional monitoring programs. In this work, we developed an equipment-free, user-friendly alkalinity analysis approach accessible to a broad demographic. Our method uses affordable commercial reagents, smartphone photography, and AI/machine learning algorithms for easy alkalinity determination. Specifically, we employed low-cost reagents to generate color changes in response to alkalinity levels in various lake waters and seawater. These images were captured with a smartphone and processed using a machine learning model to correlate color intensity with alkalinity levels. Additionally, we developed a user-friendly website where users, without prior experience, can upload images to obtain alkalinity readings for their water samples. This AI-powered, equipment-free technology represents a significant advancement in water quality monitoring, particularly benefiting developing countries.

4.01.P-Mo-069 Hydrothermal Synthesis of BN-NRGO Composites for Photocatalytic Degradation of PFOA and PFOS

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Water, the primary component of life, has seemingly been stressed by population growth and an increase in industrialization. Various synthetic chemicals, known as emerging contaminants, have been introduced to accommodate the resultant growth. However, continuous usage of these synthetic chemicals has resulted in their subsequent release into the aquatic environment and resultant adverse health implications. Among many other water remediation strategies, sustainable remediation using economical techniques is critical to addressing this problem. Advanced oxidation procedures using graphene-based semiconductor photocatalysts have shown a plausible advantage in solving these environmental challenges. In this study, hexagonal Boron nitride (BN) was synthesized using the chemical vapor deposition method, and to further improve its photocatalytic properties, nitrogen-doped reduced graphene oxide (NRGO) was composited while varying the ratios by hydrothermal synthesis at 180◦C for 12 hours to form BN-NRGO composites which was later used in the photocatalytic degradation of PFAS. These composites were characterized using FTIR, SEM-EDX, UV-VIS, and Raman spectroscopy to investigate their physiochemical and optical properties. The as-synthesized BN-NRGO (1-3) photocatalyst ratio achieved 56% removal efficiency of perfluorinated compounds PFOA and 99% PFOS after 150 mins of irradiation with a UV-visible lamp. The efficiency of the photocatalyst was determined by optimizing catalyst dosage and pH. Ultimately, the optimum degradation of PFOA (87%) was achieved at pH 2 and decreased drastically (about 27%) when the PH was increased to 8 and the catalyst dosage was kept at 100 mg. The phytotoxicity of the degradation products showed no phytotoxic effects on Lactuca Sativa. Thus, BN-NRGO can be used as a potential photocatalyst for the sustainable remediation of persistent organic micropollutants in wastewater.

4.01.P-Mo-070 Small-Scale Mercury Mining on Seram Island, Mollucus Province, Indonesia -Suppling the Small-Scale Gold Mining Industry

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Mercury is in demand for use in small-scale gold mining across many countries. Yet mercury supply is limited as The Minamata Convention on Mercury requires the 141-member Parties (including Indonesia) to phase out existing mercury mining. This has led to the occurrence of small-scale mercury mining in areas of mercury rich mineralogy, accounting for as much as 80 percent of the mercury now produced worldwide. Through logistical challenges we investigated the environmental distribution and characteristics of mercury in soils and sediments associated with cinnabar mining on Seram Island, Indonesia. The extraction process on site uses sluice boxes and large quantities of uncontained water. Panning in the ocean was also observed in the local community. Mercury in waste rock was up to 5630 mg/Kg although bioavailability was relatively low (<0.04%) but still 2 mg/Kg. There was localised enrichment around Hulong (Total Hg 811 mg/Kg) and Mount Tembaga (Total Hg 825-838 mg/Kg) and 225 mg/Kg). Sediments in creeks and estuaries contained up to 55mg/Kg and bioavailability was variable. Once collected, the cinnabar is transported to Java for processing into elemental mercury which is sold to small scale gold mining operators in the region. Elemental mercury is used to extract gold from ore through the formation of a mercury-gold amalgam. The amalgam is then heated to vaporise the mercury and leaves the recovered gold. Case studies on Buru Island, Mollucus Province, describe the follow-on processes of mercury distribution to the environment from gold mining.

4.01.P-Mo-071 Ecological and Human Health Risks due to Potentially Toxic Metals in Major Mining Areas in Ghana

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Anthropogenic activities such as mining generate a large quantity of waste containing potentially toxic elements that could, adversely affect the environment, plants and human health. The generated toxic elements become mobile and can be absorbed into food crops and build up to high concentrations. This study focused on the potential health risks associated with exposure to potentially toxic elements (As, Pb, Cd, Cr, Cu, Hg, Mn, Ni and Zn) through the ingestion of food crops (cassava, cocoyam, and yam) and water in two mining communities (Konongo and Amansie West) and a non-mining community (Mampong) in Ghana. Elemental concentrations

were determined in 303 soil samples, 208 food crops samples and 80 water samples using X-ray fluorescence (XRF) for soil as well as food crops and Microwave Plasma-Atomic Emission Spectrometer (MP-AES) for water. A Lumex analyzer was used for Hg analysis. Quality assurance/quality control included the use of NIST 2711a for soil and NIST 1575a for the food crops and replicates. The average concentrations of Hg (0.01 ± 0.006 mg/L) and Pb (0.11 ± 0.002 mg/L) in water, as well as As (30.15 ± 24.65 mg/kg), Cd (87.96 ± 11.50 mg/kg) and Cr (80.62 ± 16.38 mg/kg) in soils were higher than their international regulatory limits, such as WHO, FAO, and the Canadian Council of Ministers of Environment guidelines. The human health risk assessment indicated that the hazard indices for all metals in the root tubers were >1, indicating that children and adults may be susceptible to heavy metals health risks. Carcinogenic risk (CR) value for adults and children revealed that the inhabitants may likely develop cancer risk from the contaminants in their lifetime for As, Cd, Cr, Ni and Pb as their CR values were $>10^{-4}$. Consumption of water poses negligible non-carcinogenic risk to adults and children. It is recommended that appropriate mining technology and proper mining waste disposal practices are employed to reduce the availability of these heavy metals in food crops.

4.01.P-Mo-072 Emerging Contaminants in Philippines Rivers: Addressing the Data Gap in Developing Countries

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The occurrence of emerging contaminants such as microplastics and per- and polyfluoroalkyl substances (PFAS) in environments in the Asia-Pacific region is reported to be increasing, including in rivers. This is concerning as tropical river basins hold much of the world's biodiversity and are an important source of drinking water for communities. However, most studies of emerging contaminants in tropical rivers report occurrence and concentrations in urban and industrialized regions with high population densities. As a result, the risks of emerging contaminants to ecosystems and humans in low population, tropical river basins are largely unknown. Here, we report findings from an investigation of microplastics and PFAS occurrence in river water and sediments across ten major Philippines river basins. Unexpectedly, we did not detect PFAS in river basins with low population densities and urban extents. Conversely, microplastics were detected in river water and sediment in all the river basins studied, irrespective of population density. Polymer compounds detected were indicative of a range of sources including the chemicals and manufacturing industries, clothing, and food packaging. We explore several hypotheses to explain these results and highlight the considerable data gaps in emerging contaminants occurrence in developing countries. Our findings highlight the need for more representative regional and national-scale sampling of emerging contaminants in Philippines rivers, and more broadly throughout the Asia-Pacific region, to accurately assess ecosystem and human health risk and to better constrain regional and global fluxes.

4.02.A.T Advanced Monitoring and Assessment Approaches for Improved Treatment of Contaminants of Emerging Concern and PFAS in Wastewater

4.02.A.T-01 Moving Toward a Comprehensive Open-Source Software and Data-Visualization Platform for General Non-Targeted Analysis using LC-HRMS2: Application to Various Waste Streams

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We have developed a framework for processing non-targeted analysis data, especially covering classes with repeating motifs such as polymers (PolyMatch), Per- and Polyfluorinated Substances (PFAS) (FluoroMatch), and lipids (LipidMatch). Users can simply drag vendor files into the software, select parameters in the interface, and click run. Data-processing covers vendor file conversion (utilizing MSConvert), deconvolution of All Ions data, peak picking (utilizing MZmine 2 and 3), blank filtering, homologous series detection, annotation, confidence reporting and visualization. Annotations incorporate all evidence available in LC-HRMS. Annotation leverages sophisticated formula prediction algorithms using isotopic pattern, accurate mass, and numerous filters to retain only sensical molecular formula, MS2 based annotation using spectral matching to community databases, rules-based fragmentation libraries, predicted fragmentation based on fragment-structure relationships, and fragment screening, and homologous series evidence. Visualizations allow for thorough validation of annotations and unknown discoveries. Visualizations include Kendrick mass defect plots with series color coded, Kauffman plots, extracted ion chromatograms, isotope labeled MS1 spectra, annotated MS2 spectra, theoretical isotopic spectra from predicted formulas versus experimental spectra, and statistical visualizations. 3D visualization and CCS matching are also available for related LC ion mobility software. All visualizations are presented in a user-friendly, shareable (via html link) interactive dashboard. This presentation will explore the extensive functionalities of current iterations of the software, focusing on FluoroMatch (PFAS NTA), highlighting various applications to municipal leachate, sewage, and wastewater. We will then discuss current and future applications and advances in the software for tackling general non-targeted analysis, with an example of monitoring chlorinated species in sewage. This new software will empower researchers to delve deeply into annotation evidence and share interactive non-targeted data for collaborative investigations.

4.02.A.T-02 Analysis of Potentially Unrecognized Fluorine-Containing Compounds in Environmental Samples using LC-MS and 19F NMR

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Per- and polyfluoroalkyl substances (PFAS) have gained attention in the scientific community with their ubiquity in manufactured products, leading to contamination in the environment. Because some PFAS are resistant to degradation processes, these chemicals can be found in

biosolids (post-treatment sewage sludge fertilizer) and bioaccumulate in organisms. With rising concerns regarding elevated PFAS levels, robust techniques to detect, identify, and quantify PFAS are needed. In this research, I use nuclear magnetic resonance (NMR) spectroscopy with the fluorine nucleus (19F) to screen biosolids for novel fluorinated chemicals and create human exposure profiles of different areas in Ontario, Canada. Since C-F bonds are largely devoid in the environment, 19F NMR offers a unique advantage in providing unbiased analyses (e.g. no matrix effects) when searching and quantifying total PFAS. Complementary to NMR spectroscopy, liquid chromatography mass spectrometry (LC-MS) is used as a tool to confirm the presence, identities, and concentrations of PFAS in both biosolids and soil samples. Additionally, these results can provide information on the potential migration of known PFAS from biosolids to soil and crops. Preliminary NMR data shows high levels of fluorinated aromatic compounds present in different biosolid samples, suggesting possible pharmaceutical exposure. For LC-MS, there were minimal differences in levels of various PFAS between pre and post application of biosolids on farm soils and the control soils (background PFAS levels from rainwater deposition). It is hypothesized that further investigation of new soil samples from the same land will show variations in PFAS concentration following greater dispersion of the biosolids.

4.02.A.T-03 Advanced Micropollutant and Emerging Contaminant (CEC) Treatment Assessment with Real-Time Wastewater Surveillance

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Recent adoption of advanced treatment sees the use of efficient techniques for CEC elimination at WWTP outlets, such as granulated activated carbon (GAC) filters and ozonation. Most evaluation of such treatment relies on grab sampling measurements for only a select group of chemicals. Given the temporal variability associated with CEC discharge, treatment now requires real-time monitoring strategies to characterize micropollutant fate at times of micropollutant surge. ToxMate online biomonitoring, using multispecies (Erpobdella, Gammarus, Radix) organism avoidance behavior as a biomarker, offers effluent characterization using an effect-based non-targeted approach. The objective of the communication is to present results and variability in advanced treatment performance over a continuous 18-month surveillance period, as well as current laboratory work on fingerprinting for the identification of CECs.

ToxMate biomonitoring was used at an advanced treatment site (120,000-population equivalent WWTP) where biological treatment was followed by ozone treatment and then GAC tanks, one ToxMate after each respective treatment for comparative monitoring (3 total). Parallel sampling of model micropollutants showed moderate elimination after ozonation and good overall elimination (post GAC). However, there was both temporal and molecule dependent variability in the elimination. Our results from the long-term monitoring highlight this variability, where residence times are used to estimate correlation in micropollutant surge, in some cases showing persistence of the outgoing pollution or even the appearance of avoidance reactions, perhaps related to treatment byproducts (i.e., metabolites). Lab studies for repeated spikes tested for over 40 micropollutants were modelled with a functional approach, grouping responses into clusters known as behavioral fingerprints. Our current research looks to reveal structural similarities within fingerprints, that could improve identification of CEC nature at sites such as the described WWTP. Coupling this method and the rare insight into the characterization of pollutant fate in advanced treatment provides an opportunity for real-time alerting in WWTPs in the case of more persistent micropollutant surge, perhaps indicating temporary periods where applications such as re-use should be avoided. It also suggests the need for a review of current chemical analysis used to evaluate overall micropollutant elimination in advanced treatment.

4.02.A.T-04 Polystyrene Microplastics Reduce Sulfamethoxazole Filtration Efficacy in Fe-Mn Modified Biochar Columns

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Emerging contaminants, particularly antibiotics and microplastics (MPs), present significant challenges in wastewater treatment and pose large ecological risks. This study evaluates sulfamethoxazole (SMX) removal using Fe-Mn modified biochar (BFM) in filtration columns, focusing on polystyrene microplastics' (PS-MPs) impact on SMX removal in water and wastewater systems at different pH levels. Batch sorption results show that 10 mg/L SMX in 50 mL water can be completely removed by 100 mg BFM sorbent. The Bed Depth Service Time model indicated the BFM column is feasible in scaled-up continuous flow operations, while the Yan model suggested the adsorption mechanisms include external mass transfer and intraparticle diffusion. The present of PS-MPs (pH ≈ 5.6) significantly reduced SMX retention due to competitive sorption. However, at pH 3.2, competitive sorption became negligible due to electrostatic interactions driving the PS-MPs sorption, while neutral charged SMX bound through hydrogen-bonds or π-π EDA interactions. Elevated pH shifted both PS-MPs and SMX sorption to non-electrostatic thus intensifying sorption competition. In wastewater, SMX filtration was slightly inhibited by PS-MPs in BFM columns, whereas PS-MPs removal remained unaffected due to the high ionic strength and alkaline pH. These findings underscore MPs' role in pollutant filtration efficacy, crucial for biochar wastewater treatment enhancement.

4.02.A.T-06 Identification of Potential Groundwater Transport Pathways and Coastal Contaminant Migration from a Terrestrial Waste Disposal Area: Naval Harbor Case Study

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Navy Environmental Restoration (ER) programs are intended to identify, assess, characterize, clean and/or control pollution from past terrestrial hazardous waste disposal sites and operations. These programs were established by the Department of the Navy (DoN) to comply with EPA cleanup requirements and regulations which involve the characterization of the nature and extent of risk posed by these sites. A majority of these sites are located next to harbors, bays, and

coastal environments, which by its nature, intrinsic to naval installations and operations. It is estimated that several of these Navy landfills have groundwater infiltrating the wastes. As part of its permanent commitment and obligation to environmental stewardship, the US Navy has conducted extensive and multi-phase investigations to determine if emerging and/or legacy contaminants (VOCs/PFAS) are migrating into marine systems at levels which could pose a threat to the environment. Based on previous and ongoing studies, it was determined that a likely migration pathway for these contaminants is by means of groundwater exchange between the site and nearby water bodies. To address the potential ecological and human health risk, the acquisition of continuous groundwater discharge data is necessary to refine the understanding of the interactions between the groundwater-surface water interfaces. This case study focuses and highlights a proven environmental assessment technology developed by Naval Information Warfare Center (NIWC) Pacific which was intended to screen, map, and collect discharge-containing, sediment porewater. Based on the results, the Trident Probe was an indispensable tool and key to the direct quantification and coastal migration of contaminated groundwater from a legacy hazardous waste site located within an active naval base and its eventual discharge to the adjacent marine environment.

4.02.B.T Advanced Monitoring and Assessment Approaches for Improved Treatment of Contaminants of Emerging Concern and PFAS in Wastewater

4.02.B.T-01 An Expanded PFAS Toolkit for Waste Occurrence and Treatment Monitoring

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As PFAS moves towards regulation in wastewater, monitoring of PFAS for occurrence and fate during treatment becomes more and more important. While standard methods such as EPA 1633 are critical for regulation and will be used in NPDES permitting and many other use cases, it is well understood now that target analysis methods will fail to catch most of the PFAS flowing through a waste system. This becomes critical when looking at beneficial reuse such as biosolids, where unmonitored/unregulated PFAS can act as a long-term source if applied based on results from standard reference data alone. In this study, we looked at data from literature and internal data based on multiple PFAS measurement approaches for wastewater treatment plant and landfill leachate wastes. The internal methods used were 1633 to establish reference, an extended target list method that added 40 more targets including 6:2 Di PAP, short chain sulfonamides, ultrashorts including trifluoroacetic acid, and more, the TOP assay, organic fluorine methods including adsorbable organic fluorine (AOF) EPA 1621, and non-target suspect screening approaches. In addition, the TOP assay was extended to include ultrashort PFAS. Results show that most of the extended target list was amenable to a 1633-extended approach except ultrashorts PFAS that needed separate procedures. TOP results consistently showed large increases post-TOP indicating high precursor presence. The expanded TOP assay showed significant increases in trifluoroacetic acid and pentafluoropropionic acid (PFPrA) especially landfill leachate. Suspect screening by HRMS in biosolids showed high area counts/intensity levels for phosphate-based PFAS such as 6:2 DiPAP and short-chain sulfonamides. Work on expanding this data set is ongoing and will be presented. This and other work shows that our understanding of PFAS in waste will take a large tool kit of methods with high-resolution mass spectrometric methods playing an increasing role.

4.02.B.T-02 Advanced Monitoring and Assessment Approaches for Improved Treatment of Contaminants of Emerging Concern and PFAS in Wastewater

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PFAS are water soluble and resistant to biodegradation due to the exceptional stability of C-F bonds. The physicochemical properties inherent to their structures have made them attractive to be used in many industrial applications. Their inability to be removed by traditional wastewater treatments and the health risks associated with exposure to PFAS have brought the need to better understand its fate and transport in the environment. Once these compounds are released, they follow different transport mechanisms and might infiltrate groundwater and surface water, which are usually drinking water sources. Wastewater facilities and septic tank discharges are considered PFAS point sources of contamination. This year the Environmental Protection Agency defined the Final PFAS National Primary Drinking Water Regulation which will require public water systems to continuously monitor PFAS in water sources, anticipating a high volume of samples. PFAS pretreatment methods normally consist of at least 6 hours of analysis, including a Solid phase extraction (SPE) process and then evaporation of the eluted extract, followed by a reconstitution step. Therefore, the need for a method with less analysis time, high sensitivity, accuracy, and high throughput is a priority. Here we developed a new method involving the injection of 10 mL of sample in an online SPE system using a weak anion exchange (WAX) column, which is eluted and immediately injected into an Altis Plus (Thermo) liquid chromatography-tandem mass spectrometry (LC-MS/MS), using a Hypersil gold PFP column; the total analysis time per sample is 28 minutes. The method includes PFAS analytes listed in the EPA method 1633, and isotopically labeled standards were used for quantification. The method was validated in terms of linearity, detection limit, precision, and accuracy, with an instrument detection limit as low as 0.3 ppt for Perfluorooctanoic acid (PFOA) and 1 ppt for Perfluorooctanesulfonic acid (PFOS). Lastly, the method was applied to wastewater samples from Miami-Dade, Florida, that receive landfill leachate, including raw, treated, and septic tank discharges.

4.02.B.T-03 Per- and Poly-fluoroalkyl Substances Fate During Wastewater Treatment

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Per- and polyfluoroalkyl substances (PFAS) are commonly found in municipal wastewater. PFAS originate from industrial and municipal sources, including paper production, textile mills, and down-the-drain discharges. These compounds include commonly studied chemicals such as PFOS and PFOA, but also short-chain PFAS and polyfluorinated compounds, posing challenges for removal in wastewater treatment plants (WWTPs). Despite uncertain and variable removal efficiency previously reported, understanding PFAS fate in treatment processes is crucial, especially considering their potential transformation into the more stable PFOS and PFOA. As part of a comprehensive monitoring study of multiple WWTPs, extensive sampling was conducted at each WWTP . We focus on one WWTP to characterize efficacy of treatment and short-term and long-term variability. Samples were collected concurrently during each sampling event from locations within the WWTP to isolate unit operations. Samples were analyzed with

EPA method 1633 and an adapted version of laboratory-specific EPA method 537.1 (LS-Method 537) and total organofluorine (TOF). Quality assurance and control samples were incorporated in each sampling event. This presentation describes our findings including: PFAS composition, variation in concentrations and mass discharge and the challenges of monitoring and analyzing PFAS in wastewater treatment plants. The diverse composition of PFAS compounds and the complexities in their detection and quantification across matrices presented unique analytical challenges. Finally, we will discuss the implications for managing PFAS in WWTP operations and recommend opportunities for cost-effective PFAS removal strategies.

4.02.B.T-04 Occurrence of Per- and Polyfluoroalkyl Substances in Wastewater Treatment Plants Serving Rural Communities

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Per- and Polyfluoroalkyl Substances (PFAS) are a class of emerging contaminants defined by their carbon-fluorine bond which makes them incredibly recalcitrant in the environment. Because there are serious human health concerns surrounding exposure to these compounds, regulation of environmental emissions is likely. It is well known that effluent from wastewater treatment plants can serve as a point source while land application of biosolids can serve as a nonpoint source. Often PFAS precursors are transformed through the wastewater treatment process, long-chain compounds are segregated into sludge, and short-chain compounds are introduced into the environment through discharges into waterways. Little research is available on PFAS occurrence in wastewater treatment plants serving small communities. Smaller rural communities tend to employ different technologies to larger treatment plants such as sequencing batch reactors. Here, we adapt EPA Draft Method 1633 to quantify 40 PFAS through influent, effluent, and biosolids collected from multiple rural wastewater treatment plants in Nebraska. Modifications to the method include evaluation and control of potential contamination sources during processing and validation of detection limits for multiple matrices. It is hypothesized that similar trends of PFAS release will be observed in rural wastewater treatment plants across Nebraska.

4.02.B.T-05 Transformation of Per-and Polyfluoroalkyl Substances During On-site Septic System Treatment

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Approximately 25% of homes in the United States treat their wastewater using on-site septic systems rather than centralized wastewater treatment plants. Innovative/alternative (I/A) septic systems have been designed to reduce the amount of nutrients released into the environment. Some communities facing nutrient issues are making decisions to either require residents to install I/A systems or connect to centralized wastewater treatment. Data assessing co-beneficial removal of contaminants of emerging concern (CECs) will inform current and future watershed management initiatives and support community and residential decision making. Recent work in partnership with the Massachusetts Alternative Septic System Test Center (MASSTC) has explored CEC concentration changes within two traditional and three I/A on-site septic systems. Preliminary results indicated that while many pharmaceuticals and other CEC parent compounds were partially-to-totally removed in both traditional and I/A systems, the concentrations of the monitored per- and polyfluoroalkyl substances (PFAS) increased in the effluents relative to the influent wastewater. Additional sampling events included a wider suite of targeted PFAS, as well as methods to measure total oxidizable precursors (TOP) and adsorbable organic fluorine (AOF). The use of multiple methods provides greater insight to the fate of PFAS during treatment than any one individual method. The expanded testing verified that the increased concentrations post-treatment were not due to matrix interference but conversion of unidentified PFAS precursors to the PFAS intermediates/end products that were measured by the targeted analytical methods; that is, the study documented transformation of the precursors, not the addition of more PFAS.

4.02.B.T-06 Fate of PFAS at a Dedicated Land Disposal Field After Four Decades of Biosolids Application

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Fate of per- and polyfluoroalkyl substances (PFAS) was assessed in a dedicated land disposal field were biosolids have been applied over 38 years as a waste management strategy. Soil cores (1.8 m, divided into 30-cm sections), groundwater from four monitoring wells, and biosolids applied in 2022 were sampled, extracted and analyzed using liquid chromatography high resolution mass spectrometry. A total of 54 PFAS were targeted. Suspect screening was also applied to the samples identifying and semi-quantifying 17 additional PFAS. Total PFAS concentrations decreased with soil depth, ranging from 1700 ng/g to 2.06 ng/g. Perfluoroalkyl acid (PFAAs) precursors and intermediates were predominant in the 2022 biosolids (60 mol%). Long-chain PFAAs dominated the surface soil (67-76 mol%), indicative of precursor degradation after biosolids application, with PFOA (18% of SPFAS) and PFOS (38% of SPFAS) being the most predominant. Semi-quantified intermediates present in surface soil further confirmed precursor degradation. Long-chain PFAAs decreased with depth, while short-chain PFAAs increased, reaching up to 94 and 91 mol% of short-chain PFAAs at the bottom soil layer and groundwater, respectively. PFAS distribution with depth was consisted with chain-length specific sorption and transport, directly influenced by the air-water interface and the high organic carbon content (OC) on the surface soil (15.2% OC), that decreased with depth (~2-3% OC at > 60 cm). Elevated OC in the soil was most likely due to the high rate of biosolids application over 40 years. PFAAs branch-linear distribution in soil did not match with the reported in previous studies, showing that it may not be possible to use it to predict PFAS precursors in an area impacted by PFGAS for such a long period of time. The present study can be potentially considered as a potentially worse-case scenario where biosolids are land applied. Despite the site-specific environmental characteristics, the results are consistent with previous studies and therefore are likely transferable to assess PFAS fate at other sites.

4.02.P-Th-041 Target, Suspect and Non-Target Screening of Per- and Polyfluoroalkyl Substances (PFASs) in Wastewater Treatment Plant Effluents in South Korea

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This study employs a comprehensive approach, combining target, suspect, and non-target screening, to assess the presence of per- and polyfluoroalkyl substances (PFASs) in domestic and industrial wastewater treatment plants (WWTPs) in South Korea. Target analysis quantified 20 PFASs from WWTP effluents. Total concentration of PFASs ranged from 69.1 to 79.6 ng/L, and the levels of PFBA (mean: 15.6 ng/L, median: 14.9 ng/L) and PFOA (mean: 14.7 ng/L, median: 12.7 ng/L) were relatively higher than those of other PFASs. Compared to 2010, there is an overall upward trend in perfluoroalkyl carboxylic acids (PFCAs), particularly perfluoroheptanoic acid (PFHpA), which has seen nearly a 10-fold increase, with current concentrations reaching 9.5 ng/L. Suspect and non-target screening utilized ion mobility spectrometry-mass spectrometry (IMS-MS) to identify additional PFASs based on exact mass, collision cross-section (CCS), and MS/MS fragmentation patterns.. Among these, 20 compounds were identified through suspect screening at confidence level (CL) 3 or higher, while 2 compounds including n:2 fluorotelomer-based non-polymers and bistriflimide were identified through fragment-based non-target screening. Semi-quantification of unknown PFASs revealed that the concentration of PFASs identified from suspect screening and non-target screening was higher than that of the target PFASs, especially in industrial wastewater effluents.

4.02.P-Th-042 An On-Site, On-Demand Approach for PFAS Removal from Freshwater Using 3D Printed Natural Materials

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Per- and polyfluoroalkyl substances (PFAS) are widespread in the environment due to their use in a large variety of commercial and military products, including flame retardants and firefighting foams. PFAS can be found in water, air, biota, sediments, and soils and have been linked to harmful health effects in biota. The sequestration and treatment of PFAS in contaminated freshwater has been a focus of the military to reduce environmental and human impacts. The goal of this study was to evaluate the reduction of PFAS concentration in water by sequestration in a zeolite resin combined into natural stoneware clay. Zeolite resin (24% total mass) was combined with clay and 3D printed using a clay 3D plotter into a treatment column. The 3D printed zeolite/clay column was placed into a 3D printed cylinder that allows the contaminated water to pass through it. Contaminated freshwater was spiked with a mixture of PFOA (493 µg/L) and PFOS (504 µg/L) and passed through the 3D printed cylinder at 1.3 mL/min for 150 minutes. After 30 minutes, 98% PFOA and 97% PFOS were removed from the water. This study provides a proof-of-concept design for an environmentally friendly method leveraging natural materials and harnessing the design freedom of 3D printing to reduce PFAS concentrations from freshwater. Future work will consider scale up for various use scenarios focusing on the civil and military mission space for the reduction of additional contaminants.

4.02.P-Th-043 Screening Private Well Water and Tap Water in Rural Missouri and Illinois Communities for PFAS and Heavy Metals

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Per and Polyfluoroalkyl substances (PFAS), contaminants of emerging concern, are synthetic polymers used in numerous products, known for their persistence and toxicity. As of February 2024, 5,021 United States locations are confirmed contaminated with PFAS, with blood serum levels among Americans ranging from 0.02 to 33.4 µg/L. Individuals are exposed to PFAS through drinking water and consumer products. The Illinois EPA (ILEPA) has detected PFAS in community drinking water supply sources above health-based guidelines in Collinsville, Wood, and East Alton in Madison County. These communities are near St. Charles County, Missouri, where PFAS data is limited. Since 2013, the Missouri Department of Natural Resources has monitored PFAS in drinking water systems. Over 400,000 private wells in Missouri provide drinking water to more than 1.4 million residents, with over 5,000 wells in St. Charles. This study assessed PFAS and heavy metals in private well water in Portage, Missouri, a rural area influenced by agriculture with minimal industrial activity. Using survey responses and water samples, PFAS concentrations were quantified with Cyclopure test kits, and heavy metals (cadmium, copper, mercury, nickel, lead, selenium, and zinc) were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Preliminary findings indicated that while PFAS levels generally remained below health advisories, lead concentrations in six sampled wells exceeded the EPA's maximum contaminant level goal of zero, posing health risks, especially to children. Additionally, analysis of four tap water samples from Collinsville and Granite City revealed PFAS compounds at the following levels: PFOA at 19.4 ng/L, PFOS at 7.2 ng/L, PFBA at 2.6 ng/L, PFBS at 10.0 ng/L, PFPeA at 9.3 ng/L, PFHxA at 11.7 ng/L, PFHpA at 8.0 ng/L, PFHxS at 9.4 ng/L, and PFPeS at 1.7 ng/L. These concentrations surpass health advisory thresholds, highlighting regional water quality issues. These results underscore the need for regular water quality monitoring and public awareness. This study highlights the contamination status of water sources in rural and peri-urban areas of Missouri and Illinois, emphasizing the need for vigilant monitoring and public awareness regarding water quality.

4.02.P-Th-044 Assessment of RO Concentrate and UF Backwash Impact on Biological Reactors in WWTPs: Enabling Safe Direct Potable Reuse Waste Stream Sewer Disposal

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The need for alternative sources of water has increased as the climate becomes hotter and drier, coupled with the growing populations globally. Direct potable reuse (DPR) is one of the major strategies for sustainable water distribution in public water systems because it is drought-proof and relies on a steady water source: domestic wastewater. Advanced treatment for DPR typically involves multi-barrier processes such as a combination of ultrafiltration (UF), reverse osmosis (RO), and advanced oxidation processes (AOP) to remove pathogens and trace pollutants. UF generates a waste stream due to backwash and chemical cleaning of the membranes, and RO generates concentrate with high amounts of dissolved solids and trace contaminants. Combined, these waste streams represent up to 30% of the advanced treatment inflow, and they must be safely managed to prevent environmental pollution. Current management of the waste streams produced during advanced purification includes discharge into surface waters (seas, estuaries, and rivers), well injection, evaporation ponds, zero liquid discharge, and sewer disposal. However, some of these alternatives are not available for inland communities such as those in the U.S. Southwest. Thus, sewer disposal, which consists of the discharge of advanced purification waste streams (APWS) into a sewer network, may be considered a viable alternative. APWS may have inhibitory effects on biological processes in wastewater treatment plants (WWTPs). Thus, this study aims to determine how waste streams generated during advanced purification for DPR can be discharged into a sewer network or combined with the inflow of a WWTP without affecting the efficiency of its different conventional microbial processes, such as heterotrophic activity, nitrification, and denitrification. For this, standardized microbial inhibition assays will be conducted to assess the inhibitory effects of APWS diluted in raw domestic wastewater on these different microbial groups. In addition, the effects of acclimation on the long-term performance of biological reactors treating APWS diluted in raw domestic wastewater will be tested using a continuous-flow reactor. This study aims to inform strategies to enable sewer disposal of advanced purification waste streams as a safe management strategy.

4.02.P-Th-045 Method Development and Validation for the Analysis of Emerging Organic Contaminants (EOCs) in Water

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Emerging organic contaminants (EOCs) are synthetic chemicals that pose a growing threat to the environment and human health, they can be found within personal care products, pharmaceuticals, and cleaning products. The high consumption of these products and how they are discarded create an exposure pathway to the environment and result in the accumulation of concentrated contaminants. EOCs are known to have negative ecotoxicological and human health effects, affecting the endocrine, immune, and reproductive system, and leading to neurodevelopment issues. These pollutants are introduced to the aquatic environment and are usually not fully degraded through wastewater treatment as the process used in treating wastewater is not completely effective, it is important to note that treated water only meets 87 of the 93 federal drinking water standards without further advanced oxidation processes. The EOCs that are recalcitrant and remain after water treatment range from hormones, plasticizers, pharmaceuticals, disinfection byproducts, to per- and polyfluoroalkyl substances (PFAS). In this study we have developed a comprehensive analytical method for the quantitative analysis of representative compounds from different chemical classes; the hormone ethynyl estradiol, endocrine disruptor bisphenol A, disinfection byproducts such as dibromochloromethane, and trichloroacetic acid, the pharmaceutical sulfamethoxazole, and multi-toxicant perfluorooctanoic acid (PFOA), using high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) and gas chromatography mass spectrometry (GC-MS). We validated the method in terms of linearity and application range, method detection limit, precision, recovery, and accuracy for all compounds analyzed. This methodology will be later applied for the assessment of organic chemicals removal rate after treatment with high entropy alloys (HEA) materials, which has the potential to generate hydroxyl radical oxidants that can mineralize residual organic contaminants. The reduction of EOCs is essential for the prevention of environmental accumulation and reduction of chemical exposure, improving overall water quality after treatment.

4.02.P-Th-046 Harnessing the Power of Mass Spectrometry and Automation to Reduce Sample Size, Sample Preparation Time and Increase Laboratory Efficiency

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U.S. Environmental Protection Agency, Method 1633, has become the foundational method for analysis of PFAS in non-potable water matrices, soils, biosolids and tissues in the United States. The method consists of sample preparation using weak anion exchange (WAX) solid phase extraction (SPE) with graphitized carbon black (GCB) clean up. This U.S. Environmental

Protection Agency method is a performance-based method allowing for changes to be made as long as data quality equivalency is demonstrated. With this method becoming very much in demand, laboratory throughput and efficiency must be considered for maximum laboratory success. Automation and instrument sensitivity are investigated as avenues to decreasing required sample size and therefore increasing laboratory efficiency. A highly sensitive LC-MS/MS system was utilized for this analysis, allowing for reduction in sample volume size while still achieving equivalent results and method detection limits to the method as written. Sample volumes tested ranged from 500 mL (the recommended volume in EPA 1633) down to 50 mL. An automated solid phase extraction (SPE) system was utilized for sample preparation of various types of water samples to eliminate the need for human interaction during the sample preparation process. To make the sample preparation process fully automated, a stacked dual layer cartridge containing both WAX and GCB chemistries was utilized instead of having to perform the GCB step manually. Equivalency had to first be established for any deviations being made to the method. To establish equivalency, all quality control guidelines must be demonstrated including retention time stability, ion ratio stability, ongoing calibration verification, and recovery. Equivalency of the automated SPE system and stacked dual layer cartridge were demonstrated individually previous to this work, and in series as a complete workflow in this project. The sample volume was successfully reduced to 50 mL. A method detection limit study was performed to demonstrate equal or lower MDLs using the automated, reduced sample volume approach. All data quality guidelines were also demonstrated with the reduction in sample volume. Additionally, a wastewater certified reference material was processed in an equivalent reduced sample volume and was within all certified levels for the 40 PFAS covered by EPA 1633.

4.03.P-Mo Advances in Pesticide Application Technologies: Evolving Benefits and Environmental Challenges

4.03.P-Mo-073 A Biological Option for Control of Varroa Mites in Honeybee Hives

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Varroa mites are a serious pest of western honeybee colonies and a primary reason behind the large colony losses reported by beekeepers in recent years. Varroa mites are an external parasite that feed on adult bees and developing honey bee larvae. In addition to directly affecting bees, the mites are also vectors of several viruses that can kill bees. The traditional method for varroa mite control has been to hang plastic strips impregnated with synthetic pesticides in the hive (e.g., fluvalinate, amitraz, coumaphos). Repeated use of these pesticides has led to mite resistance, decreasing their effectiveness. Other options for controlling varroa mites such as mechanical methods (e.g., drone-brood trapping), use of mite-tolerant honeybee stocks, and natural pesticides (e.g., formic acid) may be helpful but have not provided consistent protection. Recently, a double-stranded RNA product, EP15 (vadescana), was developed to control varroa mites in honeybee hives by blocking the expression of calmodulin protein in reproductive mites through the RNAi mechanism. EP15 has a nucleotide sequence specific to the calmodulin messenger RNA of varroa mites and thus exhibits little to no cross-toxicity to other organisms. EP15 is formulated as a sugar solution in a perforated pouch that is placed inside the honeybee hive in the spring and fall. We conducted a risk assessment that demonstrated that EP15 poses negligible risk to honeybees in treated hives. The available fate and ecotoxicity studies showed that EP15 residues outside the hive are at very low concentrations, and what little EP15 is available outside the hive is rapidly degraded. We thus conclude that the EP15 formulation is a safe and effective tool for control of varroa mites in honeybee hives.

4.03.P-Mo-074 Watershed Scale Occurrence, Fate, and Potential Ecotoxicity Of Pesticide-Treated Seed Residues Resulting from Ethanol Production Waste Product Release

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Between 2015 and 2021, an ethanol plant in Eastern Nebraska used treated seed corn as feedstock for production. The plant produced significant amounts of wastewater as well as distiller's grains (wet cake) both of which contained high levels of neonicotinoid insecticides and fungicides. The wet cake and wastewater were annually land applied to cropland in the surrounding areas. Approximately four million gallons of wastewater was accidentally released into an ephemeral stream in February 2021 leading to plant shutdown. This study aims to understand the environmental pesticide loading to the environment from ethanol production using treated seed. Subsequent monitoring of local streams focused on the occurrence of twenty-two pesticide residues and degradation products in two streams that were near the ethanol plant and evaluated the potential ecological risk by determining risk quotients (RQ). Passive samplers were installed upstream and downstream of application areas, and monthly grab samples were collected from April 2021 to November 2023. All samples were extracted and analyzed using liquid chromatography/tandem mass spectrometry (LC-MS/MS). Eighteen out of the twenty-two target residues were detected with concentrations ranging from below detection (<DL) to 344 µg/L. Results revealed varying detection frequencies and concentrations of pesticides, with clothianidin (98.6 %), thiamethoxam (97.2%), and imidacloprid desnitro (95.3%) being the most frequently detected compounds. The spatial distribution shows highest levels were downstream of the wastewater spill, along with storm run-off likely from wastewater and wet cake field application areas. The risk quotient analysis shows that, except for the most upstream (nonpoint source) location, at least one sample from each site posed elevated exposure risk for three different trophic levels (Algae, *Daphnia Magna* and Fathead Minnow). Acute and chronic aquatic toxicity benchmarks set by US EPA for aquatic invertebrates were exceeded due to elevated concentrations of imidacloprid, clothianidin and thiamethoxam in both waterways. Exceptionally high levels of neonicotinoid degradation products, including imidacloprid desnitro were measured in both waterways. Findings from the study highlight the importance of monitoring to assess ecological risks and potential impact of elevated pesticide residues originating from the use of treated seed in ethanol production.

4.03.P-Mo-075 Pesticides in Streams Impacted by a Bioenergy Production Facility Receiving Pesticide Coated Corn Seeds

***Michelle L. Hladik**[1], Dana Kolpin[1], Matthew De Parsia[1], Daniel D. Snow[2], Shannon Bartelt-Hunt[3], Brenda Densmore[1], Laura Hubbard[1], David Rus[1], Jonathan Spurgeon[1], Brittany Grace Perrotta[1], Karen A. Kidd[4], Johanna Kraus, Carrie Givens[1], Christopher Kotalik[1] and David Walters[1], (1)U.S. Geological Survey, (2)University of Nebraska, (3)Civil and Environmental Engineering, University of Nebraska—Lincoln, (4)McMaster University, Canada*

Pesticide seed coatings have become increasingly prevalent in conventional agriculture in the last

several decades with many treated corn seeds containing both insecticides and fungicides. For a six-year time span (2015 to 2021) the AltEn bioenergy plant (Mead, Nebraska, USA) generated ethanol from almost 100% unused/expired treated corn seeds. The use of treated corn seeds for ethanol production resulted in accumulation of large amounts of contaminated wastewater and solid residue at the plant, a portion of which was applied to surrounding farmland. To better understand the potential long-term environmental effects from the processing of treated seeds at the AltEn plant, five nearby stream sites were sampled in 2022; sites included one upstream of the plant, two sites impacted by wastewater from the plant through the surface-water flow path, and two sites downstream of the plant. Water and bed sediment were collected in March (prior to crop planting) through July with algae and fish samples being collected in July. Overall, 59 pesticide compounds were detected including 15 herbicides, 23 fungicides, 20 insecticides, and 1 bacterial growth inhibitor. Pesticide results (maximum detection frequency, maximum concentration) varied substantially by environmental compartment: water (100%, 4,800 ng/L), algae (100%, 240 ng/g), bed sediment (47%, 50 ng/g) and fish (20%, 2.2 ng/g). Pesticide concentrations in stream water increased at all five sampling sites in May following a storm runoff event after most fields had been planted. Pesticides associated with treated corn seeds (e.g., the insecticide clothianidin) were higher in water and algae from the AltEn-impacted sites. In contrast, surface-applied pesticides (e.g., the herbicide atrazine) were higher in water and algae at the stream site upgradient of the AltEn plant. Study results indicate that pesticide concentrations have remained higher downstream of the AltEn plant even after the plant's closure. Pesticide concentrations in the water and algae were higher downstream of the AltEn plant compared to stream sites impacted by conventional agriculture.

4.04.T SETAC-A4 Special Session: Alternatives Assessment: An Evolving Landscape of Methods, Practice and Policy Supporting the Informed Substitution of Hazardous Substances

4.04.T-01 Alternatives Assessment 101

***Lauren G. Heine**, Retired*

4.04.T-02 Nanomaterials: Methods and Practice Needs to Get Ahead of Potential Regrettable Substitutes

***Molly Jacobs LeFevre**, University of Massachusetts, Lowell*

4.04.T-03 Addressing Environmental Justice Considerations in Alternatives Assessment

***Xiaoying Zhou**, California Environmental Protection Agency*

4.04.T-04 Connecting Alternatives Assessment and "Safe and Sustainable by Design"

***Colleen McLoughlin**, SciVera*

4.04.T-05 Using Product Certifications Standards: Benefits and Challenges for the Assessment of Hazard, Social Impacts and Sustainability

***Margaret H. Whittaker**, ToxServices*

4.04.T-06 Alternatives Assessment in Regulatory Policy: Current Landscape and Lessons Learned

__Catherine A Rudisill__, Safer Chemistry Advisory

4.05.P-Tu-131 Ion Mobility Filtering for Non-Targeted Analysis of PFAS from Environmental Samples Collected at a Ski Resort

Sarah E. Dowd[1], Kari Organtini[1], Marian Twohig[1], Frank Dorman[1], Jenifer Lewis[2], Naren Meruva[3], __Lindsay Hatch__[1] and Jean Carlan[4], (1)Waters Corporation, (2)Environmental, Waters Corporation, Milford, MA, (3)Food, Environment, Cannabis Markets, Waters Corp, Milford, Massachusetts, (4)Department of Chemistry, Dartmouth College, Germany

Perfluoroalkyl substances (PFAS) are a group of commonly used compounds, known particularly for their hydrophobic, non-stick properties. Their unique chemistry led to their use in ski waxes. While competition rules have recently banned the use of PFAS-containing ski wax, the persistence of PFAS means they could still be detected for years. Given the hazards and concern about PFAS contamination, we investigated if PFAS could be detected at a ski area that supports a high-level race program. While previous studies have looked at targeted analysis for known PFAS, this study used high-resolution mass spectrometry (HRMS) and ion mobility to look for new and unexpected PFAS. Samples were collected from a variety of locations within a ski area in New Hampshire to investigate the trends of PFAS in this type of environment. Data collection was done with ion mobility enabled data-independent acquisition on a SELECT SERIES™ Cyclic™ IMS (Waters Corporation). The added dimension of ion mobility can be used for spectral clean-up, which aids in the elucidation of unknown compounds as well as confirmation of known target compounds. Detected peaks were first compared to an internal HRMS PFAS library for identification. In the extracts, several legacy PFAS including perfluoroalkyl sulfonates, perfluoroalkyl carboxylic acids, and fluorotelomer sulfonates were detected. Unknown peaks were selected for further scrutiny based on their detected drift time in the ion mobility dimension. Previous studies have shown that the CCS values of per- and poly-fluorinated compounds are lower than compounds of similar *m/z*. An ion mobility filter was created based on this knowledge and applied to a list of detected peaks to select for possible PFAS. Using this filter, a series (C9-C24) of polyfluorinated carboxylic acid compounds with one hydrogen substitution in the carbon chain was tentatively identified. The distribution for this series of compounds was not consistent, with longer chains found in samples from the base of the ski slopes and the shorter chains in the snowmaking retention pond. While authentic standards were not available for many of the tentative identifications, two standards were purchased and compared with experimental data to confirm proposed structures.

4.05.P-Tu-132 Utilizing Ion Mobility to Enhance Targeted and Non-Targeted Analysis of Per- and Polyfluoroalkyl Substances (PFAS) from a Landfill Leachate Sample

Cecile Pinto[1], Sarah E. Dowd[2], Kari Organtini[2], Jenifer Lewis[3], __Naren Meruva__[4] and Lindsay Hatch[2], (1)Waters Corporation, France, (2)Waters Corporation, (3)Environmental, Waters Corporation, Milford, Massachusetts, (4)Food, Environment, Cannabis Markets, Waters, Milford, Massachusetts

Per- and polyfluoroalkyl substances (PFAS) are a group of industrial compounds that have been identified as a class of environmental contaminants of high concern due to their negative health effects and widespread presence in the environment. As new regulations on the use and manufacture of legacy PFAS are imposed, alternative replacement PFAS compounds have emerged. Current targeted methods focus on a short list of legacy and emerging PFAS and are therefore not comprehensive enough for measuring the potential PFAS contamination in environmental samples. Non-targeted analyses (NTA) with high-resolution mass spectrometry (HRMS) is a promising technique for the discovery and identification of new PFAS. Here, HRMS was coupled with liquid chromatography and ion mobility separation on the SELECT SERIES™ Cyclic™ IMS QTOF mass spectrometer (Waters Corporation) to improve the number of detected features in a complex environmental sample: landfill leachate. As PFAS may be present at low levels in complex samples, a data independent acquisition was utilized to get as much information as possible in a single injection. Detected peaks were first compared to an internal HRMS PFAS library for identification. Identifications were filtered based on metrics like accurate mass, retention time error, diagnostic fragments and measured collision cross section (CCS). In the sample, several legacy PFAS including perfluoroalkyl sulfonates, perfluoroalkyl carboxylic acids, and fluorotelomer sulfonates were identified. For the discovery of PFAS not in the suspect library, IMS adds an additional advantage as this class of compounds have lower CCS values compared to other compound classes of similar *m/z*. In this study, filtering peaks based on their observed drift time and *m/z* revealed polyhalogenated species that were in the leachate sample even at low intensity levels. A series of PFAS previously unreported were tentatively identified in the leachate samples. As authentic standards for new PFAS are difficult to find, the certainty of proposed structures benefit greatly from the data generated with LC-IMS-HRMS.

4.05.P-Tu-133 Submicron IR (O-PTIR) <500nm Used for PFAS Detection in MPs

***Jay Anderson**, Mustafa Kansiz and Eoghan Dillon, Photothermal Spectroscopy*

Microplastic or Nanoplastic (MP/NP) contamination has been recognized as a global environmental problem. MP/NP particles are found globally in water, air, and soil. These particles are regularly ingested via contaminated water, beverages, food, and by breathing airborne particles. Many MP/NP particles contain PFAS's which are known to exhibit harmful effects on organisms. We will use a new infrared(IR) microscopy technique to measure for PFAS contamination. A new approach to IR micro spectroscopy, called "Optical Photothermal Infrared (O-PTIR)" spectroscopy has demonstrated a unique ability to generate submicron IR spectra without common IR scatter artifacts. O-PTIR uses an infrared pump laser to excite the sample and a visible probe laser to measure the absorbed IR providing a 50X improvement in IR spatial resolution. O-PTIR provides mm to sub-micron size MP/NP characterization, with IR chemical specificity, in a non-contact, reflection geometry, and is not affected by fluorescence. O-PTIR can be used at visible powers of 1/10th of the power needed for Raman, eliminating the potential of burning samples. The IR spectra measured are high SN, transmission quality, that are easily interpreted and searchable. O-PTIR has proven to have increased in IR sensitivity even at the submicron scales. We will use O-PTIR to contrast variances in the IR spectra of virgin vs weathers MP particles. We will determine at what levels, if any that O-PTIR can detect PFAS additives in these MP's ranging in size from 50-300µm. These measurements will then be correlated to comparative studies using pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS).

4.05.P-Tu-134 Forensic Fingerprinting of the Unseen: Revealing the Dark Secrets of PFAS with High-Resolution Ion Mobility

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As a topic of growing concern in both the public and regulatory agencies, the persistent and ubiquitous distribution of per and polyfluorinated alkyl substances (PFAS) is an emerging challenge. Due to their slow breakdown, PFAS accumulate in the environment over time and the need for comprehensive analysis is driven by the desire to accurately assess the potential impact of these materials as well as the growing importance of environmental characterization and regulation. The high chemical diversity of these species, which includes numerous isomeric forms, makes comprehensive identification and quantitation difficult to impossible with existing techniques. Commonly, the technique to monitor the concentration of PFAS in collected samples is a targeted LC-MS/MS experiment, which presents numerous shortcomings including loss of information regarding PFAS excluded from the targeted list, discrimination of isomeric and isobaric species and retrospective data mining processes. This challenge is underscored by the lack of sufficient informatics tools for characterization. The objective of this study was to explore the use of novel analytical tools for PFAS forensic fingerprint analysis, specifically high-resolution ion mobility (HRIM) coupled to High Resolution Mass Spectrometry (HRMS), to illustrate an unparalleled combination of analytical techniques for understanding PFAS distribution, impact, and possible mitigation. HRIM augments mass spectrometry's capabilities by separating molecules based on their shape and charge, enhancing separation in complex matrices and removing the challenges of reliance on liquid chromatography (LC) for specificity not achievable by LC-HRMS alone. By rapidly separating compounds before mass detection, HRIM considerably cuts down analysis time, solvent use and heightens throughput without losing specificity. Additionally, the structural information provided by HRIM dramatically improves both targeted and non-targeted workflow performance. Here we showcase multiple advantages, including the separation of branched isomers and the addition of CCS as a complementary identifier. The conformational space map serves as a visual guide, facilitating the prioritization and annotation of unknown compounds by highlighting structural characteristics. The combined use of LC, HRIM, and HRMS emerges as a potent toolset, capable of unveiling the broader spectrum of PFAS in the environment aimed at identifying and attributing sources of PFAS.

4.05.P-Tu-135 Multivariate Forensic Analysis Enables Aqueous Film-Forming Foam Formulation Attribution by Type, Manufacturer, and Year Using 1H and 19F NMR

Lya Carini[1], *Esteban Eustacio Hernandez*[1], *Corey De La Cruz*[1], *Amergin McDavid*[1], *Andre Schaum*[1], *Derek Muensterman*[1], *Patrick Reardon*[1], *Christopher G. Heron*[1], *Jennifer Field*[1] *and Gerrad Jones*[2], *(1)Oregon State University, Corvalis, United States, (2)Biological and Ecological Engineering, Oregon State University*

Aqueous film-forming foams (AFFFs) are significant sources of per- and polyfluoroalkyl substances (PFAS) to the environment. Widespread use of AFFFs has resulted in extensive PFAS contamination near fire-fighter training areas on military bases and commercial airports. The PFAS contained in AFFFs derive from two distinct synthetic process: electrochemical fluorination (EFC) and fluorotelomerization (FT). The objective of this study was to analyze 212 AFFFs by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) from categories including MilSpec, non-MilSpec, and alcohol resistant AFFF that were

manufactured by eight AFFF manufacturers over a 36-year period along with four NIST standard reference materials, using multivariate forensic analysis to characterize formulations by type, manufacturer, and year.

4.05.P-Tu-136 Assessing Trophic Transfer and Movement Trends of Per- and Polyfluoroalkyl Substances in Aquatic and Terrestrial Food Webs

***Heather Parsons** and Xiaoyu Xu, University of Georgia, Athens, United States*

Per- and polyfluoroalkyl substances (PFAS) are a bioaccumulative chemical group that are highly widespread, contain toxic properties, and are largely unstudied. They have been linked to the heavy and direct use of Aqueous Film Forming Foam (AFFF) during fire suppression training activities which results in contamination and bioaccumulation in wildlife. Several studies suggest a variety of factors influence uptake, bioaccumulation, and transport of PFAS, however, many questions remain unanswered on the dynamics of PFAS movement. Very few studies have simultaneously compared PFAS characterization in both terrestrial and aquatic systems through trophic levels and none have compared uptake potentials within a field-based model. This research will assess PFAS concentrations and distributions from a variety of terrestrial and aquatic taxa located on the Savannah River Site (SRS, Aiken, SC, USA). The aim of this research is to (1) assess the variables (i.e., aquatic/terrestrial, abiotic factors, compound structures) which impact trophic transfer and biomagnification of PFAS, (2) to measure and characterize which PFAS compounds bioaccumulate from aquatic and terrestrial media, and (3) to create a quantitatively predictive model for PFAS trophic transfer. We hypothesize that the highest PFAS concentrations will consist of long-chain compounds and reside in organisms possessing the highest trophic status. Additionally, we expect to see a difference in the characterization and concentrations of PFAS found in terrestrial than in aquatic systems**.** Determination of specific contaminant concentrations in wildlife populations from a variety of taxa will allow us to build an accurate and predictive model for use in environmental management and will indicate which species are most at-risk of toxicity effects. Addressing the many uncertainties pertaining to PFAS is critical for understanding the ecological risks they pose and for developing environmental management protocols. Overall, this research will explore environmental factors, movement trends, and uptake capabilities of PFAS providing evidence needed to improve regulatory and ecological remediation efforts on SRS.

4.05.T Bridging the Gap Between the Unknown and the Known for PFAS Analysis

4.05.T-01 Multivariate Forensic Analysis Enables Aqueous Film-Forming Foam Formulation Attribution by Type, Manufacturer, and Year Using 1H and 19F NMR

***Lya Carini**[1], Esteban Eustacio Hernandez[1], Corey De La Cruz[1], Amergin McDavid[1], Andre Schaum[1], Derek Muensterman[1], Patrick Reardon[1], Jennifer Field[1] and Gerrad Jones[2], (1)Oregon State University, Corvalis, United States, (2)Biological and Ecological Engineering, Oregon State University*

Aqueous film-forming foams (AFFFs) are significant sources of per- and polyfluoroalkyl substances (PFAS) to the environment. The PFAS contained in AFFFs derive from two distinct synthetic process: electrochemical fluorination (EFC) and fluorotelomerization (FT). Current analytical methods that provide quantitative measures of individual PFAS in AFFFs are often complicated, time-consuming, and expensive, while methods that quantify total fluorine provide relatively little information on the chemical nature of the PFAS. Rapid and inexpensive analysis methods such as Nuclear Magnetic Resonance (NMR) spectroscopy are needed to quantify total fluorine and characterize complex AFFF formulations for forensics purposes. The objective of this study was to analyze 212 AFFFs by ^{19}F and ^{1}H NMR from categories including MilSpec, non-MilSpec, and alcohol resistant AFFF that were manufactured by eight AFFF manufacturers over a 36-year period along with four NIST standard reference materials, using multivariate forensic analysis to characterize formulations by type, manufacturer, and year. As an extension of this research, these same formulations are also being analyzed via liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS).

4.05.T-02 Data Processing Workflow Challenges for Non-targeted Analysis (NTA) of Per- and Polyfluoroalkyl Substances (PFAS)

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Workflows for the non-targeted analysis (NTA) of per- and polyfluoroalkyl substances (PFAS) present a variety of challenges from sample preparation to data processing and communicating results. A critical part of the PFAS NTA workflow is the high-resolution mass spectrometry data processing using a variety of techniques and tools to clean-up, organize, and prioritize features for compound identification. Advancements in open-source data processing tools specifically designed for PFAS NTA allows for improved identification of new and related PFAS structures that may have previously been unreported, while vendor-supported data processing workflows within specific data-processing software provide different tools to support NTA. However, there remains a need for additional development and harmonization of PFAS NTA tool functionality to address ongoing challenges, including but not limited to semi-quantitative analysis, positive electrospray ionization data, suspect libraries, data sharing, and confidence reporting. A comparative analysis of several PFAS NTA data processing workflows will be presented. The aim is to highlight the limitations encountered when identifying non-targeted or unknown PFAS in environmental samples contaminated with PFAS using currently available tools. Additional tools that address some of these PFAS NTA data processing challenges may also be discussed.

4.05.T-03 Transport and Transformation of Poly- and Perfluoroalkyl Substances in Aqueous Film-Forming Foam Impacted Soil Under Unsaturated Conditions

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Aqueous film-forming foam (AFFF) has been extensively applied in firefighting activities since the 1960s, resulting in release of per- and polyfluoroalkyl substances (PFAS) substances in the vadose zone and groundwater beneath former fire training areas. The composition of PFAS in AFFF includes a range of well-characterized perfluoroalkyl acids (PFAAs) and polyfluoroalkyl substances, some of which are PFAA precursors. Ability to elucidate precursor composition in AFFF and AFFF-impacted media is a relatively recent development in PFAS state of the science. Limited, recent studies have used high resolution mass spectrometry (HRMS) to evaluate precursor leaching and found that precursors remaining after leaching experiments were 61-100% cationic and zwitterionic. These precursors could be mobilized from impacted soils by using heat activated persulfate to transform these precursors to more mobile, anionic PFAAs. This suggests promise for persulfate as an aquifer flushing technique to support more efficient PFAS recovery during pump and treat remediation, but investigations are needed under variably saturated conditions typical of precursor source zones. In this study, 1-D column experiments at

40%, 60%, and 100% saturations over 14 days were conducted to evaluate transport of AFFF-relevant precursors under conditions representative of the vadose zone. At 40% and 100% saturation, columns were repeated with the addition of a heat-activated persulfate flush. Column soils (pre- and post-leaching) eluants were evaluated using targeted analysis, total oxidizable precursor assay, and HRMS. Total PFAS recovery for all treated and control columns was within the target range of 70-130% suggesting a closed mass balance. Retarded breakthrough curves from unsaturated columns confirmed the presence of PFAS retention in air-water interface (AWI). Correlation of saturation and carbon chain length to PFAS retention in AWI was observed within a homologous series of PFAS. In columns at 100% saturation, 10% more total PFAS was mobilized from persulfate-treated columns vs. untreated columns resulted in over 95% of total PFAS recovered and 110% more total PFCAs transformed in eluants. Overall, this study develops a more comprehensive understanding of PFAS precursors fate and transport in the vadose zone and provides both conceptual and quantitative foundation for studies of PFAS precursors.

4.05.T-04 Assessing Exposure of Osprey in the Chesapeake Bay and Delaware River Basin to Per- and Polyfluoroalkyl Substances Using Suspect Screening and Non-Targeted Analysis Tools

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Per- and polyfluoroalkyl substances (PFAS) are a diverse family of chemicals that are persistent in the environment and have been associated with a wide range of adverse health effects. Development of substitute PFAS compounds for the legacy long-chain PFAS (e.g., PFOA & PFOS) has resulted in further environmental exposure to new sub-classes of PFAS. Using nontargeted analysis (NTA) high-resolution mass spectrometry (HRMS) techniques, researchers have attempted to annotate formulas and likely structures for molecular features in environmental samples. Recently, HRMS techniques have been used to identify novel chlorinated PFAS compounds in soil samples collected in southern New Jersey near the Delaware River. Both the Chesapeake Bay and Delaware River Basin represent important habitats for many animal or avian species, including one of the largest breeding populations of osprey. Effective and accurate environmental monitoring of osprey is critical to understanding exposure and bioaccumulation of PFAS in these ecosystems. Targeted analysis has been previously conducted to examine exposure of osprey eggs in these ecosystems to different contaminants, including PFAS. This study aims to retrospectively examine exposure of osprey in the Chesapeake Bay and Delaware River Basin to novel PFAS using suspect screening and NTA techniques. Here we reanalyzed archived osprey plasma samples collected as part of studies conducted in the Chesapeake Bay in 2011 and in the Delaware River Basin in 2015. The samples cover 15 sites on the Delaware River and 4 sites in the Chesapeake Bay. Suspect screening and NTA data were processed through Thermo Scientific^{TM} Compound Discoverer and NIST DIMSpec to annotate potential novel PFAS features present in the osprey plasma. Initial results indicate the bioaccumulation of several chloro-perfluoropolyether carboxylates in osprey serum samples collected from the Delaware River Basin that are not part of the targeted PFAS method and were previously detected via HRMS in New Jersey soil samples. The results of this study will provide historical information on the potential PFAS exposure of osprey and other migratory

shorebirds in the Atlantic Flyway. Additionally, information will help to expand the list of potential PFAS to be monitored for in the Chesapeake Bay and Delaware River Basin ecosystems.

4.05.T-05 Broad Investigation of PFAS in Songbird Eggs from a Belgian Hotspot by Target Analysis and Untargeted Approaches: Suspect Screening, Non-Target Analysis, and Total Oxidizable Precursor Assay

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The Flanders region (Belgium) has been identified as a major hotspot of PFAS in Europe due to the presence of several industries using and producing PFAS, including the 3M factory that has caused extensive pollution in the surrounding area of Antwerp. Previous studies near this hotspot revealed some of the highest PFAS concentrations ever reported in soils, invertebrates and songbirds. However, these studies mainly focused on a set of legacy PFAS analysed with a target LC-MS/MS approach. Since bird eggs can be used as a biomonitoring tool for exposure assessment of PFAS-contaminated areas, the main objective of this work was to combine target analysis with untargeted approaches for a comprehensive investigation of PFAS bioaccumulation in bird eggs. Employed untargeted methods included suspect screening, Non-Target Analysis (NTA) and the Total Oxidizable Precursor Assay (TOPA). In addition, the different isomeric forms of some PFAS were investigated through ion-mobility coupled with high-resolution mass-spectrometry. In total, 29 great tit (*Parus major*) eggs and 10 blue tits (*Cyanistes caeruleus*) eggs were collected one egg per nest at three different sites within a 0-1.5 km radius of the 3M plant in April-May 2022. LC-MS/MS analysis was performed for the target analysis of 42 PFAS, and LC-QTOF analysis for suspect screening and NTA. For the TOPA, direct oxidation of homogenized samples was performed prior to extraction and then target analysis was performed. Two suspect lists—with a total of >10,000 PFAS—were used to identify suspect features, and FluoroMatch software was used to process NTA data. Target analysis showed that PFOS was predominant in the eggs, with concentrations ranging from 14 to 67,920 µg/kg ww (mean = 6780 µg/kg ww) and in general, long-chain PFCAs and PFSAs were primarily detected in the eggs. Suspect screening revealed the presence of several classes of emerging PFAS, such as pentafluorosulfide PFSAs and ether-substituted PFCAs. Several compounds were identified at confidence level 2 and 3. NTA allowed the determination of additional emerging and homologues compounds not identified through suspect screening. Direct TOPA revealed an increase of several short-chain PFAA after the oxidation (*e.g.,* PFBS +460%) suggesting the presence of short-chain precursors in the eggs. The study of isomers revealed the presence of different isomers, primarily including PFOS (5 isomers), PFDS (3 isomers), PFHpS, PFNS, PFTrDA, and PFTeDA (2 isomers each).

4.05.T-06 Unravelling PFAS Precursors in Background Soils Using Non-Targeted Analysis Techniques Pre- and Post-TOP Assay

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For a comprehensive picture of PFAS exposure, target analysis is often complemented with the total oxidizable precursor (TOP) assay and/or high resolution mass spectrometry (HRMS)-based approaches. It has been shown that the conventional TOP assay, looking at the difference in concentrations of ≥ C4 perfluoroalkyl carboxylic acids (PFCAs) pre- and post-oxidation, can underestimate the unknown PFAS fraction, because it does not capture untargeted oxidation products and unoxidized precursors. However, only a few studies have embedded HRMS-based approaches in the TOP assay and applied suspect screening and non-targeted analysis to both pre- and post-TOP samples. In the current study, more than 300 top soil samples were collected in rural areas without known PFAS point sources across the German state of North Rhine-Westfalia. Based on results from target analysis and TOP assay (including C2 and C3 PFCAs), samples from ten sites were selected for embedding an HRMS-based approach. Pre- and post-TOP methanolic top soil extracts were analyzed by liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QToF), employing electrospray ionization in positive and negative mode. Suspect lists were created based on land use categories and potentially associated sources. In addition to PFAS-characteristic filters, a comparison of pre- and post-TOP samples was included in the data evaluation workflow. Along with well-known precursors, such as the phosphate esters 6:2 diPAP and diSAmPAP, four pesticides containing a C-CF_3-group were identified in pre-TOP samples from sites with agricultural land use. This included diflufenican, fluazinam, flufenacet and fluopyram. Non-targeted analysis showed that these pesticides were among the most intense features in the corresponding samples. While the C-CF_3-containing pesticides were not detected in post-TOP samples, the concentration of trifluoroacetate (TFA) significantly increased compared to pre-TOP, pointing to formation of TFA as an oxidation product. Diflufenican is applied in comparatively high quantities in various countries, including the US, Chile, Kenya, Europe and China. This underlines its potential role as a significant TFA precursor worldwide. As both the ultra short-chain transformation product and the identified PFAS pesticide precursors would have been overlooked in a conventional TOP assay, the study highlights the added value of embedding non-targeted analysis techniques in the TOP assay.

4.06.A.T Challenges in PFAS Analyses and Detection

4.06.A.T-01 Analysis of PFAS in Consumer Products by extraction and LCMSMS

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Per- and Poly Fluorinated Alkyl Substances (PFAS) in consumer products is becoming a source of concern. From intended use to end of life, PFAS contained in consumer products may find their way into the environment. Methods for quantifying PFAS in consumer products are in their infancy and range from simple to complex: various extraction methods and several analytical approaches can be applied. Further, consumer products can be treated as a class, with multiple sub-classes: polymers, textiles, papers, and cosmetics may all contain different forms of PFAS. This presentation aims to summarize work done on multiple consumer product matrices using a

co-solvation extraction followed by targeted analysis via Liquid Chromatography Mass Spectrometry (LCMS). Other sample preparation and extraction methods will be used for comparison. Total fluoride and extracted fluoride results will also be discussed in comparison to LCMS results.

4.06.A.T-02 Escaping the Matrix: Eliminating Matrix Effects in Lipid-rich, Full-Fish Homogenates

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Extracting PFAS from dirty matrices is a challenging task. With the finalization of the EPA method 1633 (Analysis of Per- and Polyfluoroalkyl Substances in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS), the scientific community is closer to reaching that goal. However, the basic pH method in EPA 1633 is not suitable for some of the most complicated matrices, including lipid-rich, full-fish homogenates. Basic methods are lipid-limited and there is an increased likelihood of incomplete separation of PFAS due to significant matrix interferences. This poses a challenge for analyzing PFAS in whole fish (as opposed to fillets or organs) which can provide valuable information about both human and animal exposure through consumption. In addition, many methods focus on the analysis of PFAS in fish fillets with the primary purpose of informing human consumption advisories. However, this is likely not representative of the PFAS burden consumed by aquatic predators, who generally eat most of the fish, if not all. Some internal organs, such as the liver, have higher concentrations of PFAS than the fillet. Analyzing the homogenate of a whole fish provides a holistic view of animal exposure through consumption, as well as exposure for the fish itself. We developed an extraction method that can be used to analyze linear perfluorocarboxylic and perfluorosulfonic acids in lipid-rich, full-fish homogenates. Recoveries of PFCAs and PFSAs were between 73 and 104%, which is comparable, if not improved from, the acceptable range in EPA 1633. This method allowed us to analyze homogenized Pacific Herring and Chinook salmon samples. EPA method 1633 was tested on these samples, but there was no recovery of target analytes. Using the method in this presentation, linear PFCAs were detected 54% of the time with an average concentration and SEM of 5.2 ± 1.9 ng/g ww. Linear PFSAs were detected in 73% of samples with an average concentration of 1.6 ± 0.2 ng/g ww. As with most other biological samples, the profile of linear PFSAs was predominantly PFOS, which had a detection frequency of 73% and an average concentration of 1.4 ± 0.2 ng/g ww. While this method does have trade-offs with EPA method 1633, including a smaller amenable suite of analytes, it can be used to determine trophic magnification of PFAS more accurately. Additionally, this method can be used on banked fish samples that have been homogenized before storage, providing an opportunity for historical sample analysis.

4.06.A.T-03 A Straightforward Method for the Extraction, Cleanup, and Quantitative Analysis of 45 PFAS in Whole Fish

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Analytically, per- and polyfluoroalkyl substances (PFAS) can be challenging to quantify due to the complex matrices in which they are found and/or due to their physical chemical properties which can vary widely within and between homologous series. Recently, EPA method 1633 was applied to whole-fish homogenates which resulted in substandard precision and accuracy even after various modifications. This study introduces and validates a new method for extraction and cleanup of PFAS from whole-body fish tissue. The new method uses lipid removal technology to rapidly and efficiently remove undesirable matrix interferents from the extract without the need for solid-phase extraction or evaporative concentration and solvent exchange. The analyte recovery of the presented method is high (average of 86 ± 13%) and the data produced are accurate and precise (96 ± 9% compared to spiked concentration). Twenty-three of the PFAS exhibit no matrix effects; the highest degree of matrix suppression is a reduction in signal of 48%. Additionally, 43% of the method detection limits are at or below the those outlined in EPA method 1633; only seven compounds have detection limits that exceed 3x of those reported in 1633. Lyophilization was evaluated as a preservation technique for biological tissue, which results in the loss of only a few volatile compounds. The method outlined here was demonstrated on a taxonomically diverse set of whole-fish composites from Lake Michigan collected in 1994 that were part of a larger study on environmental contaminants. The results reveal that in this set of samples the magnitude of PFAS contamination in fish is driven by collection location, while the distribution of PFAS is driven by species type. Stable isotope data and redundancy analysis demonstrate that sulfonates and sulfonamides are more common with lower trophic level fish while long-chain carboxylates are more common fish with offshore habitat use. Additionally, this method is being applied to an archive Lake Trout that has been annually collected in the Great Lakes from the 1970s to the current day and results of that analysis will also be presented. *This work does not necessarily reflect U.S. Environmental Protection Agency views or policy and any reference to commercial products does not imply an endorsement.*

4.06.A.T-04 Enhanced Passive Sampling Devices for PFAS Monitoring in Surface Water: A Modified Approach based on US EPA Method 1633

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Per- and polyfluoroalkyl substances (PFAS) pose significant environmental and human health concerns due to their widespread distribution and potential adverse effects. This research aims to advance the field by developing novel passive sampling devices (PSDs) tailored for precise PFAS analysis in natural water systems. Drawing upon principles of equilibrium passive sampling, traditionally applied to hydrophobic organic chemicals, the study investigates various polymer supports such as polydimethylsiloxane (PDMS), agarose (AG), and cellulose acetate (CA), augmented with embedded sorbents including activated carbon and serum albumin, to optimize PFAS sorption characteristics. The Research represents a significant stride forward in PFAS analysis, promising a novel method for monitoring PFAS contamination in water sources. Promising prototypes have undergone rigorous sorption isotherm experiments to elucidate their binding and desorption kinetics. Subsequently, the research will transition to field testing of selected passive samplers in the Potomac River, targeting sites impacted by wastewater discharge, military installations, airports, and landfills. Utilizing liquid chromatography-tandem mass spectrometry (LC-MS/MS) in tandem with EPA Standard Method 1633, with a particular focus on key variants such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). Adopting passive sampling devices offers a promising avenue for measuring freely dissolved PFAS concentrations, addressing the imperative for precise risk assessment in environmental and public health contexts. This research aims to contribute significantly to ongoing efforts to mitigate the environmental and human health risks associated with PFAS contamination.

4.06.A.T-05 Enhancing PFAS Analysis Efficiency in Solid and Liquid Matrices Using Automated Online SPE and LC-MS/MS: Application to EPA Method 1633 Compound List

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The environmental impact of per- and polyfluoroalkyl substances (PFAS) is a significant concern due to their persistence, bioaccumulation, and potential health risks. Accurate measurement of PFAS in environmental, biological, and food samples is crucial for assessing exposure, risk and fate. Liquid chromatography with mass spectrometry (LC-MS/MS) following solid-phase extraction (SPE) is the prevailing method for detecting PFAS compounds. To enhance analysis efficiency, reduce errors, and promote sustainable practices, we evaluated a fully automated online SPE coupled with LC-MS/MS system for analyzing PFAS listed in EPA method 1633 across environmental liquid and solid samples. Our automated approach allows simultaneous analysis of soil samples (following EPA method 1633) and aqueous samples (using the co-solvation approach, e.g., EPA method 8327, coupled to the online SPE approach) with the same instrument and calibration curve. This integration significantly improves laboratory productivity and reduces labor, solvent costs and eliminates human error. We discuss the benefits and challenges, including comparing to current method specifications, estimating savings, and exploring the feasibility of running different methods and matrices on a single instrument. Our findings provide valuable guidance for resource optimization and streamlined workflows in comprehensive PFAS analysis, addressing critical environmental and health concerns.

4.06.B.T Challenges in PFAS Analyses and Detection

4.06.B.T-01 Measurement of Volatile Per- and Poly-Fluoroalkyl Substances from Whole Air and Headspace Using Selected-Ion Flow-Tube Mass Spectrometry

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Per- and poly-fluoroalkyl substances (PFAS) are widely used in consumer products to improve durability and provide various properties, including non-stick or stain coatings and water resistance. Understanding their presence in products is important because these molecules not only persist in the environment but are toxic and accumulate in the bodies of humans and animals. Testing for the presence of low-concentration PFAS impurities in consumer products using direct measurement techniques can be challenging due to the presence of other high concentration volatiles, resulting in reagent ion depletion and saturation effects. Selected-ion flow-tube mass spectrometry (SIFT-MS) can overcome these saturation issues by utilizing some of the negative reagent ions, which do not typically react with the more common volatile organic compounds (VOCs). This work utilizes Syft Technologies Tracer instrument operating on helium carrier gas and a modified polyether ether ketone inlet. Standards were prepared for eight unique PFAS compounds into Nalophan bags filled with zero air. Full scans from mass to charge 10-400 were run on the instrument to create library entries for each standard. Consumer products were

decanted into 250 milliliter glass bottle. Samples were heated to 40 degrees centigrade for 10 minutes, and the sample bottle septum was connected directly to the instrument inlet. Zero air flowed into the bottle to equalize the pressure during sampling. Full scans were again collected for each sample. Data was reprocessed using a targeted method for the eight PFAS. The positive SIFT-MS reagent ions showed signs of saturation from other VOCs present in most of the commercial products tested. We were able to successfully utilize three of the negative reagent ions to measure PFAS from numerous personal care and household products. The unique specificity and selectivity of the O2-, NO2- and NO3- reagent ions for fluorinated species mitigated any matrix effects caused by the more abundant volatiles present in these products, allowing for selective, high-sensitivity quantitation of PFAS species without the need for dilution of the products. The eight reagent ions available with the Syft Tracer provide enhanced selectivity of low concentration PFAS, because three negative reagent ions can measure low concentration of PFAS without any ion suppression effects. This prevents the need for sample dilution or pre-concentration, facilitating rapid sample screening.

4.06.B.T-02 Volatile PFAS: Head Space—Solid Phase Microextraction—GC/MS Analysis with Minimal Sample Preparation

__Ruth Marfil-Vega__, Andy L. Sandy, Evelyn Wang and Yoshiyuki Okamura, Shimadzu Scientific Instruments

Monitoring Per- and polyfluoroalkyl substances (PFAS) throughout their life cycle is crucial for mitigating their risk. Historically, priority has been given to the analysis of PFAS by liquid chromatography- mass spectrometry. However, there are several classes of volatile PFAS used in the manufacturing of industrial and consumer products that can migrate from those products to humans and different ecosystems. In this work, a Head-Space Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (HS-SPME GCMS) analytical method was developed to analyze six classes of volatile PFAS using two types of mass spectrometers: single quadrupole and triple quadrupole. These methods can be applied to a variety of PFAS-impacted sample types, including liquids and solids, to determine occurrence and fate and transport in the environment (e.g., volatilization studies) and commercial products (e.g., leaching from food contact materials). Thirteen target compounds were included in the method and quantitation was performed by an internal standard calibration. Laboratory blanks were analyzed during the experiments to ensure the absence of contamination from the laboratory and consumables used. In addition, the evaluation of carryover from the highest calibration standard was conducted by running a blank after this standard. The final methods were selective and provided high sensitivity to measure targeted PFAS compounds at trace concentrations. Calibration curve (each with a minimum of seven calibrators) results showed a good linear fit for all compounds with $R^2 \geq 0.993$ and RF %RSD < 20. Linear range varied for the target compounds. In this study, none of the target PFAS in the laboratory blank samples showed quantifiable results. The results from the carry over analysis showed < 0.2 % carryover effect. Furthermore, PFAS in the blank were below the quantitation range (2.5 to 25 pg/mL), depending on the target compound) of this method. In this presentation we will discuss how HS-SPME is suitable for the analysis of volatile compounds from liquid and solid matrices (drinking water, beverages, food packaging, etc.). We will also share the outcomes from method development experiments and performance results for the analysis of the selected classes of PFAS.

4.06.B.T-03 Bridging Methodological Divides: Comparative Analysis of Total Organofluorine Techniques in AFFF-Impacted Water

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Multiple poly- and perfluoroalkyl substances (PFAS) are present in aqueous film-forming foams (AFFF) used for firefighting activities. Currently, no single analytical technique provides a complete accounting of total PFASs or total organofluorine content in AFFF-contaminated samples. To provide insight into the performance of existing methods, we compared ten PFAS measurement techniques. In AFFF-amended tap water, U.S. EPA methods 533 and 1633, adsorbable organic fluorine with particle induced gamma emission spectroscopy (AOF-PIGE) and fluorine-19 nuclear magnetic resonance (^{19}F-NMR) provided similar estimates of total fluorine. The total oxidizable precursor (TOP) assay, suspect screening, and adsorbable organic fluorine with combustion ion chromatography (AOF-CIC) yielded estimates of total organic fluorine that were about two to three times higher than the other techniques. Proximate to AFFF sources, suspect screening and modified EPA method 1633 yielded higher results, while the TOP assay results were between the other two sets of analyses. Further from sources, suspect screening, modified EPA method 1633, and the TOP assay yielded similar results that were higher than targeted quantification methods. These results are consistent with expectations about PFAS behavior and inform the selection of analytical techniques used for PFAS contamination characterization efforts.

4.06.B.T-05 Colloidal Fluorinated Side-Chain Polymer Nanoparticles Are a Significant Source of PFAS Contamination in Textile Wastewater

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Fluorinated side-chain polymers (FSCP) based on per-and-polyfluorinated substances (PFAS) are used to create water-repellant and stain-resistant fabrics on textiles. The recent discovery of unexplained C_4—C_8 perfluorocarboxylic acids (PFCAs) at concentrations ranging from 50-1,000 ng/L in public drinking water supplies downstream from textile industry discharges in North Carolina initiated an investigation into the source of these PFAS. Analysis of raw industrial wastewaters in an upstream municipality revealed short-chain (C_4-C_7) PFCAs at levels insufficient to explain the extent of contamination. However, elevated concentrations of short-chain PFCAs were found in the corresponding wastewater treatment plant (WWTP) effluent relative to its influent, suggesting the presence of unknown PFAS precursors in raw wastewater. We employed the total oxidizable precursor (TOP) assay to assess PFAS precursor concentrations in textile wastewaters entering the WWTP. PFAS precursor concentrations near 10,000,000 ng/L were found in raw textile wastewaters after TOP assay, revealing the primary source of PFAS to the WWTP. A mass balance of WWTP influent and effluent revealed that over 90 percent of FSCP entering the WWTP was removed to solid waste. TOP fingerprinting indicated that textile-associated precursors contained primarily 6:2 fluorotelomer-based PFAS functionality, but the application of high-resolution mass spectrometry (HR-MS) failed to yield the identity of these precursors. Ultrafiltration prior to TOP assay revealed that PFAS precursors in textile wastewater were colloidal in nature, as they passed through a 0.2 μm filter but were quantitatively retained by a 100 kDa ultrafiltration membrane. Size separation and characterization of these isolated colloidal fractions using asymmetric field-flow fractionation

(AF4) revealed them to be consistent with FSCP nanoparticles used in fabric finishing. In addition to wastewaters, FSCP from WWTP biosolids were extracted by sonication in a surfactant solution and were characterized by ultrafiltration and the TOP assay. The presence of colloidal PFAS in wastewater and their potential to degrade to molecular PFAS during transport poses significant challenges for receiving waters, downstream drinking water, and areas where biosolids are applied.

4.06.B.T-06 Capturing the Full Residual Profile from Fluoropolymer Manufacturing: Ultra Short Chains to Novel Polyfluorinated Residuals

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Fluoropolymers are essential to a variety of industries including clean energy, advanced electronics, and electronic vehicles. Manufacturing fluoropolymers, however, has the potential to generate fluorinated residuals not intentionally added during the manufacturing process. The responsible manufacturing of fluoropolymers and fluorinated small molecules, therefore, requires analytical methods appropriate to monitor analytes spanning a broad range of concentrations, molecular weights, structures, and functionality. While analytical technologies have progressed, a single measurement tool capable of measuring all fluorinated organic components and characterizing unknown analytes down to relevant concentrations, i.e., parts per billion, parts per trillion, or lower, is not available. Multiple methods and sample preparation schemes, which depend on the sample matrix being interrogated, are required to characterize the full fluorinated residuals profile of a product or aqueous sample with considerations given to the chemistry, additives, and surfactant technologies involved. Liquid chromatography with tandem mass spectrometry offers with the selectivity and sensitivity to measure residuals to the appropriate concentration, where choice of experimental configuration and detection scheme can probe analytes ranging from ultra short chains (< C4) to novel polyfluorinated residuals not captured on standard targeted lists. Ultra short chains offer the challenge of limited retention using conditions suitable for longer chain analytes while novel polyfluorinated species can be difficult to detect depending on their ability to ionize but are observed when using hydrocarbon processing aids. A stepwise approach is utilized in this work first screening for total dissolved organic fluorine, using Liquid Chromatography with Triple Quadrupole Detection (LC/QqQ) to measure known targeted analytes including ultra short chains, and Liquid Chromatography with Quadrupole Time of Flight (QToF) or Orbitrap to capture remaining fluorinated residuals allowing the broadest range of analytes to be interrogated. While each of these methods offers unique challenges, when coupled together and with appropriate data processing tools and workflows this approach can comprehensively interrogate the sample matrix, help validate abatement strategies, and prevent exposure to the environment to allow responsible manufacturing of fluoropolymers.

4.06.P-We-088 Method Performance Using Dual WAX/GCB and GCB/WAX Formats for PFAS Analysis Using EPA Method 1633

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The U.S. Environmental Protection Agency recently released method 1633 “Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-

MS/MS," This method involves a two-step sample prep approach using a weak anion exchange (WAX) SPE cartridge and graphitized carbon black (GCB) clean-up in a powder format, known as dispersive Solid Phase Extraction (dSPE). For water samples, GCB is added after WAX extraction, but for soil samples the GCB is added before the WAX SPE step. The purpose of the additional GCB clean-up step is to eliminate matrix that can cause interference and reduce bias. GCB has been shown to remove organic acids (such as humic and cholic acids), which can suppress ionization and lead to low bias on the recoveries (especially for PFOS). However, limitations of using GCB are well known in that this media can bind to longer chain PFAS compounds and lead to lower recoveries. This is stated in the EPA method 1633, "…It is important to minimize the time the sample extract is in contact with the carbon." Besides these practical limitations, adding GCB in a dSPE step is very labor intensive and therefore not practical due to the extra time needed to add, mix, and centrifuge for each sample, especially in high throughput laboratories. In addition, due to the vague guidelines on using GCB for dSPE listed in the 1633 Draft Method, this step can also lead to higher RSD values. To sort out this challenge, Strata™ PFAS cartridges were developed as a single cartridge stacked with Strata-X-AW and Strata GCB sorbents that function as a traditional SPE cartridge with a built-in polishing step to meet the method guidelines. We have demonstrated the utility of the Strata PFAS stacked SPE format for PFAS analysis following DOD QSM 5.2/Table B15 for various water matrices. We have shown that using a single, stacked WAX/GCB is cheaper, easier, and ultimately yields better recoveries for PFAS analytes from various water samples. PFAS compounds are not all the same and include differences in carbon length and additional functionality. This poster will present a comparison of SPE cartridges varying in functionality for the extraction of a wide range of PFAS compounds in water. Data will show spike recovery comparisons between WAX + dSPE and a WAX/GCB stacked column.

4.06.P-We-089 Column Chemistry Considerations for Full Coverage of Sample Matrices and Analyte Ranges in PFAS LC-MS/MS Workflows

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LC-MS/MS workflows for PFAS analysis in environment drinking water and solid waste samples cover a wide range of sample preparations, target analytes and analyte ranges. As the required analytes continue to increase in number—including PFAS replacement compounds (GenX) and short-chained PFAS - laboratories running strategies of overlapping PFAS compounds, with poor resolution are often overlooked. The chromatographic separation of PFAS compounds in currently validated methods typically involves a reversed phase mechanism using a C18 or Phenyl column in an acidic-methanol eluent. For example, EPA method 537.1 uses a C18 column (5 µm, 2.1 x 150 mm C18) and EPA method 533 was validated using a C18 Phenomenex Gemini® column (3 µm, 2 x 50 mm). Conversely, ASTM D7979 and EPA 8327 were validated using a Phenyl-Hexyl column (1.7 µm, 2.1 × 100 mm), ISO 21675 used a C18 column (5 µm, 2 × 50 mm) and the Department of Agriculture CLG-PFAS 2.01 method used a C8 column, Phenomenex Luna® C8(2) (3 µm, 2 x 50 mm). Whereas many of these overlapping peaks can be successfully resolved by the mass analyzer, the potential presence of isobaric homologues and unresolved matrix interferences point to the continuing need for good chromatographic separation to assure reliable identification and quantitation. Although the problem may be manageable for today's small analyte lists, the challenge will inevitably grow as new PFAS compounds are added for investigational or regulatory purposes. We will show recent updates to our previous column comparisons while also discussing modifications to eluents and gradients. We will show that the use of alternative stationary phases of varying surface chemistry and eluents of varying polarity can significantly alter the sorption-elution characteristics of different classes of PFAS compounds. This orthogonal approach to PFAS HPLC chromatography should serve as a fruitful approach to future method development. As analyte lists increase in size and complexity, a variety of HPLC column chemistries and eluent compositions will be needed to accommodate the wide range of PFAS related compounds that might be encountered such as polar acids, non-polar acids, esters, amides, sulfonamides, and telomer length, all of which can be complicated with branched vs. linear isomers. The work presented here should be considered a starting point for column chemistry and mobile phase considerations for PFAS HPLC methodology.

4.06.P-We-090 A Multifaceted Evaluation of Stability and Extraction Methods Targeting Novel PFAS within Complex Biological Matrices

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Quantitation of per- and polyfluoroalkyl substances (PFAS) is commonly achieved by employing chromatographic techniques in tandem with mass spectrometry. A plethora of solvent extraction and clean-up methods are found in literature and are often modified based on sample type and suspected analytes. The majority of these methods were derived for perfluoroalkyl acids (PFAAs). As novel PFAS garner research and regulatory attention, translating existing preparation and measurement methods to PFAS beyond PFAAs has become a salient need. The stability of many understudied PFAS in solvents is unknown or questionable. For example, ether-containing PFAS like GenX have been shown to degrade in acetonitrile and dimethyl sulfoxide (DMSO) in laboratory experiments, while little or no data describes the stability of other ether acids. Here, we conducted a multifaceted method development experiment to (1) observe compound stability of diverse ether acids in organic solvents, (2) evaluate extraction method performance between two clean-up techniques, and (3) investigate efficacy of extraction methods across a variety of biological tissue matrices. Experiment I involved a 15-day experiment to measure compound stability in various dilutions of pure solvents versus solvent containing biological sample matrix. High performance liquid chromatography tandem mass spectrometry was used to identify and report concentrations of perfluorinated ether acids (amongst additional perfluoroalkyl acids and fluorotelomers) at d0, d1, d3, d7, and d15. In Experiment ii, extraction methods incorporating the optimal solvent ratio from Experiment i were subjected to two different clean-up steps to compare efficacy of solid phase extraction cartridges designed to remove phospholipids vs graphitized carbon clean-up. The optimal clean-up strategy was then applied in Experiment iii to multiple matrices, including whole fish homogenate, fish embryos, bird egg, whole blood, fish liver, and fish muscle. These results inform our understanding of how non-PFAA PFAS behave in routinely used sample preparation and measurement approaches, allowing users to appropriately tailor their laboratory approaches to best accommodate their analytes of interest.

4.06.P-We-091 Per- and Polyfluoroalkyl Substances (PFAS): Validation of Methodology for the Determination of Residues in Fruit, Fruit Processed Commodities and Fish Tissues

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Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic organofluorine compounds that have become a concern due to their persistence in the environment following extensive use over the last few decades. Due to their high degree of chemical stability, as well as their widespread use, PFAS chemicals now are increasingly being detected in the environment and appear to persist for extended periods of time. Furthermore, exposure to these chemicals in everyday objects, such as packaging and cookware, as well as within certain areas of industry, has led to the apparent bioaccumulation in certain areas of the population. Because of the concerns over health and environmental issues associated with PFAS chemicals, there has been a need to be able to monitor the occurrence of these materials in food commodities. There are numerous approaches to analyzing a range of these chemicals in a number of different matrix types, and this area of analytical chemistry is constantly evolving as new PFAS chemicals are identified and added to the list of those where methods already exist. The work described in this poster was a project to develop and validate an LC-MS/MS method for the analysis of a "typical" suite of PFAS chemicals in the fruit (strawberries and grapes), fruit processed commodities (jam and wine) as well as in fish tissues. A simple and robust method was required, that could be easily applied to routine analysis at low concentrations, using commonly available equipment and approaches. There was also a requirement to be able to add additional PFAS analytes to expand the method as the need arises. The method development work for the analysis of PFAS chemicals in the tested matrices is still ongoing and is based on common residue analysis approaches such as the use of QuEChERS procedures with quantitation performed by LC-MS/MS. This poster will present the work performed to date. The resulting method will be validated for a range of PFAS analytes in the sample matrices at a limit of quantitation (LOQ) of 10 ppb. The validations will be performed in accordance with the SANTE/2020/12830 guidelines to ensure that current and future performance requirements were met. Further work will include additional analytes to the PFASs already validated, as well as to investigate approaches to reduce the LOQ of the method to increase its sensitivity.

4.06.P-We-092 Monitoring Produced Gases from PFAS Removal Technologies Using Thermal Desorption Coupled to Gas Chromatography/Mass Spectrometry

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Many per and polyfluoroalkyl substance (PFAS) removal technologies seek to breakdown the compounds into small components which can be captured or neutralized. Incineration of PFAS, and PFAS containing products like aqueous film forming foams (AFFF), is a common disposal method and aims to degrade PFAS species into HF, CO_2 and water. However if this process does not happen under the correct conditions products of incomplete destruction (PIDs) are created. This is also true of other novel techniques which are now being researched and used to try and managed PFAS. Often PIDs are smaller fluorinated species and have their own detrimental effect upon the environment such as acting as greenhouse gases or ozone depleting substances. This means that PFAS destruction must be monitored in a meaningful way. Thermal desorption coupled to gas chromatography and mass spectrometry (TD-GC-MS) is commonly applied to monitoring of hazardous organic compounds in ambient air. The technique is well suited to

monitoring ultra-volatile species and enables detection of compounds at low ppt levels from canister or online samples. In this study we will share data generated using a Markes UNITY-CIA Advantage-xr preconcentrator system and the 8890/5977 for GC/MS analysis. The matrix from incineration-based sources can be challenging due to high levels of CO2 and water which is a challenge for sample injection, separation and compound detection. The data will show that canister sampling offers a robust approach to performing monitoring of ultra-volatile perfluorocarbon species which are expected to be produced at destruction sites and enable detection limits as low as 1 pptv.

4.06.P-We-093 LC-MS/MS Robustness: A Real-World Case Study of PFAS Testing

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Robustness can be defined as the ability to withstand or overcome adverse conditions or rigorous testing. Taken into the context of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), robustness then relates directly to the fidelity of analyses day-over-day, week-over-week and month-over-month. Demonstrating an LC-MS method is robust is laborious, as it requires a critical mass of longitudinal data, on a system running real-world samples to truly understand method performance within the broader content of expected intra-laboratory variability. This study demonstrates 15 months of robustness for the SCIEX 7500 system in a laboratory analyzing per- and polyfluoroalkyl substances (PFAS) in ground, surface and drinking water samples. The resulting control charts show acceptable response variability in quality control samples across 26 PFAS compounds with minimal instrument maintenance performed over the course of the study.

4.06.P-We-094 Extending Standard Methods for Universality: A Case Study on EPA 1633 in Drinking Water

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As PFAS moves from emerging to regulated contaminant in the US, standard reference methods will become the basis of regulation and enforcement. There is now a diversity of US EPA reference methods for PFAS due to the structure and frameworks of US environmental policy. EPA 537.1 and EPA 533 are specifically validated under the Safe Drinking Water Act (SDWA) to measure a total of 29 PFAS between them with significant, but not complete overlap in targets. EPA 1633 is currently in the process of promulgation under the Clean Water Act to measure 40 PFAS in non-potable water, soils, wastewater, biosolids, leachates, and tissue. EPA 8327 is a direct injection screening-level method for 24 PFAS in aqueous matrices under the SW-846 series methods for the Resource Conservation and Recovery Act (RCRA). This policy fragmentation means there is no single standard method that measures PFAS in all relevant environmental compartments from source to exposure. Countries such as Canada that do not have the same policy framework, but frequently use US EPA reference methods as the basis for their methods need further work to resolve this enforcement challenge. In our study, we extended the use of EPA 1633 to finished drinking water to provide jurisdictions with one reference method that could be used for all sample types while reporting the same standard list of PFAS. We tested the effects of chlorine on the 1633 targets, and three different preservatives/dechlorinating agents Trizma (EPA 537.1), Ammonium Acetate (EPA 533) and Sodium Thiosulfate (most common dechlorinating agent). Tap water samples were spiked at least in triplicate at a level close to the method reporting limit, and analyzed at two different time points (2 and 14 days except for chlorine-fortified samples which only had a 2-day timepoint). All samples were compared against control reagent water. 35 of 40 1633 PFAS showed no effects under any of the experimental conditions. Sodium thiosulfate worked best for all PFAS at both timepoints. Chlorine resulted in a loss of two sulfonamides MeFOSA and EtFOSA by day 2 and a concomitant increase in PFOSA indicating a chemical transformation. Both Trizma and ammonium acetate showed significantly lowered recovery that increased with time for 11Cl-PF3OUdS (F-53B constituent) and PFDoS. Our results show that 1633 can be used consistently to measure drinking water samples preserved with thiosulfate and represents a universal method for PFAS where policy permits.

4.06.P-We-095 Distribution and Modeling of Fluorotelomer Alcohols and Perfluorinated Carboxylic Acids in Soils

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Fluorotelomer polymers (FTPs) are used by industries to treat textiles, carpets, and paper. In the soil environment, FTPs can be transformed into fluorotelomer alcohols (FTOHs), and subsequently to the environmentally persistent perfluorinated carboxylic acids (PFCAs). PFCAs can be accumulated and distributed in soil due to physicochemical properties such as sorption and partitioning affinity. In this study, distribution and transformation of per- polyfluoroalkyl substances (PFAS) in soils receiving sludge from a textile mill are estimated using mathematical modeling, and partitioning characteristics of FTOHs and PFCA are investigated. Targeted PFCAs (perfluorooctanoic acid; PFOA, and perfluorodecanoic acid; PFDA) and FTOHs (8:2 FTOH, 10:2 FTOH, 7:2 secondary FTOH, and 9:2 secondary FTOH) were extracted using methanol and methyl tert-butyl ether, respectively. To quantify analytes, negative electrospray ionization, liquid chromatography with tandem mass spectrometry was used for PFCAs, and positive chemical ionization, gas chromatography with mass spectrometry was used for FTOHs. The accumulated FTP mass at the surface was estimated from reported applied sludge quantities. Distribution and transportation of PFCAs and FTOHs in soil depth profiles were estimated with a mass balanced model coded in R. FTOH concentrations were significantly higher at the surface compared to subsurface environments, regardless of any soil properties such as water content or total organic carbon. Modeled PFCAs distribution showed low mobility along the shallow profile in soil as reflected in a shallow subsurface maximum concentration, with increasing concentrations over time. In the environment, different partitioning properties affect leaching and sorption of contaminants. As FTPs at the surface continuously degrade and provide a source for FTOHs and PFCAs, the estimated concentrations of FTOHs and PFCAs, as precursor and product, will be helpful for elucidating the impact of contaminants in soil and groundwater systems.

4.06.P-We-096 A Method Development Study to Comparatively Measure Diverse PFAS in Wet and Freeze-Dried Sediment

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Per- and polyfluoroalkyl substances (PFAS) are anthropogenic chemicals found in all environmental media. Some PFAS preferentially partition into soils and sediment due to their limited solubilities. Because of variable water and mineral content, measuring PFAS in soil and sediment matrices can be difficult. Current measurement techniques, like EPA 1633, primarily focus on legacy PFAS like perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA); however, there is little published information detailing how these extraction techniques work for other types of PFAS like polyfluoroalkyl ether acids (PFEAs) or non-perfluoroalkyl acids (non-PFAAs). Our research aim was to evaluate an extraction procedure for a wide range of non-PFAAs including GenX and larger analogs, chlorinated ether acids, and fluorotelomer carboxylates in sediments with variable moisture concentrations to determine the impacts of water content on PFAS extraction performance. The proposed method includes a solvent extraction in basic methanol, ultrasonic bath, and extract clean-up using graphitized carbon. Precision, accuracy, matrix effects, and method detection limits (MDLs) will be calculated for all compounds in both the wet and freeze-dried matrices, which will be used to make recommendations for future characterization of diverse PFAS in sediment.

4.06.P-We-097 Assessment of Types and Levels of Per- and Polyfluoroalkyl Substances (PFAS) in Electronic Waste

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Per- and polyfluoroalkyl substances (PFAS) are well known for their durability and resistance to degradation. They are extensively employed in many consumer products across different industries, including the electronics industry, where they serve to provide coatings and protective layers for electronic devices. However, the disposal of electronics—whether through improper methods, incineration, or recycling—can release PFAS into the environment, where they can accumulate and persist for many years, potentially endangering ecosystems and human health. This research aims to investigate the occurrence and concentration of PFAS in discarded electronic wastes and the health risks associated with the exposure. In this study, e-waste components, including cables, circuit boards, mobile phone screens, computer monitors, electronic keyboards, and batteries, were collected at FIU Surplus and the STS Electronic Recycling Center in Miami. These materials were disassembled, shattered, and sieved and run through leachate experiments for 30 days to elucidate potential runoff and partitioning from the e-waste. The e-waste leachates obtained were extracted and pre-concentrated through solid-phase extraction (SPE) and analyzed through Liquid Chromatography-Mass Spectrometry (LC-MS) following the EPA method 3316, which targets 40 PFAS. The data from this research will reveal the type of PFAS, concentration levels, and potential environmental health-related implications associated with e-waste recycling and disposal. It will further raise public awareness about the

risk of PFAS in e-waste and inform proper management practices to prevent their release and protect the environment and public health.

4.06.P-We-098 Total Fluorine Analysis in Textiles and Leather Treatment Products, and Carpets as a Tool for Screening PFAS

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Per- and polyfluoroalkyl substances (PFAS) are a complex group of synthetic chemicals used in various consumer products due to their water and oil repellent properties. In addition to industrial processing agents, PFAS compounds have been used in food packaging materials, cookware, clothes, carpets, and firefighting foams. Due to the strong carbon-fluorine bond, PFAS molecules are persistent and have been detected in the environment, indoor dust and serum. PFAS exposure occurs through the environment, by consuming PFAS contaminated water and food, and by using products that have been treated with PFAS. Research links many of these compounds to have adverse health effects including childhood obesity, altered metabolism, reduced immunity, suspected infertility, endocrine disruption and cancer. The California Department of Toxic Substances Control listed carpets and rugs with PFAS and treatments containing PFAS for use on converted textiles or leathers as Priority Products under the Safer Consumer Products Regulations. To assess compliance with these regulations, we developed a method to analyze the total fluorine (TF) content in various consumer products using Combustion Ion Chromatography (CIC). We followed ISO 17025 non-standard method development procedure. Quality control samples (blanks, check compound standard (CCS), continuous calibration verification (CCV), laboratory control samples (LCS)) and matrix spiked (MS) samples tested at mid-level of our calibration curve showed excellent precision within acceptable error ranges (80%-120% for CCV and LCS, 70%-130% for CCS, and 50%-150% for MS). This method measures the TF at concentrations ranging from 3ppm to several thousand ppm with a Method Detection Limit (MDL) of 3ppm and Limit of Quantification (LOQ) of 5ppm. Seven carpet samples and 11 textile and leather treatment products including 1 wipe, 3 gel, and 7 spray samples were analyzed for the method validation. Carpet samples were studied as whole carpet as well as fiber, backing, and mesh materials separately. The TF concentration in whole carpet ranged from 218ppm to 437ppm with the highest amount found in backing materials in 4 samples while in fibers in other 3 samples. The TF levels in 7 textile and leather treatment samples were measured less than MDL while the other 4 samples ranged from 678ppm to 3,551ppm. Additional collection of TF data will help to assess the degree to which PFAS are being used in textile and leather treatment products.

4.06.P-We-099 Comprehensive Analysis of Side-Chain Fluorinated Polymers in Stain Repellent Products

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Per- and polyfluoroalkyl substances (PFAS) have long been utilized in a wide range of consumer

and industrial applications due to their unique properties. Non-polymeric PFASs with low molecular weights have been the predominant focus of research and regulatory bodies. However, polymeric PFAS comprise the bulk of PFAS products but have been little studied. Side-chain fluorinated polymers (SCFPs) are of significant concern because studies have shown the degradation of SCFPs to their corresponding perfluoroalkyl acids in the environment. Many SCFPs are embedded in consumer products as a thin coating or sprayed on demand. Impregnation sprays from the 3M trademark Scotchgard™ products have been known to contain SCFPs. Limited research has shown the presence of two of these SCFPs in sediments and agricultural soils augmented with biosolids. The SCFPs were found to be more abundant than the sum of all other commonly monitored PFAS. However, little is known about their polymer size, chemistry, and structure. Also, only two types of Scotchgard samples have been studied so far, pre- and post-2002. Nevertheless, years of Scotchgard usage in household items is a major source of PFAS exposure to humans, warranting further investigation into the nature of these SCFPs. In this study, the chemical characteristics and polymer chemistry of Scotchgard products were determined through various analytical techniques. A total of 11 commercially sold Scotchgard products from 1987 to 2022 were evaluated, including fabric cleaners, fabric protectors, carpet cleaners, carpet protectors, and outdoor fabric water repellent. Total fluorine was determined via combustion ion chromatography and compared to the total oxidizable precursory assay. Both 1-dimensional and 2-dimensional 19F NMR elucidated the structural information without prior knowledge of the types of C-F bonds or functional groups present.1D NMR provided information about the chemical environments of fluorine atoms, while 2D NMR offered more detailed structural information by elucidating the molecular connectivity, stereochemistry, and conformational information between fluorine atoms. Mass spectral information of the SCFPs in the Scotchgard was obtained using LC-HRMS (Orbitrap and QTOF) and pyrolysis-GC-HRMS. Our findings emphasize the need for comprehensive regulatory measures and further research to fully understand the environmental and health implications of SCFPs and other polymeric PFAS in consumer products.

4.06.P-We-100 Identifying Perfluorocarboxylic Acids in Common Consumer Items and Their Relation to Fluorinated High Density Polyethylene Plastic Packaging

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High density polyethylene plastic is a commonly used packaging material for consumer items due to its structural integrity and its ability to be recycled. This plastic can be fluorinated to decrease permeability to the air, useful for a variety of consumer items including highly flavored or fragranced items. The process of fluorination inadvertently produces a sub-class of per- and polyfluorinated substances (PFAS) known as perfluorocarboxylic acids (PFCAs), fragmented from the plastic bottle, which can migrate into the contained product. Exposure to PFCAs disrupt many systems in the body and poses long-term threats to health due to their ability to bioaccumulate with long half-lives. To avoid increasing exposure to PFCAs, a greater understanding of their sources from consumer products is required. To accomplish this, consumer items such as shampoo and cleaning products held in HDPE containers will be tested for the presence of PFCAs and correlated with packaging fluorination. To measure PFCAs, extraction techniques are currently being developed, utilizing a modified QuEChERS extraction to account for complex sample matrices, followed by LC-MS/MS analysis. We are also developing ^{19}F-NMR techniques to screen for PFAS in these complex matrices, without needing extensive sample clean-up that can result in analyte loss. To measure plastic fluorination, we are using attenuated total reflectance (ATR)-infrared spectroscopy. We hypothesize that products held directly in fluorinated containers should contain the highest level of PFCAs, whilst products which have encountered fluorinated containers elsewhere during their formulation should have comparatively lower levels. This research will further the current understanding of exposure sources to PFAS and increase consumer transparency and accuracy of estimations regarding total exposure compared to safe exposure levels set by the EPA, Health Canada, and other international regulatory agencies.

4.06.V Challenges in PFAS Analyses and Detection

4.06.V-01 Evaluation and Comparison of Extraction Efficiencies for Four PFAS Extraction Methods in Four Aquatic Organisms

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Per- and polyfluoroalkyl substances (PFAS) have garnered tremendous concern due to the widespread contamination in waterways. Accompanying studies have found that biota can accumulate high concentrations of PFAS, depending on functional group, chain length, and species It is vital to quantify the concentration of PFAS within aquatic organisms, specifically fish and bivalves, to understand potential deleterious effects as well as quantify exposure in human populations. Thus, the current study aims to assess four different PFAS extraction methods on four different aquatic organisms (mahi mahi, mackerel, bass, and oysters) to determine the method with the highest extraction efficiency for the specific organisms. Four methods were utilized. Samples were spiked with native and isotope labeled standards either before (n=3) or after (n=3) extraction. The native standard included 31 PFAS in total. All methods used solid-liquid extractions with two methods using an additional solid phase extraction (SPE) step. Samples were analyzed on a UHPLC coupled with a triple quadruple mass spectrometer equipped with an electrospray interface (LC/ESI/MS/MS). Results indicated differential efficiency for the different species indicating the role of physiology in the extraction process. While three of the methods had high recovery for most of the analytes measured, the method where samples were placed on an orbital shaker for 8 hours had 0%-low recoveries for all the organisms tested as well as all the analytes analyzed. While there were differences in extraction efficiency between species as well as individual PFAS recovery within species, the method utilizing a mixture of TBA, MTBE, and sodium carbonate as the extraction solvent had the highest efficiency for a majority of the analytes across fish species and bivalve.The results of this study will help research in determination of the best method for future studies quantifying PFAS in aquatic organisms.

4.07.P-Mo Data Curation Approaches: Collecting, Organizing, and Validating Chemical Information to Ensure Its Accuracy, Reliability, and Usefulness to Build Qsars in Order to Support Private-Public Regulatory Partnerships

4.07.P-Mo-077 Development of Quantitative Structure Activity Relationship Models for U.S. EPA's Cheminformatics Modules

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To assess the likelihood of adverse health effects from exposure to hazardous chemicals, EPA needs an array of physicochemical property and toxicity data. Experimental data for these properties can be collected from public domain databases and peer-reviewed literature. Despite data collection efforts, there will always be experimental data gaps. QSAR (quantitative structure-activity relationship) models are often employed to fill these data gaps. The Predict module of EPA’s Cheminformatics Modules has been developed to enable users to readily access EPA QSAR models using a single web site. The Predict module utilizes a PostgreSQL database to store raw experimental data, modeling datasets, and QSAR models. A database centered approach allows one to version various aspects of modeling and to deploy new models more rapidly. The creation of new models involves the following steps: extraction / curation of raw experimental data, filtering the raw data to generate the data set, splitting the data set into training and test sets, developing model(s) from the training set, and validation of the model(s) using the test set. Important considerations for each of these steps will be outlined. The Predict module provides model documentation via Excel summary and QMRF (QSAR Model Reporting Format) reports. In addition, HTML reports are provided for each real time prediction. Results will be presented for new QSAR models for physicochemical properties (e.g. boiling point and water solubility) based on expanded data sets.

4.07.P-Mo-078 Dataset Curation and Development of a Machine Learning QSAR for the Prediction of Generalized Wastewater Treatment Removal Rates

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The Chemicals Management Plan (CMP) is a Government of Canada initiative aimed at reducing the risks posed by chemicals to Canadians and the environment. Under the CMP, priority chemicals currently in commerce as well as new substances entering the Canadian market undergo risk assessment and, as appropriate, risk management. The extent to which a substance is removed during wastewater treatment is one among many important elements that inform the potential for exposure, and ultimately, risk assessment decisions. Mechanistic models to estimate the removal of substances during wastewater treatment are well known and have been used for many decades. The goal of this project was to curate a sufficiently large collection of measured wastewater treatment removal data (e.g., influent and effluent concentrations) and explore development of an alternative machine learning-based quantitative structure-activity relationship (QSAR) approach to predicting substance removal. Dataset curation, endpoint and descriptor selection, and performance of the Canadian Artificial Intelligence Removal Rate Estimator (CAIRRE) will be discussed, as well as challenges encountered. Partnerships to expand the size and quality of the training set could further improve the CAIRRE model.

4.07.P-Mo-080 Characterization of Structural Similarity and Mode of Action of Chemicals with High Reproductive Toxicity to *Daphnia magna*

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Prediction methods for chronic ecotoxicity of chemicals using in silico analysis, such as quantitative structure-activity relationship (QSAR), are expected to be used to prioritize chemicals which should be tested or as an alternative method for chronic ecotoxicity test. However, it is difficult to predict chronic toxicity of reactive chemicals and chemicals with specific mode of action (MoA) using QSAR. To improve the prediction accuracy, it is necessary to develop a model considering structural descriptors and MoA in test organisms. Since chemicals with known MoA in daphnids are limited, the QSAR classes for daphnid chronic toxicity are based on characteristic substructures and are not currently associated with MoA in daphnids. In this study, to identify the characteristics of structural similarity and MoA for highly toxic chemicals to daphnids, we collected toxicity data for *Daphnia magna* and extracted chemicals with high reproductive toxicity (i.e. NOEC ≤ 0.1 mg/L) and high acute chronic ratio (ACR ≥ 10). Dataset includes industrial organic chemicals from training dataset used in model development in the ecotoxicity QSAR system by National Institute for Environmental Studies, "KAshinhou Tool for Ecotoxicity" (KATE), insecticides from Pesticide Ecotoxicity Database by US EPA office of pesticides programs, and pharmaceuticals from database collected by National Institute of Health Sciences, Japan. The KATE dataset contained 57 chemicals with NOEC ≤ 0.1 mg/L and ACR ≥ 10 for daphnids, and these chemicals were predominantly in the two QSAR classes, CNO_X amine primary reactive group and amine primary unreactive aromatic group. Pesticides with high reproductive toxicity and ACR to daphnids were mostly found in the following Insecticide Resistance Action Committee (IRAC) MoA classifications: pyrethroids, spinosyns, glutamate-gated chloride channel allosteric modulators, and juvenile hormone mimics. Based on the anatomical therapeutic chemical (ATC) classification for pharmaceuticals, antineoplastic agents and psycholeptics were more likely to be highly toxic to daphnids. These highly toxic pharmaceuticals were mostly categorized as "unclassified Daphnid chronic" in KATE classification, and their toxicity was underestimated or unpredictable by KATE. These results will help to develop structural alerts associated with the MoA classification for daphnid chronic toxicity.

4.08.P-We Domestic, Agricultural, Landfill, and Industrial Waste: Occurrence, Fate, and Effects of Contaminants

4.08.P-We-101 PAH Characterization of an Offshore Industrial Dumpsite

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The San Pedro Basin was a dumpsite for an array of industrial wastes during the 1940's through 1970s. In addition to DDT and its metabolites which have been measured at concentrations well-above those at the nearby Palos Verdes Shelf superfund site, evidence of multiple petroleum distillates has been identified. PAHs are a class of compounds that are found in petroleum

products and are formed during the incomplete combustion of organic matter. Many are carcinogenic and/or mutagenic in nature and are thus of particular interest. Ongoing efforts are aimed at characterizing the contaminants present and the spatial extent of contamination within the basin. Here we present the first basin-wide survey of PAH concentrations and distributions within the sediments from the San Pedro Basin. Through analysis of previously collected sediment cores we aim to improve our understanding of the extent and diversity of contaminants at the dumpsite.

4.08.P-We-102 Chemical Speciation of Trace Metals and Microplastics Presence in Biosolids for Land Application

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The application of treated biosolids from wastewater treatment plants to agricultural land contributes to the introduction of macro-nutrients (e.g., nitrogen and phosphorous), with the disadvantage of also carry contaminants, such as microplastics and trace metals. Microplastics can sorb and release contaminants like persistent organic pollutants, thereby acting as a continuous source of contamination to the surrounding environment. Trace metals, on the other hand, can be absorbed by crops (bioavailability), leach into groundwater (mobility), and/or accumulate on the surface. Metal's bioavailability, mobility, and toxicity strongly depends on the metal speciation, which can be influenced by soil physicochemical properties. This work aims to generate a detailed evaluation of trace metals and microplastics present in biosolids produced with a novel, low energy, alkaline hydrolysis/electrochemical process. To achieve this, we analyze samples prior and post alkaline hydrolysis/electrochemical process treatments by a multi technique approach that includes sequential extraction and passive sampling for metals characterization, and microscopic and spectroscopic techniques for microplastics identification. This evaluation provides essential information to better understand the effect of the electrochemical treatment on the metals' bioavailability and mobility.

4.08.P-We-103 Environmental Transport of Per- Polyfluoroalkyl Substances (PFAS) from Biosolid-Amended Soils

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Per and polyfluoroalkyl substances (PFAS) do not degrade in conventional wastewater treatment processes and are found in wastewater effluent and biosolids. In 2022, 6.5 million tons of biosolids were generated in the United States where 23% of produced biosolids were land applied in agricultural settings. There are increasing concerns regarding the fate of PFAS in biosolids that are land applied as agricultural fertilizers. Therefore, this work evaluates PFAS behavior in two biosolids amended soils using column experiments. The objectives of this work were to identify the leaching behavior of PFAS from biosolids amended soils using high-resolution mass spectrometry (HRMS). Second, the effect of a PFAS stabilization technique, polyDADMAC amendment, is evaluated to identify how PFAS leaching behavior is altered by this approach. lastly, PFAS leaching behavior is modeled using HYDRUS 1-D to identify transport parameters relevant to PFAS leaching from biosolids-amended soils. Source biosolids

applied to soil were ~80% known PFAS with ~20% unknown precursors. Leaching experiments indicated that 11 and 44% of all PFAS were eluted from in both biosolids amended soils. The majority of this was comprised perfluoroalkyl carboxylic acids (PFCAs) (73.0 - 80.33%). Up to 93% of PFSAs of unknown precursors were retained on both biosolids amended soils. Chain length dependent leaching was observed, with short chain PFAS eluting first. However, the majority of total PFAS will be retained in biosolids amended soils. The application of polyDADMAC resulted in a 73.5% reduction of total PFAS detected in unmodified column eluent. These results indicate short chain PFAS will be released from biosolids amended soils. Further, polyDADMAC amendment can prevent or slow the release of PFAS from biosolid amended soils which may reduce impacts to groundwater. Ongoing HYDRUS 1-D modeling efforts will be used to obtain transport parameters from 1D column experiments and apply them to predictions of transport at field relevant scales.

4.08.P-We-104 Quantification of Organic Fluorine in Landfill Leachate Using Combustion Ion-Chromatography and Inductively Coupled Plasma Mass Spectrometry

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Landfills are the repository for many per- and polyfluoroalkyl substance (PFAS) containing products. PFAS have been detected in landfill leachate at concentrations up to the hundreds of, and the handling and disposal of these wastes is an understudied PFAS release pathway. Total PFAS concentrations in leachates are typically identified by a nontargeted analytical approach by using liquid chromatography high-resolution mass spectrometry (LC-HRMS). Non-targeted analysis is costly and time intensive for both data collection and processing. Therefore, there is a need for rapid, low-cost screening methods to identify total PFAS in landfill leachate. This work evaluates and compares two analytical techniques for identifying total organic fluorine (TOF) concentrations in landfill leachate samples: combustion ion-chromatography (CIC) and inductively coupled plasma mass spectrometry (ICP-MS). TOF is assumed to approximate total PFAS concentration, and inorganic fluorine is separated from TOF in leachate samples using solid phase extraction before analysis by both analytical approaches. The TOF detection limit for landfill leachate samples spiked with PFOA and adjusted to 10% MeOH was 0.25 mg/L. This indicates that CIC is applicable for analysis of PFAS in landfill leachate samples, however the approach is not very sensitive relative to HRMS and may not be applicable to leachates where TOF is in the µg/L range. ICP-MS analysis of TOF is accomplished by supplying excess Ba^+ during sample introduction to form and detect BaF^+ as a surrogate for measuring TOF. The formation of this polyatomic ion in the ICP-MS plasma is variable, and the conditions which allow for reproducible formation and detection of BaF^+ are investigated in this work. The ICP-MS variables which are optimized include but are not limited to sampling (Z) distance, argon gas flow, Ba^+ concentration, and reaction gas (O_2) flow. Initial findings indicate that TOF from PFAS can be observed as low as 50 PPB relative to blank samples, though the current working range is between 1-100 mg/L. Work is ongoing for both CIC and ICP-MS analysis, including analytical optimizations and evaluating different sample matrices and PFAS mixtures. Optimized methods will be applied to a suite of landfill leachate samples which have been fully characterized using LC-HRMS.

4.08.P-We-105 Elucidating the Environmental Impact of Neutral PFAS in Waterproofing Sprays in the Japanese Market

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PFOS, PFOA, and PFHxS are regulated by the Stockholm Convention. Specifically, PFAS such as PFOS and PFOA have been shown to have a deleterious impact on living organisms. It is speculated that PFAS are also used in household products such as waterproofing sprays. These PFAS would then be released into the environment upon the disposal of waste associated with these products. However, little information is available regarding the type of PFAS used. Accordingly, we investigated the presence of PFAS in waterproofing sprays obtained from the Japanese market. Twenty samples of previously purchased waterproofing sprays and eighteen samples purchased in 2022 were used as test samples. The target compounds for analysis were nine neutral PFAS that can be analyzed using GC-MS. In the past samples, at least one PFAS was detected in 10 of 20 samples. The highest detection rate was eight out of 20 samples for 1H,1H,2H,2H-perfluorodecan-1-ol (8:2 FTOH), with a detection frequency of 40% (i.e., at a concentration of 1.87–214 µg/g). 1H,1H,2H,2H-perfluorooctan-1-ol (6:2 FTOH) had the next highest detection rate, detected in 7 of the 20 samples. The detection range was 1.26–8.79 µg/g. Most of the samples were dominated by 8:2 FTOH, whereas some samples were dominated by 1H,1H,2H,2H-perfluorooctyl methacrylate (6:2 FTMAcr) and 6:2 FTOH. In contrast, in the samples purchased in 2022, one or more PFAS were detected in 14 of the 18 samples. The highest detection frequency was 78% for 6:2 FTOH and 56% for 6:2 FTMAcr. PFAS with C_8F_{17} as part of the structural formula, such as 8:2 FTOH and 1H,1H,2H,2H-perfluorodecyl acrylate (8:2 FTAcr), were the main components in the past samples, whereas 6:2 FTOH and 6:2 FTMAcr with C_6F_{13} were the main components in the sample purchased in 2022. Based on PFOA having been shown to be formed from 8:2 FTOH, we speculated that many manufacturers use 6:2 FTOH and 6:2 FTMAcr instead of 8:2 FTOH. The PFAS load released into the environment when one can of a waterproofing spray is used or disposed of ranged from 0.60 to 71 mg. The persistence and behavior of these PFAS in the environment should be studied, facilitating a more stringent control of its use and disposal in the future.

4.08.P-We-106 Assessing Perfluoroalkyl Substances (PFAS) in Aquatic Ecosystems in the Grand River, Ontario

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The Grand River watershed in southern Ontario, Canada is highly influenced by urban development, municipal wastewater effluents (WWEs), urban stormwater and run-off. This study examines PFAS in water and in biota exposed to WWE. In 2019, we investigated PFAS in river water and effluent from a single treatment plant, and in situ bioaccumulation in caged fish (Rainbow Trout (*Oncorhynchus mykiss*), Rainbow Darter (*Etheostoma caeruleum*)), and freshwater mussels (Flutedshell mussel [*Lasmigona costata*]). In 2021, we expanded the number of sites for monitoring PFAS in wild-caught fish (Greenside darters [*E. blennioides*]) and mussels (*L. costata*). Health assessment endpoints were measured in both fish and mussels. Total PFAS concentrations measured in river water in 2019 were 18-26 ng/L, similar to the wider survey conducted in 2021 (10-50 ng/L). In both years, PFAS were largely comprised of C4 to C8 PFCA. Maximum concentrations of total PFAS in treated WWE were higher than those measured in river water. PFAS were detected in relatively low concentrations in the caged fish compared to other studies. In trout, the highest PFAS concentration corresponded to perfluorooctane sulfonate (5.38 ng/g w.w. PFOS) while the 7:3 FTCA (fluorotelomer acid) was highest in mussels, 0.16 ng/g w.w.. In wild biota, total PFAS was on average 5.5 to 9 ng/g w.w., similar to the caged biota and the PFAS profile was dominated by PFOS. In wild mussels, total PFAS concentrations were lower and ranged from 0.6 to 1.8 ng/g w.w.. The PFAS composition was much more diverse in mussels and was predominantly long chain perfluorocarboxylates (C12-C18 PFCAs) as well as the C4 and C8 sulfonamides. Biological responses (health indices, gene expression, oxidative stress) in all exposed biota were limited and not consistent among fish or mussel tissues. This research demonstrates value in sampling water and multiple taxa to evaluate the quality and extent of PFAS exposure in the receiving environment.

4.08.P-We-107 The Ins and Outs of Per- and Polyfluoroalkyl Substances in the Great Lakes: The Role of Atmospheric Deposition

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Like for other legacy compounds, such as polychlorinated biphenyls (PCBs), atmospheric deposition could be a significant environmental pathway for delivering PFAS in the environment. PFAS are washed out from the atmosphere by wet and dry deposition and the gas absorption process, of which precipitation is generally considered the dominant mechanism. To date, the mass transfer flow of PFAS via atmospheric deposition to the Great Lakes was not well addressed. In this study, precipitation (n = 207) and air (n = 60) from five sites and water samples (n = 87) from all five Great Lakes were collected in 2021-2023 and analyzed for 41 per- and polyfluoroalkyl substances (PFAS) as part of the Integrated Atmospheric Deposition Network (IADN). These measurements were combined with other available data to estimate the mass budget for 4 representative compounds, PFBA, PFBS, PFOS, and PFOA for the basin. The Σ_{41}PFAS concentrations in precipitation across the five sites ranged between 2.4 and 4.5 ng/L. The median Σ_{41}PFAS concentration in lake water was highest in Lake Ontario (11 ng/L) and lowest in Lake Superior (1.3 ng/ L). The median Σ_{41}PFAS concentration in air samples was highest in Cleveland at 403 pg/m^3 and lowest at Sleeping Bear Dunes at 150 pg/m^3. The net mass transfer flows were generally negative for Lakes Superior, Michigan, and Huron and positive for Lakes Erie and Ontario, indicating that the three most northern lakes are accumulating PFAS and the other two eliminating PFAS. Atmospheric deposition is an important source of PFAS, particularly for Lake Superior.

4.08.P-We-108 Optimizing an Alternating Water Source Scheme: Minimizing Plant Accumulation of Emerging Contaminants from Treated Wastewater Irrigation

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The use of treated wastewater (TWW) for agricultural irrigation is essential in coping with depleting freshwater resources and advancing sustainable agricultural production. However, this practice can introduce contaminants of emerging concern (CECs) into agroecosystems, posing a potential risk to environmental and human health as CECs bioaccumulate in the edible tissues of food crops. Previous studies have indicated that a simple change in irrigation scheme—alternating TWW irrigation with freshwater irrigation—is effective in reducing the accumulation of many CECs in food crops via reduced chemical input, back-release from roots, plant metabolism, and plant growth dilution. This study aims to refine the optimal time for the water source switch when using an alternating irrigation scheme, as well as better evaluate the roles of plant metabolism and growth dilution on the reduction of CEC accumulation in plant tissues. To achieve this, lettuce (*Lactuca sativa* L.) and tomato seeds (*Solanum lycopersicum*) were cultivated in a hydroponic system under four different treatments based on their estimated time to maturity. The plants were grown in CEC-spiked solution containing eight perfluoroalkyl acids (PFAAs) and three pharmaceutical and personal care products (PPCPs) for either the full (${}^4/_4$) growth period, ¾ of the growth period, ½ of the growth period, or ¼ of the growth period, followed by clean solution for the remainder. The treatments received the same total CEC loading based on their respective exposure durations, per the total growth period. The roots, shoots, and edible portions (leaves for lettuce and fruits for tomato) were analyzed at the water source switch point and the end of the growth period to observe differences in accumulation between treatments. Additionally, reductions from growth dilution, back-release, and plant metabolism were calculated for each treatment. The results of this study will provide insight as to the optimal irrigation schedule to minimize CEC accumulation in the edible tissues of food crops to reduce human exposure risks, while maximizing the period of irrigation with TWW to advance sustainable water management in agricultural systems.

4.08.P-We-109 The Presence, Concentration, and Potential Ecological Impacts of Trace Metal Contaminants in the James River Near Multiple Anthropogenic Contamination Sources (Bremo Bluff, VA)

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This study examines the spatio-temporal distribution and biological impacts of trace metals in the James River near the decommissioned Bremo Power Station (Bremo Bluff, VA). Over six million yd^3 of coal combustion residuals (CCRs) are stored in its proximal, unlined North Ash Pond. Industrial CCRs contain numerous toxic trace metals which can infiltrate surface waters via accidental spills, authorized discharge, and/or leaching from impoundments. Existing research emphasizes major release events over chronic exposure. In addition to potential CCR leaching, tailings from historic gold mines near Bremo Power Station may also contribute to metal contamination in both surface water and sediments. Surficial sediments, sediment cores, surface water, and adult panhandle pebblesnails (*Somatogyrus virginicus*) were collected from the James River upstream, downstream, and adjacent to the plant. Sediment samples were extracted using aqua regia ($3H_2O:3HCl:1HNO_3$). Cores were divided into 2 cm intervals and analyzed for trace metal content. Surface waters were acidified with HNO_3 and then filtered. Snail body tissues were removed from shells and pooled into groups of three before extraction with 65% HNO_3 and 30% H_2O_2. Sediment, water, and tissue samples were analyzed for 13 CCR-associated elements (Al, As, B, Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Se, Zn) using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Additional surface water samples were collected and assessed for species richness by Jonah Ventures (Boulder, CO) using eDNA MiFish primers. Our data indicates enriched Al, Cu, and Pb in surficial sediments downstream from the station as well as increased B and Mn bioaccumulation in adjacent *S. virginicus* tissues. Environmental DNA analyses have identified the presence of twenty-four fish species, six of which are commonly consumed by recreational anglers. As such, our results provide novel insight into the dynamics and fate of trace metal contaminants in waterways near CCR repositories, with implications for both ecosystem and human health.

4.08.P-We-110 Wastewater Treatment Wetlands: Microplastic Source or Sink?

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The potential for microplastic (MP) movement through porous media is an important consideration for management of waters that have been found to contain MPs, such as wastewater effluent, urban runoff, or surface waters used for artificial groundwater recharge. However, the risk of water resource contamination and the potential consequences are not yet understood. Research is needed to characterize how MP particles interact with the soil environment and what hydrologic conditions and mechanisms influence particle movement between surface and groundwater systems. In this study we hypothesize that wastewater treatment wetlands contain higher concentrations of microplastics compared to natural wetlands, and that these plastic particles can move vertically through fine textured sediments over time, potentially reaching shallow groundwater systems. This is investigated through analysis of the vertical distribution of MP's in the surface water, sediment, and shallow groundwater of an established wastewater treatment wetland (with nearly 40 years of use) compared to an adjacent, groundwater fed natural wetland system. Chemical identification of microparticles is accomplished using micro Fourier Transform Infrared Spectroscopy (µFTIR) with exploration of nanoparticle identification via time of flight mass spectrometry.

4.08.P-We-111 Effects of Soil Amendments on PPCPs Mobility in Soil and Uptake by Potatoes Grown Under Wastewater Irrigation

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Wastewater use for irrigation could partly fulfill water demand and solve disposal problems. In several developing countries, knowingly or unknowingly, partially treated and/or untreated wastewater is used to irrigate food crops. However, the presence of contaminants, including (e.g., pharmaceuticals and personal care products [PPCPs])in wastewater, may represent a major risk as these contaminants may be harmful to the environment and human health. Therefore, low-cost simple treatment methods are needed to reduce contaminant transmission to crops. A field lysimeter experiment was conducted for two years to investigate the effect of mixing barley straw biochar and compost in soil on the mobility and plant uptake of PPCPs from wastewater to potatoes. Potatoes were planted in sandy soil and irrigated with synthetic wastewater containing organic contaminants (e.g., caffeine, carbamazepine, DEET, diclofenac, ibuprofen, and triclosan). In general, the highest (3%) biochar application, alone or in combination with compost, significantly reduced ($p \leq 0.05$) the soil mobility of PPCPs compounds (CAF, CBZ, and DEET). Results are discussed in the context of the organic contaminants (e.g., PPCPs) physicochemical parameters, the surface morphology of soil amendments, and their potential attending interactions.

4.08.P-We-112 Land Application of Biosolids: Bioaccumulation of Unregulated Organic Chemicals (UOCs) in Vegetables

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Biosolids, the nutrient-rich organic materials resulting from the treatment of sewage sludge, can be land-applied to improve plant health and soil quality in addition to offering benefits over detrimental disposal methods such as landfilling and incineration. However, biosolids contain numerous unregulated organic chemicals (UOCs) leading to concerns that may impede public perception of the benefits gained from land application, potentially hindering the expansion of its beneficial use. Therefore, understanding the broad human and environmental health risks of biosolids-borne UOCs is imperative for the safe use of biosolids in agriculture. In this study, biosolids were land-applied to vegetable crops (sweet corn, cucumber, potato) at environmentally realistic rates for one growing season. Thin-film passive samplers were deployed in the field plots as a biomimetic tool to estimate the bioavailability of biosolids-borne UOCs. Passive samplers, soil samples, and the edible tissues of various vegetables were collected and analyzed for 20 “priority” UOCs. The concentrations quantified in the samples evaluate the bioavailability, uptake, and presence of the priority UOCs in food crops. In future work, biosolids will be applied to fruit trees at environmentally realistic rates to determine the bioaccumulation of UOCs in the edible portion of the fruits. These completed studies will contribute to holistic human health and ecological risk assessments.

4.08.T Domestic, Agricultural, Landfill, and Industrial Waste: Occurrence, Fate, and Effects of Contaminants

4.08.T-01 Toward Engineering Microbial Communities for Enhanced Chemical Removal in Wastewater Treatment Systems

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Contaminants of emerging concern present significant challenges in wastewater treatment systems, often being unregulated or poorly understood with potential risks to human and environmental health. Microbes play a crucial role in degrading organic compounds in conventional treatment processes, yet their specific impact on chemical composition remains unclear. We hypothesize that different microbial communities have differential effects on the chemical composition in wastewater. We predict that microbial communities could be engineered to maximize biological treatment in conventional wastewater treatment systems. Our goal is to identify differences in microbial community composition among different biological treatment systems test whether microbial communities can be used to predict changes in the overall chemical composition. We quantified the microbial community composition as well as the suspect and non-target chemical composition from three conventional biological treatment types (activated sludge, biological nutrient removal, and facultative lagoons). Samples were collected before and after biological treatment from 12 wastewater utilities in Oregon. Microbial communities were profiled via 16S rRNA sequencing, while chemical analyses employed high-resolution time-of-flight mass spectrometry. Using multivariate tools, we report a distinct chemical and microbial composition between lagoon effluent as compared to activated sludge and biological nutrient removal (i.e., non-lagoon systems). While the overall change in the chemical composition was similar between the three systems, suggesting equivalent treatment capacity, lagoon and non-lagoon systems exhibit statistically distinct clustering. Phenol ethers and flavonoids emerged as significant drivers separating lagoon from non-lagoon systems. Notably, genera such as *Aliarcobacter* and *Dechloromonas* were associated with specific chemical composition clusters. Our findings suggest that different biological systems have differential ability to catalyze chemical reactions but controlled experimentation is needed to identify the microbial communities and mechanisms responsible for chemical transformations. This research enhances our understanding of how microbial communities alter the chemical composition in wastewater and suggests that specific wastewater treatment systems could be engineered to treat different families of emerging contaminants by cultivating specific microbial communities.

4.08.T-02 Probing the Impact Threshold of Perfluoroalkyl substances (PFAS) Mixtures Simulated from Land-Applied Biosolids: A Case Study Using a Soybean Model

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In recent years, investigations into the impact of perfluoroalkyl substances (PFAS) on ecosystems have highlighted a dose-dependent effect influenced by PFAS functional groups and chain lengths. Existing studies predominantly focus on individual or a mixture of a limited number of well-known PFAS, such as PFOA, PFOS, PFBS, and GenX, demonstrating toxicity mostly commencing at parts per billion levels. Yet, in environments where biosolids are used, more than ten PFAS compounds are typically detected, prompting questions about whether impact thresholds derived based on single or limited PFAS mixtures are sufficient to understand the broader impacts of PFAS in the environment. This study contributes to addressing this gap by quantifying PFAS levels in soil, runoff ditches, and ponds from a farm with a history of biosolid application. Utilizing this data, a mixture of ten PFAS compounds, prepared into five concentration groups (1, 10, 50, 100 and 1000 ppt per PFAS), were respectively used to irrigate soybeans for 80 days (50 mL every other day). Plant phenotypes and stress biomarkers are being collected, alongside monitoring the larval-adult transition of mid-3rd instar fall armyworm larvae post-dietary exposure to leaves collected at day 50 of the plant exposure experiment. Preliminary findings indicate significantly stunted growth in exposed plant groups (except the 10-ppt) and elevated levels of total reactive oxygen species (ROS) in the 100 and 1000-ppt groups at day 25 post-exposure compared to controls. By day 50, ROS levels increased across all exposed plant groups, while activities of superoxide dismutase and catalase decreased compared to controls. Larvae feeding on leaves from the 10- and 50-ppt plant groups exhibited stimulated weight gain and a subsequent 66.7% larval-pupal transition at day 8 post-exposure, compared to 16.7% in controls. Ongoing analyses involve quantifying PFAS accumulation in leaves, pod formation statistics and quality, root phenotypes, and stress biomarkers. Additionally, bioaccumulation of PFAS, stress biomarker analysis, and expression levels of development-regulating genes are being carried out on the exposed larvae. When compared to studies on single PFAS, these preliminary findings suggest a possible decreased impact threshold associated with environmentally relevant concentrations of PFAS mixtures arising from biosolid application on soybean health and insect pest-plant interactions.

4.08.T-03 City-Scale Impacts of PFAS from Normal and Elevated Temperature Landfill Leachates on Wastewater Treatment Plant Influent

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This study systematically evaluated the occurrence and implications of per- and polyfluoroalkyl substances (PFAS) in landfill leachates and wastewater treatment plant (WWTP) influents at a city-wide scale. To gain a deeper understanding of the sources, fate, and transport of PFAS, we assessed the occurrence of PFAS and their precursors in both normal and elevated temperature landfill (ETLF) leachates and delineated their influence on WWTP influent. We employed targeted and total PFAS analysis, covering 70 PFAS compounds and 5 fluorotelomer alcohols (FTOHs), alongside the total oxidizable precursor (TOP) assay and adsorbable organofluorine (AOF) analysis. Our sampling strategy was designed to encompass various scenarios, including normal leachate, ETLF leachate, municipal-only wastewater and WWTP influent with and without leachate impacts. Our findings revealed elevated concentrations of PFAS in both normal and ETLF leachates, with ultra-short-chain (<C4) and short-chain (C4-C6) PFAS exhibiting the highest levels. The detection of additional PFAS precursors in municipal wastewater and WWTP influent, like 6:2 polyfluoroalkyl phosphate diester (6:2 diPAP) as well as other untargeted compounds as determined by increases in total PFCA concentrations in post- TOP assay leachate samples, suggests diverse sources contribute to PFAS emitted to the sewer systems beyond leachate inputs alone. The results of this study improves the understanding the sources and dynamics of PFAS contamination and releases from landfills in different types of leachates, and this information will aid in implementing effective mitigation measures and evaluating treatment technologies.

4.08.T-04 Diffusivity of Landfill Leachate Through Bentomat Clay and Fluorosorb Membrane Liner Material

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Per- and polyfluoroalkyl substances (PFAS) are known to be ubiquitous in landfill leachate which may pose risks to soil and groundwater if liner systems fail to retain PFAS within disposal facilities. Landfill liners typically consist of both compacted clay and geomembrane layers, but limited studies have evaluated the potential for PFAS to diffuse these materials. In this study, side-by-side diffusion cells were used to investigate the diffusion of PFAS through 2 types of geomembranes and two types of clay liners. These liners were used to separate a source cell containing field-collected leachate from a receiving cell containing deionized water, and PFAS concentrations in source and receiving cells were measured over time to evaluate PFAS diffusion (D_f, m^2/sec). Batch sorption of PFAS (K_d, L/kg) to both clay materials (bentonite and bentonite + Fluorosorb^{TM}) were also evaluated. Importantly no measurable PFAS diffusion through geomembrane materials was observed. Sorption of PFAS to bentonite was low for all PFAS evaluated (e.g., $K_d < 1$) except those with fluoroalkyl chain lengths > 9-10 ($K_d > 1$). Sorption to bentonite modified with Fluorosorb^{TM} yielded sorption coefficients an order of magnitude or higher than bentonite alone. The values of D_f in bentonite ranged from 4.8×10^{-11} for PFDA to

8.9×10^{-10} for 5:3 FTCA which corresponds with PFAS molecular weight. No trends with the PFAS functional group were immediately apparent. Evaluation of diffusion across clay modified with Fluorosorb^{TM} is ongoing. Results of this study preliminarily suggest that intact landfill liners will have similar efficacy for PFAS as for other, legacy contaminants.

4.08.T-05 Deposition of Per- and Polyfluoroalkyl Substances (PFAS) in Soil Surrounding a Municipal Solid Waste (MSW) Landfill

***Alina Timshina**, Allison Cerlanek, Ashley Lin, Timothy Townsend and John A. Bowden, University of Florida*

Municipal solid waste (MSW) landfills are passive receivers of wastes that contain per- and polyfluoroalkyl substances (PFAS), persistent pollutants of concern for human health. Through a combination of waste leaching and biotic and abiotic degradation processes, PFAS migrate into both collected byproducts of landfills: gas and leachate. Typical treatment approaches for landfill gas and leachate, such as flaring and traditional wastewater plant treatments, respectively, are not effective for PFAS treatment, with a high potential for PFAS release into the environment. Thermal evaporation systems have emerged as an alternative landfill leachate treatment option that allows for onsite management of this wastewater, reducing its volume by up to 97% while discharging water vapor and recirculating the remaining sludge residuals back into the landfill. However, a recent study found that, though a majority of PFAS partitioned into the residuals, 9-24% of PFAS entering an active evaporator were released into the atmosphere with the vapor during sampling days ($n = 3$). In this study, soil samples surrounding the same active landfill leachate evaporator stack were tested for potential deposition of 91 PFAS using ultra high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). Results showed a difference between soil collected within 45 m of the evaporator and control soil samples collected at other locations at the landfill and at a nearby state park. Interestingly, the profile of PFAS measured in soil near the evaporator suggested that the greater portion of measured PFAS may have originated from landfill gas. This preliminary assessment links these two point sources of airborne PFAS at MSW landfills to PFAS measured in surrounding landfill soil, warranting more research to ascertain the scope of potential aerial deposition of PFAS from landfill gas flares and thermal leachate evaporators.

4.08.T-06 ACH Water Treatment Residuals and Pyrolyzed Biosolids for Mitigating Perfluoroalkyl Acids Leaching from Biosolids-Amended Soil Columns

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Al-based residuals after water treatment (WTRs) have been found to be a good sorptive medium for phosphates. From our batch sorption studies both aluminum chlorohydrate residuals (ACH-WTRs) and biochar sorb PFAS from biosolids-derived porewater. Here, we evaluated the affinity of ACH-WTR and biochar (pyrolyzed biosolid) used as received to sorb per and polyfluoroalkyl substances (PFAS) from a biosolids-amended soil column operated under transient unsaturated conditions. The upper part of the soil columns were packed with a sandy low organic matter (0.6%) soil mixed with biosolids at 3 wt % plus either no sorbent (controls) or 1 wt % ACH WTRs or 1.5 wt% biochar. Both control and treatment columns were done in triplicate. Simulated rain events were invoked every 4 days for six months (~3 pore volume per week). C_4-C_{14} perfluorocarboxylates (PFCAs), C_4-C_8 and C_{10} perfluorosulfonates (PFSAs), 6:2 and 8:2 fluorotelomer sulfonates; 5:3 and 7:3 fluorotelomer carboxylic acids, and three perfluoroalkane sulfonamido derivatives were monitored in leachate after each rain event. The total PFAS concentrations that leached out weekly was seen to be lower in the treatment columns when compared to the control columns. Biochar outperformed ACH-WTR with a 41% and 32% less total PFAS in their leachate respectively. More PFCAs were observed in the leachates across all treatments than PFSAs. Even though biochar was more effective than the ACH-WTR in total PFAS mitigation, it was least effective in the short-chain PFCA group, while ACH-WTR was most effective in the long-chain PFSA group particularly PFOS. Our results support that ACH-WTR and biochar may have a promise for reducing PFAS leachate concentrations in biosolid-amended agricultural fields; thus, may be a significant mitigation approach.

4.09.P-Tu Environmental Fate of Polymer

4.09.P-Tu-137 Revealing Radical Induced Degradation of Polyacrylamide - A High Resolution Mass Spectrometry Approach

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Industrially important water-soluble synthetic polymers and their environmental degradation products are overlooked pollutants in wastewater. However, their detection is limited to bulk solution chemistry and molecular level analysis, pairing both molecular weight and end group chemistry, remains unreachable. For the first time, we assessed the feasibility of current non-target workflow using liquid chromatography-high resolution mass spectrometry (LC-HRMS) and proposed new approaches to elucidate molecular level information of free radical chain scission of polyacrylamide by persulfate under simulated hydraulic fracturing conditions. We found current non-target workflows using commercial software have very low accuracy in predicting the formula of MS^1 features. We enhanced the accuracy by constructing a database of 463,665 unique formulas of degradation products based on degradation pathways, improving the formula from 19% to 31% accuracy. Leveraging these constructed databases, the ratio of aldehyde/ketone terminating molecules was found to increase from 1-6 over 24 h degradation time, contradictory to previously proposed ratios of radical position along polymer backbone that are specific to hydroxyl radicals, likely due to the steric hindrance of sulfate radicals. We also employed theoretical fragmentation of aldehyde and ketone end groups to confirm this result with the MS^2 data. Eleven structures with confidence level 2b-3 were identified using MS^2 information. Additionally, a concentration-dependent crosslinking and precipitation of degraded polymer were observed which also impact the fate of polymers. The mechanism of this phenomenon will be explored further. This is a first attempt to resolve complex polymer degradation chemistry using HRMS that can advance our understanding of the environmental impact of overlooked water-soluble polymer pollutants.

4.09.P-Tu-138 A Weight of Evidence Analytical Approach to Understanding the Chemical Composition of Synthetic Polymers to Aid in the Interpretation of Biodegradation Results

*Takashi Sasaki, **Jared Bozich**, Ronald Gabbard, Chii-Fen Wang, Ding Jiao, Yefim Brun and Michael Popplewell, International Flavors and Fragrances*

In 2023, the European Commission officially published a ground-breaking restriction on the use of intentionally added synthetic polymer microparticles restricting their uses in a broad range of products down to 0.01% by weight. Despite a low contribution to the microparticle pool according to the European Chemical Agency, fragrance microencapsulation used in laundry care were in-scope of the restriction. Ensuring the restriction was proportional, especially for microencapsulation, the restriction included an exemption for microparticles that met specified rules for proving biodegradability, namely test methods and their associated pass criteria. Noteworthy, these rules call out specific testing requirements in case the microparticle consists of more than one polymer (i.e., a blend), whereby all components of the polymer “blend” must be proven biodegradable to avoid misleading results or false positives. Yet, the restriction did not provide guidance on how to distinguish a polymer blend from a single polymer leaving ambiguity. Here we present an approach to differentiating polymer blends from non-blends to assist those in evaluating the biodegradability of their polymeric technologies using two extensively characterized fragrance microcapsule technologies that results in similar biodegradation behavior but differ greatly in their chemical structure. Various physical and chemical analyses were used in a weight of evidence approach as means to demonstrate co-polymerization. The study highlights the value of a weight of evidence approach to verify polymer composition to inform biodegradation testing and illustrates that in-depth analysis is critical to confirming the product of the intended reaction was achieved.

4.09.P-Tu-139 An Experimental Approach to Determining the Movement of Dimethylsilanediol (DMSD) Formed in-situ in Soil

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Dimethylsilanediol (DMSD) is a major degradation product of commercially available methylsiloxanes, both volatile (VMS) and polymer (PDMS). Both VMS and PDMS are commercial products and can be found in waste streams and in biosolids used for land applications. Previously, hydrolysis rates of both PDMS and VMS were determined in different soils, and DMSD was detected in agricultural soils on which biosolids were applied. DMSD is highly water soluble and has a low air-water partition coefficient (log K_{AW} ~-7), suggesting a potential for movement to ground water. This study investigated whether DMSD produced *in situ* in loamy soil might be transferred via soil runoff or leaching. Segmented glass columns were constructed with perforated support discs that held the soil above the collected leached water, which was sampled through a septum sealed port. Each column sat inside a glass dish to contain runoff. The top half of the soil column (20 cm) was packed with soil spiked with VMS or PDMS, while the lower half (20 cm) was packed with untreated soil. A column was sacrificed at each sampling timepoint, and each segment was separated, allowing for analysis of soil at increasing depths. Volatilization loss was determined by the difference in radioactivity between the initial and remaining amounts in the soil and water samples. Environmental conditions representing three temperate climate seasons were mimicked in a laboratory setting using an environmental chamber that controlled temperature, humidity, light, and airflow. Water was added periodically to simulate rain. ^{14}C-Octamethylcyclotetrasiloxane (^{14}C-D4) and ^{14}C-polydimethylsiloxane (^{14}C-PDMS) were used. A sequential extraction and analysis scheme was used to extract, speciate and quantify parent and degradation products from soil. Unextractable radioactivity was also quantified. Leachate and runoff were filtered and analyzed directly. Under the tested conditions, most silanols formed *in-situ* from ^{14}C-D4 and ^{14}C-PDMS either remained in the soil or were removed by volatilization. Minimal concentrations of silanol degradation products or parent material were detected in leachate and runoff. Minimal downward migration of either ^{14}C-D4 or ^{14}C-PDMS in soil was observed. In this experiment, volatilization was the major removal mechanism for ^{14}C-D4 prior to hydrolysis in soil. ^{14}C-PDMS did not volatilize, but silanols were generated and removed from the test system through volatilization.

4.09.P-Tu-140 Identifying Polymer-degrading Microbial Groups and those Sensitive to Polymer Exposure under Composting Conditions

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In recent years, there has been an increase in demand for biodegradable polymers due to growing consumer calls for more biodegradable materials and increased regulatory scrutiny on polymer fate. Biodegradation is an important mechanism whereby the biodegradability of biodegradable polymers can be assessed. The current study examined the biodegradability of cellulose, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), polyvinyl acetate (PVAc), poly (vinyl alcohol) (PVOH), and walnut shell powder in three compost sources and the effects on the compost microbial communities after 45 days of incubation. Using the ASTM D5338-15 method, the biodegradability of the polymers of interest was indirectly assessed by recording carbon dioxide evolution through an open flow respirometer. The effect of the polymer biodegradation on the compost microbial community was evaluated using 16S rRNA amplicon sequencing. Cellulose reached > 90% biodegradation during the 45-day experiment in all three compost sources. PHBV was tested in two compost sources and reached > 90% biodegradation in one compost source and 77% biodegradation in the other compost source. PVAc achieved < 20% biodegradation in two compost sources and 38% biodegradation in one source. PVOH achieved < 20% biodegradation in all the compost sources over the course of the experiment. Walnut shell powder exhibited 49% biodegradation in the sole compost source where it was tested and was showing upward trend when the experiment was terminated. The above results suggested that the extent of biodegradation tested materials depended both on the compost sources and test material itself. Data analysis on the microbial communities using high-throughput sequencing is currently on-going, and the results will be presented at the conference.

4.09.P-Tu-141 Limitations and Thresholds of Cationic Charged Guars in OECD Biodegradation Screening Methods

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Cationic guar is a commonly used ingredient in personal care products, particularly hair care products, due to the cationic charge making the material a good thickener and conditioning agent. Additional benefits of using this material include a generally favorable human toxicity profile and a high ISO naturality index score. However, highly charged materials may have the potential to be inhibitory to microbes and be persistent in the environment. This study aims to investigate the behavior of various charged cationic guars and their suitability in OECD biodegradation screening assays. Eleven cationic guars with cationic degree of substitution (cat DS) ranging from 0.08 to 0.22 were assessed in an OECD 302B study. A correlation was observed between increased cationic charge and decreased percent biodegradation results. Materials with a cat DS of ≥ 0.15 reached a level of > 75% biodegradation at the 28-day timepoint, and were classified as ultimately, inherently biodegradable. Guars with higher cat DS had percent biodegradation levels ranging from 57% to 65%, below the ultimately inherent threshold of 70%. When looking at the biodegradation curve for these higher cat DS guars,

evidence of sorption was present (non-linear curve). Based on this, the threshold for cationic guars to be suitable to test in OECD 302B, and achieve ultimately inherently biodegradable, is expected to be approximately 0.15 cat DS. Following this, OECD 306 (seawater biodegradation) test was selected due to its lower microbial count and biologic content which reduces the potential for sorption during the study. Four guars (identified as either "low," "medium," "high," or "very high" cat DS) were assessed in OECD 306. The results also showed a dose dependent correlation of a decrease in biodegradation potential with increase cationic charge. All materials showed a linear increase over the course of the study, indicating that no sorbing effects were present. The percentage biodegradation was 41, 40, 28, and 19% for the low, medium, high, and very high charged guars, respectively. The low, medium, and high charged guars would be classified as "inherently biodegradable" based on these results. Therefore, it can be concluded that OECD 306 assay is better suited to cationic guars with a cat DS > 0.15 and < 0.22, and that a DS of ≥ 0.22 is likely above the threshold to achieve an inherent biodegradation classification.

4.09.T Environmental Fate of Polymer

4.09.T-01 Importance of Polymer Structural Information in Biodegradability Assessments

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The need for high quality test material information is crucial for accurately assessing the biodegradability of polymers. Before initiating a study, detailed data on the test material's structure, elemental composition, empirical formula, polymer purity, and impurity identity and quantity should be provided. This information is essential for calculating the Theoretical Oxygen Demand (ThOD) or Theoretical CO_2 yield ($ThCO_2$), which are critical for determining the biodegradability of the materials in respirometric studies. Accurate ThOD or $ThCO_2$ calculations are imperative because they directly influence the reliability of biodegradability assessments. However, challenges in identifying polymer structures have led to uncertainties in these theoretical values, highlighting the need for precise and complete information. ThOD is a crucial parameter that represents the total amount of oxygen required for complete degradation of a material into carbon dioxide, water, minerals, and acids. The accuracy of ThOD calculations is contingent upon precise structural information of the test material, as any inaccuracy can lead to significant errors in estimating biodegradation potential. Conversely, Chemical Oxygen Demand (COD) is an experimental method that can be used to estimate ThOD, but it may have limitations due to incomplete oxidation of certain compounds or interference from physical-chemical properties. Therefore, ThOD is preferred for its accuracy, provided that the structural information of the material is correct. However, obtaining precise structural information on polymeric materials is difficult due to the complex nature of polymers. Further, the presence of impurities in test materials can significantly affect the outcome of a study. Impurities such as unreacted monomers, solvents, and catalysts, that may be present in substantial amounts, can lead to skewed results if not properly accounted for. This is particularly relevant in OECD screening tests, which are not designed for complex mixtures. For accurate interpretation of biodegradation data, it's crucial to limit and characterize the quantities of these impurities.
This presentation will provide an overview of the types of information needed, why it is useful, and the potential pitfalls if the information is not available. Specific case studies where certain

structural information was not available, and the subsequent impacts on the interpretation of the biodegradation study results, will be discussed.

4.09.T-02 Aerobic Biodegradation of Polymers in Aquatic Environments: High-Throughput Methods and Machine Learning Models

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The environmental concern over synthetic polymers, including water-soluble polymers and plastics, highlights the critical role of biodegradable polymers which account for only a small fraction of polymers. Existing experimental methods for assessing polymer biodegradability are costly and time-consuming. With very few Quantitative Structure-Activity Relationships (QSARs) dedicated to polymer biodegradability, existing approaches focus on specific polymer types or singular experimental conditions, which may not capture the complexity of polymer biodegradability influenced by structural and environmental factors. To address this gap, we first developed a high-throughput closed bottle method using a fiber-optic dissolved oxygen measurement approach based on a modified OECD 301D method. The evaluation of this new method on over 40 natural, synthetic, and graft polymers indicated that it could be reliably applied for testing the ready biodegradability of diverse water-soluble polymers. We then built a novel machine learning model based on a curated dataset comprising 42 polymers from published studies, 26 polymers from our experiments, and 13 polymers identified as non-biodegradable, with biodegradation percentages based on 9 OECD guidelines under different test durations. The best regression model achieved an R-squared value of 0.70 and a two-level classification model accuracy of 0.84. This model offers a potent tool for assessing polymer biodegradability under diverse conditions.

4.09.T-03 Implementation of a CO2 Evolution Test Design in Seawater Using Natural and Synthetic Polymers

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According to some estimates, at least 8 million metric tons of plastic enter our oceans each year and that is on top of the 200 million metric tons of plastic already circulating in our oceans (Oceans Conservancy.org). At the same time the assessment of persistence of polymers and other organic chemicals is increasingly scrutinized especially in marine environments. Environmental agencies have required the conduct of standardized tests to assess chemical persistence now for decades. Several of these standardized tests assess persistence or their potential to ultimately biodegrade by following the amount of CO_2 produced after the chemical under study is in contact with an inoculated matrix. Most of the CO_2 evolution methods are used with dilute inoculums, which generate low levels of endogenous CO_2 which is useful in the experiment to reduce matrix effects that could otherwise mask biodegradation results. Such methods are especially useful when working with low water soluble and/or adsorptive chemicals. However, such CO_2 evolution techniques are generally not part of the standard suite of tests used in the OECD guidelines for testing in seawater, although there is a procedure in the ISO guidelines. Difficulties with current seawater procedures have often been related to high background matrix effects, which impact endogenous DOC concentrations, deplete O_2 content, and increase CO_2 amounts. In this presentation, we will discuss techniques that remove matrix effects but still represent the natural ocean microflora while using multiple CO_2 evolution test designs to assess the ultimate biodegradation of polymers in seawater. At least three natural polymers representing a disaccharide, protein, and fat have been used to show proof-of-concept of these procedures, and results will be presented and compared to a typical reference compound along with a more challenging polymer found in the oceans today.

4.09.T-04 Critical Review of the Environmental Lifetime of Non-Biodegradable Plastics

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Currently, there are limited or no science-based estimates of the environmental lifetimes of plastic. Such estimates are challenging to obtain due to plastic's complex multi-step and multi-mechanism degradation pathways, releasing plastic particles, dissolved carbon, and CO_2. This project aims to expand lifetime estimations limited to biodegradable plastics to non-biodegradable plastics degraded via abiotic pathways (photo/photochemical and mechanical degradation). In particular, we will calculate the specific surface degradation rates (SSDRs) derived from manually selected published studies from 2010-2023. SSDR is based on the mass loss rates of plastic, and the typical reported mass loss data includes (1) weight loss of the parent materials, (2) size and concentration of released micro- and nano-plastics, and (3) concentration of dissolved carbon, volatile compounds, and/or CO_2. First, polyethylene (PE), polypropylene (PP), and polystyrene (PS) were found to be the most studied plastics, and combined photo- and mechanical degradation was found to be the most common degradation mechanism. Initial estimates of SSDRs were found to be 0.2 - 36.3 m/yr for PE, 0.6 – 1.2 m/yr for PP, and 4.31 – 16.3 m/yr for PS. Our preliminary calculations for PS were 3-4 orders of magnitude higher than previously reported biological degradation estimates (0.001 m/yr), highlighting the importance of abiotic pathways. Second, around 50% of articles lack essential information for calculating SSDR (e.g. initial mass and/or thickness), highlighting the need for best practice of reporting degradation data for estimating SSDRs. In addition, this review aims to assess: (1) whether SSDR changes over time, (2) whether and how SSDRs of plastic films differ from that of microplastics, (3) the current practice of converting rates from lab to field, (4) knowledge gaps in plastic degradation research, (5) future directions in estimating the environmental lifetime of plastics. This research lays important groundwork on the fate and lifetime estimation of plastics for developing novel LCA tools that will inform the sustainable design of future plastics.

4.09.T-05 Mechanisms of Microplastic Generation and Reactivity in Aqueous Media from Thermal and UV-Oxidized Plastics

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This study compares the generation of microplastics (MPs) from plastics oxidized by thermal and ultraviolet (UV) radiation processes. Our group has found that open-pit waste burning in underserved areas contribute to MPs generation showing signs of thermal and UV oxidation. However, the mechanisms behind the MP generation under these two oxidation processes are not fully understood. The plastics selected in this study include low density polyethylene (LDPE), polypropylene (PP), polystyrene (PS), and rayonand were all obtained as consumer products. To understand changes in functional chemistry, MP generation and reactivity in aqueous media, thermally, ultraviolet (UV), and non-oxidized plastics were subjected to mechanical abrasion conditions that promote the fragmentation of plastic pieces into MPs. We used Attenuated Total Reflectance - Fourier Transform Infrared (ATR- FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS) to analyze the functional group and structural changes in the plastics before and after oxidation and before and after abrasion. Microplastic generation was quantified using fluorescence microscopy, and will be confirmed with pyrolysis gas chromatography/mass spectrometry (Py-GC-MS). Initial findings from this study indicate increasing temperatures weaken alkane and alkene C-H stretch (2840-3100 cm-1) and bending (690 – 1100 cm-1) signals, suggesting a breakdown of the plastics. Concurrently, C-O (1000-1260 cm-1) and C=O(1700-1820 cm-1) signals become more pronounced, indicating oxidation. Other findings indicate that the selected abrasion conditions (sand and gravel mix) generate MPs, that the oxidation processes compounded with mechanical abrasion may increase the quantity of MPs generated, and that mechanical abrasion has an effect on the oxidation and degradation of the studied plastics (since the spectra taken from the plastics before and after the abrasion process are different). Current work is focused on understanding functional chemistry, and evaluating chemical reactivity in aqueous media. Our study is relevant, as open-pit waste burning sites are an underestimated source of MPs, exhibiting a unique chemical signature that could change their reactivity in the environment.

4.09.T-06 Quantum Chemically Calculated Abraham Parameters for Quantifying and Predicting Polymer Hydrophobicity

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Plastics have become a ubiquitous part of modern life owing to their reduced costs as compared to more traditional materials and the myriad of highly tunable functional properties they exhibit. For these reasons, global production of plastics has expanded to over 450 million tons produced in 2019 alone. This massive production has led to problems associated with end-of-life (EOL) effects given that recovery and recycling rates are minimal and leakage of plastics into the environment is a common occurrence. The growing accumulation of waste plastics in the environment combined with the growing abundance of research into micro- and nano-scale plastics and their effects on ecological and human health has increased attention toward the EOL fate and effects of plastics in the environment. Hydrophobicity is a common chemical property that strongly affects the mobility of compounds through environmental media and is a significant driver of chemical partitioning to environmentally relevant compartments like soil, sediment, plants, and organisms. Additionally, the hydrophobicity of polymer micro- and nano-particles is an important mechanism behind the sorption of small molecule pollutants which can further exacerbate their toxicity in the environment. The ability to quantify and predict the hydrophobicity of polymers is thus important to understanding and mitigating the negative impacts that plastic waste can have in the environment. To this end, this study used quantum chemistry (QC) and density functional theory (DFT) methods to calculate Abraham Parameters (APs) to predict polymer hydrophobicity from molecular structure. The APs consist of five unique molecular descriptors for solvation reactions and combine to form a poly-parameter linear free energy relationship for predicting partition coefficients. The model was validated with experimental octanol-water partition coefficients (K_{OW}), solubility parameters and spectrophotometric measurements of nile red staining. Additionally, hydrophobicity predictions were used to model the sorption of organic contaminants to polymers, with the hydrophobic interactions of both the sorbates and sorbents contributing. These predictions are crucial to quantifying how unique polymers interact with the environment, to better understand the mechanisms through which they produce adverse effects, and to facilitate the design of more environmentally benign alternatives.

4.10.P-Th Late Breaking Science: Chemistry and Exposure Assessment

4.10.P-Th-144 Health Risks to the Nepalese Population Associated With Pesticide Residues in Fruits

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Studies of pesticide use on commercial fruits are limited in Nepal. We surveyed the experiences and opinions of banana farmers in Nepal and monitored pesticide residues in apples and bananas. We monitored the residual status and associated health risks of 51 multi-group pesticides using Liquid Chromatography Triple Quadrupole Mass Spectrometry. Apple and banana samples were collected from different districts, farms, markets, and seller categories (our farm to fork survey). We found a significant bivariate positive correlation between farmers' knowledge of pesticide negative effects and safety-promoting behavior among farmers. Residues of tebuconazole, difenoconazole, propargite and imidacloprid were most frequently detected. Of all the detected pesticides, propargite showed the highest concentration in apples, exceeding regulatory maximum residue limits (MRLs) in 24% of the samples. Epoxiconazole, tebuconazole, imidacloprid, quinalphos, fenpropathrin and bifenthrin in apples, and metalaxyl and imidacloprid in bananas also crossed their MRLs. Dietary exposure to pesticides among children and adults was estimated based on a hazard quotient and hazard index by using lower-bound and upper-bound approaches. Our results indicated that children consuming apples with residues of propargite are exposed to the greatest pesticide hazard.

4.10.P-Th-145 PCB Contamination of Fish Collected Near Formerly Used Defense Sites in the Aleutian and Bering Sea Island Region

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The Aleutian and Bering Sea Island (ABSI) region is a culturally and biologically unique arctic ecosystem in Alaska. ABSI residents rely on marine resources for subsistence, including marine mammals, seabirds, fish, and shellfish. The ABSI region was heavily militarized during World War II and the Cold War; more than 50 formerly used defense (FUD) sites are scattered among ABSI region islands. Few of these FUD sites have gone through any remediation. FUD sites, oftentimes collocated with subsistence communities, are hotspots of contamination of local environments, including as a source of persistent organic pollutants (POPs). The Arctic is also a hemispheric sink for POPs subject to atmospheric transport, including polychlorinated biphenyls (PCBs). Previous research showed that PCB concentrations in fish sampled from FUD sites are higher than those in fish from non-FUD sites on St. Lawrence Island. The current study compares PCB concentrations and congener-specific profiles in freshwater fish collected from FUD and reference sites at four ABSI region islands (Shemya, Amchitka, Adak, and St. Pail) and one island outside of the ABSI region (East Amatuli). PCB concentrations will be used to perform a quantitative risk analysis to determine certain health risks among subsistence residents due to consumption of PCB-contaminated fish. Preliminary results show that cancer risk due to PCB exposure exceeds EPA guidelines, and PCB concentrations are higher at FUD sites than at non-FUD sites.

4.10.P-Th-146 Contaminants of Emerging Concern in Effluent of Municipal Wastewater Facilities in Kazakhstan and Proposed Treatment Alternatives for Water Reuse

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In Kazakhstan, sewage treatment is often undeveloped and raw sewage is discharged directly onto filtration beds or impoundments. Wastewater facilities often fail to meet treatment standards prior to discharge, and the problem of treatment and disposal of sewage sludge from treatment facilities requires urgent solutions. In this study, the current state of communal sewage treatment in Kazakhstan is reviewed aiming to devise strategies for reuse. The technological characteristics of sewage disposal systems, including Almaty and Astana, are presented to highlight environmental problems and opportunities related with sewage treatment. Using polar organic chemical integrative samplers (POCIS), we report the results of wastewater monitoring from two treatment plants in summer and fall seasons. Several pharmaceuticals, including carbamazepine, paracetamol and sulfamethoxazole, are found in wastewater effluent from both municipalities. Treatment alternatives are proposed and tested for removal of pharmaceuticals from wastewater using activated carbon as filtration media. Activated carbon is a microporous form of carbon with high surface area which makes it an excellent adsorbent. It is often used in wastewater treatment, drinking water filters, and air purification systems. Commercial activated carbon is typically made from raw materials such as coal, wood and lignite. As those materials are relatively expensive and damaging to the environment, researchers are experimenting with alternative low-cost feedstock. We produced activated carbon from locally produced sawdust and sugar beet pulp through carbonization in a nitrogen filled muffle tube furnace (700ºC). The resulting carbonized materials were activated using potassium hydroxide (KOH) at various KOH/carbonized material ratios (1:1, 2:1, 3:1, and 4:1). Agrochemical analyses of soil (humus, pH, water content, and trace elements) surrounding a municipal wastewater infiltration pond are presented with a proposed strategy for onsite wastewater treatment using functionalized activated carbon. With this research we aim to demonstrate how agro-industrial wastes can provide an economic treatment feedstock, which is modified for metal supported for catalytic wet peroxide oxidation of pharmaceuticals. This strategy will form the basis for sustainable reuse of wastewater by providing cost effective treatment alternatives for elimination of emerging contaminants.

4.10.P-Th-147 Monitoring and Mitigating Micropollutant Contamination in River Waters: A Focus on Catalytic Treatment of Hospital Wastewater

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Population growth has led to the increased contamination of river waters with a variety of anthropogenic chemical compounds, posing potential risks to aquatic ecosystems. These pollutants, including personal care products, pharmaceutical compounds, and other consumer products, highlight the need for vigilant water quality monitoring and the development of scalable solutions to ensure sustainable water use. In this study, we examined the presence of human-use compounds in river water over three months, focusing on sites upstream and downstream of a wastewater treatment plant (WWTP) in northeastern Portugal. Polar Organic Chemical Integrative Samplers (POCIS) were deployed at both sites and replaced every 23 days throughout the study. Our findings revealed a significant increase in the concentration of 28 identified micropollutants downstream of the WWTP, with antibiotics and antidepressants showing the most notable post-treatment rise. An important source of these pharmaceutical contaminants was identified as hospital wastewater, which is often discharged untreated into local WWTPs. Catalytic wet peroxide oxidation (CWPO) was explored to address this issue as a potential on-site treatment solution for hospital wastewater. A simulated wastewater matrix was treated in continuous flow using composite polymeric membranes filled with carbon nanotubes (CNTs). CWPO, an advanced oxidation process, employs hydrogen peroxide (H_2O_2) to generate hydroxyl radicals that effectively degrade organic pollutants. Sulfamethoxazole (SMX), an antibiotic, was selected as the model compound for this study. The treatment was conducted with a SMX concentration of 10 mg/L and a stoichiometric amount of H_2O_2 required for complete mineralization at a flow rate of 1 mL/min. The results demonstrated that the combination of the polymeric membrane and the catalytic activity of CNTs maintained a pollutant degradation efficiency above 70% over 8 hours. In comparison, filtration alone achieved only a 9% reduction in the same timeframe. The results showed the potential application of CWPO for the degradation of micropollutants, solving part of the antibiotic contamination problem before the discharge in the water body.

4.10.P-Th-148 Identifying Potential Microplastic Polymers and Hazardous Substances in Korean Detergent Products for Exposure Assessment

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Microplastics exhibit a notable propensity for aggregation and adsorption with other materials, owing to their small size particles and the influence of Brownian motion, a phenomenon accentuated in nanoplastics. Such processes occur not only with substances inherent to the environmental system but also with other additives or polymers present in products. To address these concerns, this study examined the full ingredient disclosures of commonly used detergent products in South Korea that were voluntarily disclosed by manufacturers. Through analysis of these ingredients, focusing on hazard information, we aimed to shed light on the characteristics of the substances employed. Previous research primarily concentrated on microplastic presence in the environment and direct exposure; however, non-polymeric substances utilized in products have been overlooked. Our study thus assumes significance as it represents a foundational contribution to microplastic exposure research, offering insights into the polymers potentially serving as microplastics in detergent products, as well as identifying potentially harmful substances within these products.

4.10.P-Th-149 Preliminary Use of Bioavailability Metrics To Assess Remediation Effectiveness at Superfund Sites

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Remediation of environmental contamination at sediment sites is complex, expensive, and the clean-up procedures themselves can be an environmental stressor. Selecting a cleanup technique that balances long term effectiveness and minimal adverse environmental impacts is critical to a successful remediation. Existing data collected from contaminated sites (e.g., Superfund sites, current or archived) provides an abundance of information that can be used to evaluate remedial effectiveness. In this work, Superfund site data from historically contaminated sites is being gathered to compare bioavailability metrics with remediation events to assess remedy effectiveness. Specifically, measurements of bioavailable concentrations of contaminants associated with the water column, passive samplers, and tissues are compared to a timeline of site events to observe the effectiveness of remediation techniques. Superfund sites contain well documented procedures, logic, and quality controls that span decades. The longevity of these sites and the associated datasets allow for increased understanding of remediation effectiveness within the same complex site. For example, the Alcoa Aggregate Superfund site (Massena, NY, USA) has been investigated from 1989 to 2022 where the main dredging and capping events occurred in 2019 to 2021. Results indicate minimal deviation in bioavailable concentrations a year post-remediation compared to previous years (used as a baseline). Future work includes the addition of data from 2023 upon publication to further observe changes in bioavailable concentrations for the water column and proxy fish species. While site goals, remediation tactics, conditions, and other considerations will differ at every site, the question of common patterns in remediation effectiveness remain. Additional contaminated sites are under evaluation both for site specific interest and to determine if remediation effectiveness can be compared across sites.

4.10.P-Th-150 Climatic and Biochemical Controls on Arsenic Bioaccessibility in Mine Tailings Sites

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Health risks related to gastrointestinal exposure to arsenic (As) are frequently evaluated using *in vivo* models. However, due to the expense, ethical issues, and technical difficulties of *in vivo* models, *in vitro* bioaccessibility (IVBA) approaches—which enable the quantification of the portion of a contaminant released from a solid matrix placed in contact with a biochemical fluid—are more feasible for routine analysis. Particulate matter from sulfide ore-derived mine tailings is a common source of As exposure to human populations. As these waste materials are deposited on the landscape, they undergo weathering-induced changes in As molecular speciation as a result of climatic forcing, with poorly understood impacts of bioaccessibility. In unweathered sulfidic mine tailings, As is found predominantly as As^{1-} in the form of arsenopyrite. However, oxidative weathering in the surface layers shifts prevalence to As^{V} species adsorbed or co-precipitated with Fe^{III} (oxyhydr)oxides and hydroxysulfates, including ferrihydrite, goethite, and jarosite. We postulated that the depth of oxidative weathering would correlate with mean annual precipitation – i.e., shallow in arid and deep in humid climates – for

tailings subjected to similar weathering durations. Additionally, we hypothesized that As would exhibit lowest bioaccessibility in surface tailings where it is adsorbed to secondary Fe^{III} (oxyhydr)oxide surfaces, and highest bioaccessibility in deep, unweathered tailings where it is incorporated into primary sulfides (as observed by X-ray absorption spectroscopy measurements). Samples were collected as a function of depth (0-200 cm) through the oxidative reaction front from 13 sites spanning a range in climate across the Western U.S. The lowest As bioaccessibility was found in the intermediate zone characterized by highest As accumulation in secondary mineral phases, whereas highest As bioaccessibility was mainly found in the least weathered parent material of aridic sites and in the highly altered surface layers of humid sites. This indicates that the distinct As speciation detected at varying depths and among the mine tailings sites significantly impacted As bioaccessibility results. The data also highlight the importance of the different gastrointestinal constituents (e.g., protons, pepsin, pancreatin) in influencing As release as a result of the acidic and reductive dissolution of host minerals in gastric and intestinal bioassays, respectively.

4.10.P-Th-151 The Effects of Nano-Enabled Copper Hydroxide Fungicide on the Fate and Transport of Neonicotinoid Insecticides in a Pacific Northwest Wetland Environment

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Nanopesticides offer potential environmental benefits in agriculture, yet their fate and impact on downstream wetlands remain understudied. This study evaluates the effects of a nano-Cu fungicide and a neonicotinoid insecticide (imidacloprid) on wetland nutrient cycling using eighteen mesocosm wetlands. Neonicotinoids and copper-based fungicides are prevalent globally and now exist in nano-enabled forms, promising improved efficacy and reduced application rates. Despite their widespread use, environmental persistence and degradation of these nano-formulations are not well understood. Here we investigate the behavior of nano-enabled and conventional pesticides in wetland mesocosms. Treatments included nano-copper hydroxide, nano-Imidacloprid, and conventional Imidacloprid, with various combinations applied to mesocosms planted with local wetland flora (*Eleocharis palustris, Schoenoplectus pungens,* and *Carex obnupta)* that were allowed to establish over a 6-month time frame before exposure. In the18 mesocosms, pesticide treatments were then applied. Water samples collected during a 10-day application period are analyzed using HPLC-qTOF-MS to track imidacloprid degradation and its products. Kocide 3000 dissolution is measured using ICP-MS and spICP-MS for total, dissolved, and particulate copper quantification. Our results will enhance understanding of the fate, transport, and behavior of nanopesticides in wetland ecosystems, informing future environmental risk assessments and management strategies.

4.10.P-Th-152 Revisiting the Dock Model: Updates to Estimating Ecological Risk From Antimicrobial Pesticides Used in Pressure-Treated Wood

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The United States Environmental Protection Agency's (EPA) Office of Pesticide Programs Antimicrobials Division is in the process of updating its screening-level model used to assess risks to non-target aquatic organisms from antimicrobial pesticides used in pressure-treated wood (referred to as the 'dock model'). Currently, risk to aquatic organisms is calculated based on the leaching rate of the antimicrobial pesticide, the number of treated docks required for the environmental exposure concentration to exceed a concentration of concern, and whether that number of docks is reasonable for a 1-hectare waterbody. After evaluating the parameters that most influence the results in our current approach, EPA is proposing to update its dock model to (1) standardize the leach rate estimates from wood leaching studies used in the model, and (2) update the conceptual model assumptions related to dock surface area and waterbody volume based on geographic information system analyses and data on waterbody characteristics across the United States. In the updated approach, EPA identifies risks of concern when exposure estimates calculated using the updated conceptual model for a vulnerable canal/marina exceed toxicity endpoints. EPA will present the results of the analyses used to inform the most appropriate leach rate estimates and duration of exposure from wood leaching studies used to support the registration of wood preservatives. Furthermore, the rationale for selecting a representative waterbody and a corresponding reasonably high-end estimate of the ratio of dock per waterbody volume for estimating risks to aquatic organisms will be presented. EPA believes this updated approach will result in more transparent and consistent environmental risk assessments for antimicrobial wood treatments.

4.10.P-Th-153 The Bioaccumulation and Metabolism of 6PPD-Quinone in Rainbow Trout

***Rory Mumford** and Christopher Kirkman, Environmental Fate and Metabolism, Smithers, United Kingdom*

6PPD-quinone (6PPD-Q), a transformation product of the antiozonant and antioxidant 6PPD (N-(1,3-Dimethylbutyl)-N′-phenyl-phenylenediamine), is believed to be carried from tire and road wear particles (TRWP) into watercourses. Some publications have identified 6PPD-Q as a contributor to Urban Runoff Mortality Syndrome (URMS) of a single specific fish species, Coho salmon. The bioaccumulation potential of 6PPD-Q in rainbow trout (*Oncorhynchus mykiss*) was investigated by exposure to [^{14}C]-6PPD-Q at a single test concentration according to the OECD 305 bioaccumulation guideline. Additionally, the metabolism of 6PPD-Q was investigated in the fish tissues sampled at the end of the exposure phase. This poster will present the results of the bioaccumulation study and the metabolism of 6PPD-Q observed in rainbow trout.

4.10.P-Th-154 The Fate of 6PPD-Quinone in Soil and Water-Sediment Systems Using a 14C Radiotracer

***Rory Mumford**, Daniel T. Evans, Pete Yeomans and Christopher Kirkman, Environmental Fate and Metabolism, Smithers, United Kingdom*

6PPD-quinone (6PPD-Q), a transformation product of the antiozonant and antioxidant 6PPD (N-(1,3-Dimethylbutyl)-N′-phenyl-phenylenediamine), is believed to be carried from tire and road wear particles (TRWP) into watercourses. Some publications have identified 6PPD-Q as a contributor to Urban Runoff Mortality Syndrome (URMS) of a single specific fish species, Coho salmon. The potential mobility of 6PPD-Q in soils and sediments has been investigated using the OECD 106 test guideline (Adsorption—Desorption Using a Batch Equilibrium Method) and ^{14}C-radiolabelled 6PPD-Q. This study is used to indicate how likely a compound is to move through soils. Additionally, the transformation of 6PPD-Q has been investigated according to OECD test guidelines 307 (Aerobic and Anaerobic Transformation in Soil), OECD 308 (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems) and OECD 309 (Aerobic

Mineralisation in Surface Water—Simulation Biodegradation Test). These studies were selected to allow us to gain an understanding of the transformation and partitioning of 6PPD-Q in the key environmental compartments. Results were presented at SETAC EU (Seville, May 2024). This presentation focuses on the identification and quantification of transformation products generated during these aforementioned studies.

4.10.P-Th-155 Analysis of Contaminant-Plastic Sorption Using Nuclear Magnetic Resonance Spectroscopy

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In addition to being a contaminant in its own right, plastic pollution also plays a role in the fate and impact of many contaminants, particularly for hydrophobic organic compounds. Plastic litter and particles can act as both media for the sorption and as a vector for the transport of organic contaminants. Plastic litter ranging from macroplastics, such as littered bottles or food containers, to microplastics, including tire wear particles or fragments from larger plastics is commonly be found in roadside ditches, where it is exposed to organic contaminants from sources such as automobile exhausts and pesticide runoff from adjacent agricultural fields. Commercial plastics originate from wide diversity of polymers with different physical and chemical properties, which become further modified by environmental weathering processes, such as ultraviolet radiation and mechanical degradation. These differences can result in significant changes to their sorption behaviour, and to date, terrestrial plastics have received much less focus than their marine counterparts. This study shows how Nuclear Magnetic Resonance (NMR) spectroscopy can be used as a simple tool to quantify the sorption of organic contaminant mixtures on common plastic types found as litter. Plastics polymers and their extent of weathering are qualitatively determined using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy. These plastics are then exposed to a contaminant mixture that includes polycyclic aromatic hydrocarbons (PAH) and hydrophobic pesticides. Sorbed compounds are extracted with deuterated chloroform in a single step and directly analyzed with by NMR, enabling high-throughput and quantitative analysis with minimal sample preparation. This approach helps measure plastic-water partitioning coefficients, identify competitive sorption between different contaminants, and can be applied towards sorption kinetics and isotherm experiments. Preliminary findings have shown interesting changes in sorption behaviour of PAHs onto polyethylene (PE) compared to metallised-PE.

4.10.P-Th-156 Quantifying Clothianidin Persistence and Stability in Missouri Freshwater Sediments

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Prophylactic application of agrochemicals as seed coatings and foliar sprays has resulted in the unintentional movement of neonicotinoid pesticides into non-target ecosystems through drift and precipitation events. Physicochemical properties, such as high solubility and leachability have further contributed to the transfer of neonicotinoids into freshwater ecosystems. Detection in lake, stream, and wetland waters has been well documented in the literature, along with lethal and sublethal effects identified through laboratory aquatic insect bioassays. However, the influence of sediment-associated neonicotinoid concentrations on benthic aquatic macroinvertebrates and subsequent trophic levels remains poorly studied. Previous research found that Missouri neonicotinoid concentrations in wetland sediments were an order of magnitude greater than aqueous concentrations observed in the same wetlands. Further, an overall decrease in aquatic insect richness and abundance was associated with greater neonicotinoid concentrations in wetland water and sediments. Although neonicotinoid benchmark concentrations for aquatic insects have been determined in water, sediment toxicity thresholds have not been established; thus, the implications of contaminated sediment for aquatic insects in freshwater systems are largely unknown. To address this knowledge gap, we needed to establish the persistence and bioavailability of neonicotinoids in sediments of differing organic carbon and clay content. This initial step is prerequisite to completing laboratory macroinvertebrate toxicity bioassays using spiked sediment. We conducted a chronic sediment spiking experiment using the widely used neonicotinoid, clothianidin, and sediments collected from two distinct Missouri wetlands and one sandy-bottomed Missouri stream. We spiked at high (100 µg/kg dry weight) and low (10 µg/kg dry weight) environmentally relevant concentrations determined from the aforementioned Missouri wetland study. Throughout the study, we periodically analyzed sediment, overlying water, and porewater clothianidin concentrations, and determined half-life and partition coefficients. This work lays the foundation for establishing sediment clothianidin toxicity thresholds and further determining the importance of sediment-associated neonicotinoids as an exposure pathway to benthic aquatic invertebrates.

4.10.P-Th-157 The Utilization of Negative Chemical Ionization To Expand Nontargeted Screening of Halogenated Organics in Great Lakes Top Predator Fish

__Saly Bolis Abadir Abskhroun__, Sujan Fernando, Thomas Holsen, Philip Hopke and Bernard Crimmins, Clarkson University, United States

Non-targeted screening (NTS) of halogenated contaminants in biota is part of the routine monitoring of the Great Lakes ecosystem. NTS can give insight into new chemicals with possible persistent bioaccumulative and toxic (PBT) properties and track known PBT's degradation and transformation products. 2-dimensional gas chromatography (GCxGC) coupled with high-resolution mass spectrometer (HRMS) is one of the best techniques used for NTS. The most common ionization technique for NTS is electron impact ionization (EI) due to the consistent fragmentation patterns that can be standardized. The current research explores the utilization of electron capture negative ionization (ECNI) as a complementary technique to broaden the range of halogenated contaminants detected through NTS. This was achieved using GCxGC-HR-ToF MS with a multi-mode ion source (MMS). The MMS offers consecutive runs on EI and ECNI using the same chromatographic setup, facilitating the retention time alignment in both modes. The exact mass measurements help in identifying compounds found only in ECNI. The addition of ECNI led to the detection of 67 new halogenated features that were not detected in EI. Furthermore, ECNI aided in the identification of eight compounds detected in both modes.

4.10.P-Th-158 Modification and Application of U.S. Environmental Protection Agency Method 1633 for Per and Poly-Fluoroalkyl Substance Extraction and Quantification of Great Lakes Top Predator Fish

***Ogechukwu Ernestina Ofodum**, Thomas Holsen, Sujan Fernando, Bernard Crimmins and Adam Point, Clarkson University, United States*

The Great Lakes are a group of five interconnected freshwater bodies in North America. The lakes are ecologically diverse, support numerous plant and animal species, and play a vital role in transportation, industry, and tourism. Factors such as pollution from industrial and agricultural activities have long affected the health of the Great Lakes ecosystem, which led to the establishment of the U.S. Environmental Protection Agency Great Lakes Fish Monitoring and Surveillance Program (GLFMSP). This program monitors the levels of contaminants in the lakes, including per and poly-fluoroalkyl substances (PFAS). PFAS are a group of synthetic chemicals widely used in industrial and consumer products since the 1940s due to their unique stability, resistance, and surface tension-lowering properties. However, the ubiquitous presence of PFAS in the environment have raised concerns due to their persistence, bio-accumulative nature, and potential adverse effects on human and ecosystem health, particularly in aquatic ecosystems. Monitoring PFAS in the fish of the Great Lakes is vital to assess their presence, distribution, and potential impacts. The EPA developed Method 1633 in response to the Clean Water Act (CWA) for the determination of 40 PFAS in fish tissue, water, as well as other types of samples by liquid chromatography/tandem mass spectrometry (LC-MS/MS). This research focuses on adapting Method 1633 to fish that are part of GLFMSP and making necessary modifications to improve its performance and/or adapt the materials and resources available. The optimized extraction technique to efficiently extract PFAS from fish matrices and to minimize matrix interference and maximize PFAS recovery using solid phase extraction (SPE) will be presented. Results will focus on alkyl acids and sulfonates that have been detected in the Great Lakes over the past decade using the traditional U.S. EPA method 537.1, as well as additional PFAS precursors now included in Method 1633. The data obtained with this method will help expand the knowledge on the quantity of different target PFAS in the Great Lakes, their pattern of accumulation in fish species, and help infer potential ecosystem and human risks.

4.10.P-Th-159 Improving Targeted PFAS Analytical Technologies With Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Large Volume Injection

***Elizabeth Brown**[1], Sujan Fernando[2] and Thomas Holsen[2], (1)Civil and Environmental Engineering, Clarkson University, (2)Clarkson University*

LC-MS/MS is a highly sensitive, accurate, and established technology used to quantify water-soluble ionic per- and polyfluoroalkyl substances (PFAS). As legislation works towards lower and lower enforceable contaminant levels, improvements to current LC-MS/MS methodologies will be necessary to quantify PFAS at those levels. Large volume injection (LVI) technologies are a promising approach for increasing the sensitivities of the current LC MS/MS methods. Additionally, LVI utilizes inline sample preparation thus eliminating the need for a separate step that typically involves solid phase extraction (SPE). The aim of this research, with the overall goal of quantify PFAS contaminants in the parts-per-quadrillion (ppq) range, is to understand the effects LVI has on the limits of detection (LOD) and limits of quantification (LOQ) on already established LC–MS/MS methods and develop new methodologies to overcome issues typically experienced using LVI technologies (i.e., increased signal-to-noise ratio, poor chromatography, longitudinal diffusion).

4.10.P-Th-160 Evaluation of Solid Phase Extraction (SPE) Technologies for Quantifying Non-Targeted Per- and Polyfluoroalkyl Substances (PFAS) Using Total Organic Fluorine (TOF) Via Combustion Ion Chromatography

Elizabeth Brown[1], Sujan Fernando[2] and Thomas Holsen[2], (1)Civil and Environmental Engineering, Clarkson University, (2)Clarkson University*

As the number of non-targeted per- and polyfluoroalkyl substances (PFAS) rapidly expands advancements to non-targeted technologies need to be made. The non-target analysis approach of using combustion ion chromatography (CIC) measures the total organic fluorine (TOF) in a sample. Using organic fluorine, as proxy for PFAS, the CIC analysis returns a single representative result of the total PFAS concentration in a sample. Additionally, due to the simplicity of the analytical technology, it excels in terms of cost and time efficiency—providing short turn-around times with little data processing. In addition to lowering the limits of detection and limits of quantification to less than 50 $\mu g\ L^{-1}$ this work compared solid phase extraction (SPE) technologies including absorbable organic fluorine (AOF) and extractable organic fluorine (EOF) for the efficacy of capturing short-chain PFAS, breakthrough concentrations, and the applicability of direct combustion of the granulated activated carbon AOF cartridges.

4.10.P-Th-161 Current Landscape of Risk Assessment Relevant Knowledge and Understanding of Per- and Polyfluoroalkyl Substances

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Legacy per- and polyfluoroalkyl substances (PFAS), such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), have been extensively studied and analyzed for decades, providing adequate exposure and toxicity data, making it possible for risk to be evaluated and equivalent regulations to be implemented to protect public health. However, there are currently over 14,000 PFAS that have been identified by the U.S. Environmental Protection Agency (EPA), many of which lack the necessary data to assess risk. The goal of this study is to summarize the extent of available PFAS data and highlight key research gaps for risk assessment. We used publicly available federal and state sources for data compilation and performed literature searches to complement data gaps. Starting from over 14,000 identified PFAS from the EPA's PFAS Analytical Tools webpage, we searched for their toxicity and exposure information. We also searched for established regulatory health advisory values such as drinking water standard levels, cancer slope factors, reference doses (RfDs), and tolerable daily intakes (TDIs) for each PFAS compound. A vast majority of identified PFAS lack available data that is necessary for human health risk assessments. Out of over 14,000 PFAS, only 2,945 (19.9%) PFAS have available physiochemical property data, 354 (2.4%) PFAS have available production data, and 63 PFAS (0.4%) have biomonitoring data. In vitro, in vivo, and in silico toxicity data are available for 511 PFAS (3.5%), 186 PFAS (1.3%), and 7,715 PFAS (52.4%), respectively. Endocrine and neurotoxic high-throughput assay data are available for 15 PFAS (0.1%) and 89 PFAS (0.6%), respectively. Only 1 PFAS (i.e., PFOA), 16 PFAS (0.1%) and 4 PFAS (<0.1%) have an established cancer slope factor, RfDs, and TDIs, respectively. This study serves as a summary of the current understanding of PFAS data that are necessary for risk assessment. Highlighted data gaps should help improve research prioritization.

4.10.P-Th-162 Assessing the Physiochemical Properties and Environmental Behaviour of Non-Polar Organic Compounds Transported by Methane Ebullition in an Oil Sands Pit Lake Through GCxGC

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Syncrude's Base Mine Lake (BML), located in Fort McMurray Alberta, is the first commercial demonstration of water capped tailings technology (WCTT) in the oil sands industry. It sequesters the fluid fine tailings (FFT) by overlaying a water column combining fresh water and oil sands processed water (OSPW), with the goal of developing into an aquatic ecosystem. Current field observations indicate that methane ebullition caused by microbial activities within the FFT is of environmental concerns as it is transporting residual bitumen to the lake surface. In this study, we subsampled bitumen-coated bubbles in ice cores from BML, solvent extracted and separated them into non-polar and polar fractions by silica gel chromatography, then analyzed the non-polar fraction through comprehensive two-dimensional gas chromatography (GC×GC). While identifying specific compounds within the unresolved complex mixture present in these samples was challenging, a MATLAB model based on the work of Nabi et al. (2014) was utilized to estimate a series of physiochemical properties for compounds present in different regions of the chromatogram. This model was based on Abraham polyparameter linear free energy relationships (pp-LFER) and was calibrated by locating 16 reference compounds (*n*-alkanes & PAHs) within the GC×GC chromatogram. Some key parameters estimated included organic carbon-air partitioning coefficients ($\log K_{OA}$) ranging from 3.5 to 10, air-water partitioning coefficients ($\log K_{AW}$) ranging from -2.5 to 2.5, and octanol-water partitioning coefficients ($\log K_{OW}$) ranging from 2.5 to 6.5. Combing these parameters with estimated masses of compounds within a given region can allow the assessment of their potential environmental behaviour. For instance, compounds with $-5< \log K_{AW} <-1$, or $-1<\log K_{AW}$ & $\log K_{OA}>5$ are likely to have long range transport potential (LRTP), and compounds with $5 < \log K_{OW} < 7$ & $-4 < \log K_{AW}< -1$ likely have aquatic bioaccumulation potential (ABP). Using this approach, the environmental behaviour of compounds present in complex mixtures can be estimated despite not confidently knowing the identities. However, given the characteristics of the BML, factors like ABP may be more of concerns over the others, and with the recent effort of dredging out the bitumen mat underneath the water column, the ebullition-facilitated transport of bitumen is expected to subside, with potential environmental risks of transported compounds being mitigated.

4.10.P-Th-163 Pending PFAS Regulations and Testing for Fluorotelomer Alcohols (FTOHs) in Water

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Fluorotelomer alcohols (FTOHs) represent a major class of per- and polyfluoroalkyl substances (PFAS) and include some of the most well-known precursors of perfluorocarboxylic acids (PFCAs), including perfluorooctanoic acid (PFOA) and perfluorohexanoic acid (PFHxA). FTOHs are used in the synthesis of various surfactants and as intermediates in the manufacture of a variety of products with a wide range of applications including textiles, polymers, paints, adhesives, waxes, cleaning agents, and are also found in many aqueous film-forming foams (AFFF). Their presence in surface water, groundwater, and drinking water supplies represents a potential risk to human health and the environment. As a major precursor of common PFCAs, FTOHs may indirectly cause adverse effects to human and environmental health. Human exposure to FTOHs occurs mainly through ingestion pathways such as diet and drinking water. Because they are widely used, FTOHs have been found in various types of water sources including drinking water, wastewaters, industrial wastewater influents and effluents, and surface water. New European regulations are currently pending for 6:2 and 8:2 FTOH which propose their inclusion within a regulated sum of twenty-four per- and polyfluorinated alkyl substances (PFAS) of primary concern. Incorporation of these substances into the regulatory regimes of other regions may follow. The ALS R&D team has recently validated a sensitive, robust, and selective analytical method to quantify FTOHs to ultra-trace levels using gas chromatography with triple quadrupole tandem mass spectrometry with positive ion chemical ionization (GC-MS/MS-PCI).

4.10.P-Th-164 State of the Science Selenium Method Development at the U.S. Geological Survey Moffett Field Selenium Laboratory

Veronika Alexis Kocen*, Evangelos Kakouros and Benjamin Shawn Linhoff, U.S. Geological Survey*

Selenium is a metalloid that is easily mobilized through natural and anthropogenic activities such as mining, oil and gas extraction, and agricultural. There is considerable global concern because selenium both bioaccumulates and biomagnifies in the food chain. Selenium is known to negatively interact with tissue at the cellular level, ultimately leading to declines in wild fish and bird populations. However, selenium is notoriously difficult to analyze due to isobaric interferences and high ionization potential. The U.S. Geological Survey Selenium Laboratory at Moffett Field, CA, has developed a novel method to analyze sediment and tissue for trace quantities of selenium. By using isotope dilution hydride generation inductively coupled plasma mass spectrometry (ID-HG-ICP-MS), we regularly measure total selenium concentrations in samples with as little as 5 mg dry weight achieving detection limits of 0.01 µg/g. This method is ideal for difficult samples such as fisheye lenses, a single mayfly, and the ovaries of redside shiners (*Richardsonius balteatus*). We use an enriched selenium isotope (^{82}Se) to spike samples when measuring the dry weight. By adding the spike to the sample, we can identify the isotopic ratio between ^{78}Se and ^{82}Se, which is the ratio between the natural isotopic abundance and the known isotope spike; this isotope dilution method allows us to calculate total Se concentration. It can be assumed that both isotopes respond similarly to the sample processing that follows. A strong oxidation process uses a concentrated nitric acid and a benchtop autoclave to achieve a complete digestion; this is followed by adding hydrogen peroxide, evaporating the sample and reconstituting in 2% hydrochloric acid. We then reduce by heating with hydrochloric acid and use hydride generation to remove the sample matrix and isolate Se for ICP-MS analysis. The laboratory has also purchased a laser induced breakdown spectroscopy/laser ablation system (LIBS/LA-ICP-MS) and a lab grade microwave to develop methods for faster Se analysis. This will allow our lab to expand its range beyond selenium to include methods to analyze microplastics and new matrixes such as fish fin rays and bivalve shells. Our goal is to continue to provide state of the science selenium analyses and research while expanding our lab's capabilities to help identify and study risks to aquatic ecosystems.

4.10.P-Th-165 Microplastics in Water and Air in the Urban Environment: Using Accelerated Solvent Extraction, Visual Identification, and Pyrolysis-Gas Chromatography-Mass Spectrometry

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The increasing presence of microplastic (MP) waste in the environment is escalating by the thousands due to the exponential use of plastics. MPs are emerging persistent pollutants that are difficult to remove from the environment due to their durability and small size, posing environmental and human health hazards, especially in heavily populated areas like the Dallas-Fort Worth (DFW) and Houston metroplexes. We aim to examine the occurrence and characteristics of MPs and plasticizers in the urban environmental systems (e.g., air and water) of these metroplexes. Over the seasons, we collected water and air samples under dry and wet weather conditions. Wet depositions, surface runoffs, and stormwater in creeks were collected after precipitation events along with the corresponding meteorological records. All water samples were processed in the lab through microfiltration to isolate designated-sized particulates prior to MP analyzation. Air samples were primarily collected during the dry season using an active sampler from indoor and outdoor spaces, including through traffic by a car-equipped air quality monitoring system. The retrieved glass fiber filters (GF/F, GF/B) were visually identified through a binocular microscope (4x-40x magnification) equipped with an adjustable halogen light source, and chemically examined by pyrolysis-gas chromatography-mass spectrometry to gather both the morphological and compositional characteristics of the MPs in the samples. The MPs' seasonal occurrence, concentration levels, and wet deposition mass flux will be reported. The morphological and compositional profile of the MPs will be compared between dry and wet weather conditions and against air-water partitioning.

4.10.P-Th-166 A Low-Tech, Mass-Based Approach To Quantify Plastic Microfibers in Dryer Lint

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Clothes laundering is a known source of environmental plastic microfibers. While most research has focused on microfiber releases to water during clothes washing, studies have shown more microfiber production occurs during tumble drying. Recent work on dryer lint used tumble-dried textiles of a single material to measure microfiber production (e.g., cotton as a natural fiber and polyester as a synthetic fiber). However, dryer lint is often a complex mixture of natural (e.g., cotton, fur, hair, wool) and synthetic (e.g., polyester, acrylic) fibers. A methodology for quantifying plastic microfibers in complex dryer lint samples is needed to accurately characterize this type of microplastics. This work proposes a high-throughput method to first isolate plastic microfibers from real dryer lint samples using common laboratory reagents and then quantify the plastic microfiber concentration using a laboratory balance. Lint samples are initially digested for 24 hours in 7.5% bleach, then in 50% H_2SO_4 for an additional 24 hours. To validate this method, the digestion efficiency was evaluated using a range of proteinaceous (silk, hair, fur, wool), cellulosic (cotton, denim, rayon, Tencel, viscose), and plastic textiles anticipated to be present in household dryer lint. Preliminary results showed the bleach digestion dissolved over

97% of proteinaceous fibers, while H_2SO4 removed over 93% of cellulosic material. H_2SO4 did dissolve nylon fibers, which are common in textiles and likely to be present in dryer lint. However, nylon fibers account for a relatively small proportion of synthetic fiber production compared to polyester. Preliminary microfiber recovery was tested by spiking fluorescent polyester microfibers into real lint samples and counting recovered fibers under cyan light. Recoveries ranged from 100-103%, suggesting this method does not result in significant microfiber losses. To confirm that digested lint samples are composed of plastic microfibers and non-plastic components are effectively removed, microfibers from digested and undigested lint samples will be analyzed by Raman spectroscopy. This methodology will contribute to the improved quantification of microfibers and to a better understanding of the sources of microplastics entering the environment.

4.10.P-Th-167 Leaching of Tire Wear Particles Additives Under Controlled Physicochemical Conditions

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Tire particles, generated from everyday road use, are a significant source of non-exhaust emissions. As data increasingly reveals their widespread environmental presence, it is crucial to understand the pollutant loads inherited in these particles and those sorbed onto them. Tire wear consists of various components, including additive chemicals, aromatic oils, synthetic rubbers, and natural rubbers. Among these, 6PPD, 6PPD-quinone and Hexamethoxymethylmelamine (HMMM) are of particular concern because of its acute and chronic toxicity effects on aquatic organisms. Several precursor and transformation products of these compounds has been detected in waters bodies, particularly following road runoff after rain events, suggesting their presence in aquatic systems. This study investigates the leaching kinetics of tire wear particles and the associated chemical additives under controlled conditions. Using LC-Orbitrap Mass Spectrometry, we perform high-resolution and accurate mass measurements to enhance molecular identification and quantification, employing both targeted and untargeted approaches. Quadruplicates tire particle samples are incubated in Milli-Q water, artificial seawater and sterilized water from Lavaca River for one month. Following the incubation, the sample was extracted through solid-phase extraction, and analyzed for targeted tire additives and an untargeted chemical profile. Although the research is ongoing, our preliminary data will guide a more comprehensive study aimed at understanding the role of environmental factors in leaching of additives from tire wear particles.

4.10.P-Th-168 Microplastics in Wild-Caught Tiger Sharks: Quantification and Characterization of Polymer Types and Additive Contaminants

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Annual global demand for plastics has consistently increased, reaching approximately 245 million tons, leading to a significant rise in plastic waste and environmental contamination. Microplastics (MPs) and phthalates, widely used plasticizers in a range of consumer products, are particularly concerning due to their known endocrine disrupting potential and reproductive health effects. These additives and other chemicals of emerging concern (CECs) associated with MPs occurrence contribute to impose adverse impact to wildlife due to exposure to MPs in the environment. This study aimed to quantify the presence of eight phthalates and seven major types of MPs—Polystyrene (PS), Polypropylene (PP), Polyethylene (PE), Polyethylene terephthalate (PET), Polyvinyl chloride (PVC), Polymethyl methacrylate (PMMA), and Polycarbonate (PC)—in wild-caught Tiger sharks. Samples (n = 35) were collected by the Georgia Aquarium and International Union for Conservation of Nature and Natural Resources Center for Species Survival. Both male and female sharks were caught in Florida and Hawaii from 2020 to 2022. Whole blood samples (~10 mL) were collected with other specimen and analyzed using microfiltration (0.7 µm size cut off) and single shot pyrolysis-Gas Chromatography-Mass Spectrometry. Our preliminary results indicate that PS trimer (followed by monomer and dimer), PET-vinyl benzoate, and PC-phenol are the most abundant types of MPs in sharks. Minor background contamination (up to 15% of dynamic range) was found for MPs (PS, PP, PET, PMMA, and PC), and all values were subtracted from the raw detected results of samples.

4.10.P-Th-169 Comparative Evaluation of HydraSleeve Passive Samplers and Low-Flow Purge Methods for PFAS Groundwater Sampling

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This study systematically evaluated the performance of HydraSleeve passive samplers relative to the conventional low-flow purge method for the sampling of per- and polyfluoroalkyl substances (PFAS) in groundwater. The low-flow purge technique, though widely used, is associated with substantial investigation-derived waste (IDW) generation, requires skilled operators, and involves complex operational procedures, with the potential to generate up to 3 gallons of IDW per well. In contrast, HydraSleeves offer a more cost-effective and labor-efficient alternative, significantly reducing IDW production and operational complexity. Field sampling was conducted across monitoring wells characterized by varying hydrostratigraphic conditions to evaluate the consistency and reliability of PFAS concentration measurements obtained using both methods. Additionally, adsorption studies were undertaken to assess the interaction of PFAS compounds with HydraSleeve materials. Initial experiments using deionized water revealed selective adsorption patterns, with longer-chain PFAS compounds exhibiting higher adsorption rates, highlighting the influence of molecular structure on adsorption dynamics. Subsequent tests with site-specific groundwater demonstrated lower adsorption rates across all PFAS compounds, suggesting that the presence of competing constituents in groundwater may mitigate PFAS adsorption onto HydraSleeve surfaces. Statistical analysis, including linear regression, indicated strong correlations between PFAS concentration measurements obtained via HydraSleeve and low-flow methods, suggesting that variability in one method could reliably predict the other. However, further analysis using Bland-Altman plots revealed that while HydraSleeve measurements were comparable to those of the low-flow method at lower concentrations, increased variability, and potential biases were observed at higher concentrations. In summary, the findings suggest that HydraSleeves offer substantial potential for PFAS analysis, particularly at lower concentrations.

4.10.P-Th-170 Assessment of Microplastic Distribution and Composition in a Contaminated Tributary of the Mohawk River

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Since the beginning of mass production and consumption of plastics, the risk of their degradation has become a threat to human and ecosystem health. Microplastics pose a multitude of adverse physiological effects in the body. In waters, they can provide a vessel for other organic pollutants to accumulate on. While highly studied due to their abundance as marine debris in our oceans, it is equally important to focus on microplastics in freshwater streams. This study focuses on a freshwater stream in Upstate New York, the Hans Groot Kill (HGK), with known wastewater inputs due to failing sewage infrastructure. Microplastics in wastewater are of particular interest, as they have often made their way through the human body. To test how microplastic composition and distribution vary spatially in the HGK, sediment samples were taken in duplicate at eight different sites along the stream. Plastics found in sediment samples were sorted and counted before being subjected to chemical analysis. By using a novel pyrolysis method of Gas Chromatography-Mass Spectrometry (GC-MS), the chemical composition of plastics can be identified using a mass spectral library. Determining the most abundant types of plastics present in wastewater allows inferences to be drawn about which types of plastics are most prone to degradation, and which types of plastics are frequently ingested and excreted by humans.

4.10.P-Th-171 Assessment of Chemical Exposure induced by Post-Hurricane Harvey in Pregnant Women in Houston Area

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Hurricane Harvey, the second costliest storm in the United States, caused severe flooding in Houston's metropolitan region in 2017. Such incidents can compound natural disaster impacts by increasing residents' exposure risks to various chemicals. This study aims to investigate Hurricane Harvey's effect on exposure of environmental pollutants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and microplastics (MPs). Whole blood samples were collected from pregnant women (n = 60) who visited Baylor College of Medicine Department of Obstetrics and Gynecology and live in Houston, comprising 30 women reside within a 7-mile radius from confirmed flooded chemical sources such as landfill sites (i.e., exposed group) and other 30 women reside more than 7 miles away from those regions (i.e., non-exposed group). Based on the questionnaires, demographic profiles and socioeconomic status of participants were compiled. Samples were extracted using solid phase extraction followed by gas chromatography-mass spectrometry (GC/MS) for organic compounds, and using Microfiltration (0.7 μm size cut off) followed by pyrolysis-GC/MS. Preliminary results indicated higher levels of PAHs (ΣPAHs: 138–373 pg/μL) found in the exposed group, with Naphthalene and Acenaphthene being the most abundant, while Benzo[a]pyrene never detected. For PCBs (ΣPCBs: 21.6–327 pg/μL), indicator PCBs (tri-CB#28 and tetra-CB#52) were detected only in the exposed group. Microplastics (5 types) concentrations ranged from 294 to 840 pg/μL, with significant PS, PMMA, PET, and PC levels detected. Statistical analyses revealed a mild association between proximity to exposure sources and EDCs concentration levels and weak correlations between socioeconomic parameters (e.g., education and income) and chemical exposure impacts. This study provides insights into the environmental and health impacts of

chemical exposure followed by natural disaster through the case example of Hurricane Harvey, highlighting the need for reinforced interventions to prevent and address chemical exposures in disaster vulnerable populations.

4.10.P-Th-172 Pharmaceuticals and Personal Care Products in Estuarine Systems Across a Gradient of Effluent Contributions to Base Flow: Influences of Grab and Time Weighted Composite Sampling

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Billions of humans live within a few hundred kilometers from coastlines, but aquatic exposure information for pharmaceuticals and personal care products (PPCPs) are limited in coastal regions. This represents a key research need because PPCPs can present water quality risks, and further development and utilization of these systems for aquaculture and coastal fisheries will be required to meet future global food demand. To better understand the influence of PPCPs in effluent discharges on estuarine systems, we used a hydrological model to identify 14 estuarine systems with varying proportions of wastewater effluent contributing to base flows across the Gulf Coast of Texas, USA. We specifically utilized traditional grab sampling methods on study day 1 and study day 2, and also employed time-weighted composite sampling over a 24-hour period, and then determined surface water concentrations of 30 PPCPs by isotope dilution LC-MS/MS. When sufficient data was available, we developed probabilistic environmental exposure distributions to examine influences of sampling approaches on potential exceedances of water quality thresholds. We quantitated 18 target analytes in these coastal systems, including several benzodiazepines and glucocorticoids. Frequently detected compounds were identified at the highest concentrations in Houston, TX or Corpus Christi, TX, the two largest cities included in our sampling effort. Among the sampling methods utilized, we observed higher likelihoods of encountering temazepam, sulfamethoxazole, and trimethoprim, based on 10th centile values, using grab sampling, while for time-weighted autosamplers, alprazolam, caffeine, and diphenhydramine were more likely to be observed. These findings can inform future surface water quality monitoring efforts of PPCPs in coastal systems across gradients of urbanization.

4.10.P-Th-173 Advancing a Predictive Understanding of Per- and Polyfluoroalkyl Substance Bioaccumulation in Pimephales promelas

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Due to persistence, toxicity and ubiquitous contamination of water resources, there has been growing interest in understanding the disposition of per- and polyfluoroalkyl substances (PFAS) in the environment. However, bioaccumulation dynamics remain poorly understood for many substances within this group of chemicals, and kinetic-based information is lacking across environmental gradients. Therefore, the aim of this study was to examine the influence of differing pH (low (6.5) and circumneutral (7.5)) and salinity (low (0.3 g/L) and high (3.0 g/L)) conditions on the uptake and elimination of 19 PFAS, which were selected at low levels not eliciting standardized adverse outcomes, by fathead minnows (*Pimephales promelas*), over 14 and 7 days, respectively. The PFAS mixture consists of short and long chain perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonates (PFSAs), a sulfonamide, and fluorotelomer sulfonates. Water, whole-body tissue, and plasma were collected at multiple timepoints, and samples were analyzed via liquid chromatography tandem mass spectrometry. Kinect uptake and elimination rate constants were estimated for each PFAS with non-linear regressions for both tissue and plasma. Across all experimental conditions, rapid uptake was observed in tissue, reaching pseudo steady-state conditions with 72-96 hours of the exposure period, with uptake rates increasing with increasing chain length. Limited elimination of PFAS was observed, across each experiment, especially for the long chain PFCAs and PFSAs. Kinetic based bioaccumulation factors (BAF_ks) were estimated and increased with increasing chain length for PFCAs and PFSAs. For example, PFCA BAF_ks ranged from $1.78 – 1.67 \times 10^8$, $2.72 – 6.07 \times 10^5$, $1.16 – 2.05 \times 10^8$, and $0.81 – 1.14 \times 10^9$ L/kg for the low pH, low pH:high salinity, circumneutral pH:high salinity, and circumneutral pH experimental conditions, respectively. The PFSAs BAF_ks ranged from $1.14 – 1.24 \times 10^3$, $2.16 – 1.13 \times 10^3$, $0.93 – 9.94 \times 10^2$, and $0.67 – 7.88 \times 10^2$ L/kg for the low pH, low pH:high salinity, circumneutral pH:high salinity, and circumneutral pH experimental conditions, respectively. When comparing BAF_ks to ratio-based BAFs, a significant relationship was observed ($r^2 = 0.768$; $p = 0.01$). These ongoing efforts promise to support the development of a predictive understanding for bioaccumulation of PFAS and ionizable organic contaminants, across environmental gradients, in PFAS contaminated sites.

4.10.P-Th-174 Per- and Polyfluoroalkyl Substances in Estuarine Systems Across a Gradient of Effluent Contributions to Base Flow: Influences of Grab and Time Weighted Composite Sampling

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Texas estuaries provide significant value to humans and the environment by supporting industries, fishing, tourism, recreational activities, biodiversity, shoreline stabilization and runoff filtration. However, like other global estuarine and coastal systems, Texas estuaries face significant anthropogenic pressures. The limited global perspective on PFAS occurrence in estuaries, coupled with their presence as mixtures in complex environmental gradients, complicates the understanding of these contaminants in the estuarine systems. Therefore, we aimed to better understand the influences of effluent discharges of these compounds on estuarine systems. We employed a hydrological model to identify 14 estuaries with a gradient of wastewater effluent contribution to base flows. Additionally, we determined surface water concentrations of 24 PFAS in Texas estuaries via isotope dilution LC-MS/MS. The PFAS examined include both short and long chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonates (PFSAs), sulfonamides, and fluorotelomer sulfonates. Time-weighted composite sampling over a 24-hour period and grab samples were collected from each sampling site to evaluate differences in sampling methods for estuarine systems. Of the 24 PFAS screened, 11 were detected at least once in composite samples from the Texas estuaries, and PFBA, PFHxA and PFHpA, were detected at each site. Additionally, the short chain PFCAs and PFSAs were detected at a higher rate compared to their longer chain counterparts. For example, detected

PFBA and PFBS concentration ranges were 2.35-12.1 and 2.11-35.7 ng/L, respectively, while neither PFTeDA nor PFDS were detected above their respective detection limit. Highest concentrations of detected PFAS were mainly found in Houston, TX and Corpus Christi, TX, the two largest cities in the current sampling effort. These findings can inform future surface water quality monitoring efforts of PFAS in coastal systems across gradients of urbanization.

4.10.P-Th-175 Leveraging Public Databases to Characterize Environmental Pharmaceutical Concentrations Internationally

*Kiersten McMahon and **Phyllis Fuchsman**, Ramboll*

Global pharmaceutical use and environmental monitoring have sparked increasing concerns about active pharmaceutical ingredients (APIs) in the environment. APIs typically enter the environment through discharge from wastewater treatment plants, with the potential for effects on aquatic life and terrestrial organisms depending on API concentrations. Research agencies have created public databases of measured environmental concentrations (MECs) including many APIs. These databases can be used to estimate and analyze current API concentrations, though data availability varies geographically. We reviewed publicly available, international data on MECs of selected representative APIs. MEC values were extracted from four databases (NORMAN EMPODAT, PHARMS-UBA, NAIADES, USGS-WQP) for multiple environmental media (water, soil/biosolids, etc). Detailed examination of each database's structure and content was required to automate data quality screening, distinguish detected versus non-detected concentrations, and maximize comparability across data sources. We quantified and visualized data availability by country, as well as examined trends in global MEC levels. Additionally, we calculated predicted environmental concentrations (PECs) using regulatory methods as well as a modified approach accounting for country-specific distributions of receiving water dilution factors. As expected, PECs estimated without accounting for wastewater treatment overestimated MECs. However, the PEC results highlight the need for monitoring in arid developing countries, where wastewater treatment gaps may coincide with lower dilution capacity. The approach developed here provides an efficient means to advance ecopharmacovigilence toward understanding and addressing ecological risks from APIs in the environment.

4.10.P-Th-176 Advancing the Tissue-Specific Eco-Exposome: Distribution of Legacy Persistent Organic Pollutants and per- and Polyfluoroalkyl Substances in Fish Representing Different Trophic Positions

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Aquatic systems are routinely stressed by diverse chemical classes: however, many studies focus on only one chemical class and do not consider internal disposition of anthropogenic contaminants, which can present risks to aquatic life and human consumers. Herein, we examined muscle, liver, gill, and gonad tissues from three understudied fish species, longnose gar *(Lepisosteus osseus)*, gizzard shad (*Dorosoma cepedianum*), and smallmouth buffalo (*Ictiobus bubalus*), collected in the Brazos River, Texas, USA, below a municipal wastewater effluent discharge. Tissues were analyzed using low-pressure gas chromatography and high-pressure liquid chromatography for 573 anthropogenic compounds including 286 veterinary drugs, 252 persistent organic pollutants, and 35 per- and polyfluoroalkyl substances (PFASs). Of these 573, 28 were detected, including multiple pesticides and their metabolites, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and long-chain PFASs. Both perfluorooctane sulfonic acid (PFOS) and p,p'-DDE were detected in all samples tested with p,p'-DDE being detected at the highest concentrations ranging from 1.8—844 ng g^{-1} wet weight. Tissue specific differences were observed between liver and muscle for all compound classes in gar samples while smallmouth buffalo only had significant differences ($p < 0.05$) for pesticides. Species specific differences were observed in gonad samples for pesticides, PBDEs, and PCBs with longnosed gar having significantly higher ($p < 0.05$) concentrations. Interestingly though, PFAS was found to be significantly higher ($p < 0.05$) in smallmouth buffalo gills compared to the other species. These findings indicate that common, but understudied, fish species possess a complex exposome of diverse anthropogenic contaminants. Future efforts are needed to predict internal disposition of organic contaminants among tissues and species to advance an understanding of potential risks to aquatic life and people who consume fish.

4.10.P-We General: Chemistry and Exposure Assessment

4.10.P-We-115 Immunoaffinity Magnetic Beads for Microcystins Capture and Concentration in Biological Samples

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Cyanotoxins are a category of toxins produced by cyanobacteria, also known as blue-green algae. Under favorable environmental conditions, cyanobacteria can transform into harmful algal blooms (HABs), posing a significant health risk. Microcystins are the most found cyanotoxins and have hepatotoxic effects. The US EPA has issued health advisories for these toxins in drinking and recreational water. However, government agencies, public health labs, and academic researchers are also interested in detecting Microcystins in biological samples, primarily urine and blood serum, to assess exposure. Given the complex composition of urine and blood serum, and the potential for low levels of Microcystins, a sample clean-up/concentration is necessary to prepare the samples for downstream detection using sensitive analytical techniques such as LC-MS and ELISA. Immunoaffinity Magnetic Beads (IMB) have emerged as an effective clean-up method. These beads, synthesized by conjugating antibodies onto magnetic nanoparticles, are mixed with a biological sample. The antibodies then bind to the Microcystins, forming an IMB-target complex. This complex is then separated from the sample matrix using a strong magnet. The IMB clean-up method, with its high affinity for the target of interest, can result in a 10-fold concentration of the Microcystins, making them detectable. This kit is designed for sample pre-treatment and concentration prior to downstream analysis. It includes matrix-matched Microcystins standard curves of known concentrations of 0.025 to 0.4 ppb in synthetic urine and blood serum, used alongside unknown biological samples. Results obtained during the development and validation of the IMB clean-up kit are presented, including a novel LC-MS downstream analytical method that optimizes the detection of Microcystins following the sample clean-up, and evaluation of the method using commercially available ELISA and phosphatase enzyme-inhibition (PP2A) assay kits for Microcystins. This innovative kit can potentially revolutionize the detection and analysis of Microcystins in complex biological samples.

4.10.P-We-116 Benthic Mat Sample Matrix Extraction Kit for Laboratory and Field-Based Analysis of Algal Toxins

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Cyanobacterial benthic mats are thick layers of microbial communities that form on the bottom of bodies of water. These mats are essential in aquatic ecosystems, providing habitat and food for various organisms. However, under certain conditions, cyanobacterial mats can become dominated by toxigenic cyanobacteria, threatening water quality and public health. This study aimed to develop methods for extracting and analyzing the most common toxins produced by cyanobacterial mats (Microcystins, Cylindrospermopsin, Anatoxin-a, and Saxitoxin) using both laboratory-based ELISA and field-based lateral flow immunoassay testing formats. Similarities in the requirements for toxin stability and assay tolerances enabled the processing of Microcystins/Cylindrospermopsin and Anatoxin-a/Saxitoxin in grouped extractions. Commercial automated (bead-based) and manual extraction methods demonstrated comparable efficiencies with a 0.92 correlation coefficient. Interference studies determined the appropriate dilution factors and extraction medium necessary to remove background interference and provide acceptable spike recoveries. Based on the tolerances determined in the interference studies, the detection ranges for the lateral flow tests are 20–200 ppb for Microcystins, 20–400 ppb for Cylindrospermopsin, 40–250 ppb for Anatoxin-a, and 2-40 ppb for Saxitoxin. Considering the sensitivity differences between the lateral flow and ELISA plate methods, there was a strong correlation between the methods for all toxins in all samples. Potentially toxigenic species identified by taxonomic analysis correlate with the detected Anatoxin-a and Saxitoxin in the collected samples. This study shows that sample extraction methods can enable laboratory and field-based extraction and analysis of multiple cyanobacterial toxins and potentially contribute to developing standardized approaches to monitoring, managing, and mitigating benthic cyanobacterial proliferations.

4.10.P-We-118 Towards Fully Integrated Hydrological Fate and Transport Modeling for Plant Protection Products: Incorporating Groundwater, Tile Drainage and Runoff

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Plant protection products (PPPs) are herbicides, insecticides, and fungicides used in agriculture to protect crops from pests and disease. In the context of PPP authorization, water exposure assessments (drinking water and aquatic exposure) use numerical modeling software to simulate relevant chemical fate and transport processes. Common practice for estimating PPP leaching to groundwater, PPP loading onto surface water via tile drainage, or PPP transport via surface runoff, utilizes one-dimensional models, with separate or simplified representation of exposure pathways. Separate analysis of individual exposure pathways can result in disparate assumptions being made that represent relative worst-case scenarios for each pathway, rather than an integrated reasonable worst-case scenario for all pathways. Alternatively, the interplay between PPP retardation and degradation, leaching to groundwater, transport in tile drainage, and surface runoff can be resolved in a single model using a fully integrated groundwater—surface water (GW-SW) flow and transport approach. Accordingly, this study presents verification of functionality added to the HydroGeoSphere (HGS) fully integrated GW-SW model against other

recognized PPP fate and transport models, namely PRZM, HYDRUS, PEARL, PELMO, and MACRO. Features added to HGS for PPP simulation include: automatic irrigation; non-linear adsorption; temperature and soil water content-dependent degradation; and, solute uptake by plant roots. HGS results for leaching of PPP mass to groundwater showed the highest correlation, lowest error, and lowest bias relative to PEARL model results. Simulation of macropore flow to tile drains in HGS produced intermittent tile drain flow in summer that resulted in generally lower peak effluent concentrations compared to the MACRO model. Simulation of runoff in HGS produced higher total runoff compared to the PRZM model, attributed to lower evapotranspiration in HGS. Results demonstrate that fully integrated GW-SW modeling can, within a single simulation, reproduce the water balance and PPP mass balance across multiple transport pathways with greater consistency than multiple models that each simulate individual hydrologic pathways.

4.10.P-We-119 Occurrence of 111 Human Pharmaceuticals in River Water in Japan

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In order to evaluate the risks of human pharmaceuticals in Japanese water environment, we have developed a simultaneous analytical method of the 111 human pharmaceuticals in water samples by liquid chromatography-tandem mass spectrometry (LC/MS/MS). Then, we have conducted environmental monitoring of these human pharmaceuticals in Japanese river water. The sample preparation method, LC/MS/MS analytical conditions were developed and optimized using LCMS8050 (Shimadzu). The validity of analytical method was evaluated based on the results of the recovery test of each chemical added to purified water. Limit of quantitation for most pharmaceuticals were 1 ng/L or less. The recovery rate for most pharmaceuticals were 70% or more. Therefore, sufficient accuracy for environmental monitoring were obtained in the method development in this study. We have collected 700 river water samples in 25 prefectures in Japan during 2018 to 2022. Then, these samples were analyzed by the method developed in this study. Among the 111 pharmaceuticals investigated, 91 pharmaceuticals were detected with a maximum concentration of 15 µ/L (azithromycin). Deet (insect repellent), salicylic acid (analgesic), and crotamiton (antipruritic) were detected in almost all samples. Seasonal variations were observed in some rivers. Many pharmaceuticals showing high concentrations in autumn and winter and low concentrations in spring and summer. In order to understand the occurrence of pharmaceuticals in the water environment, it is necessary to monitor seasonally over a long period of time, taking into account seasonal variation. The results obtained from this study can be applied to environmental risk assessments of human pharmaceuticals.

4.10.P-We-120 Spatial and Temporal Assessment of Chemical and Bacterial Contaminants in Coastal Waters of Charleston, SC, USA

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Passive samplers can be a useful tool to determine the presence of organic contaminants in aquatic environments. While passive sampler derived data is not used in a regulatory manner at this time; understanding the relationship between PSD data and other thresholds linked to policy or management decisions is critical to increase PSD utility and application. In this study, we examine spatial and temporal patterns of organic contaminants (polycyclic aromatic hydrocarbons (PAH's), pyrethroids, and fipronils) at 20 estuarine sites located in and around

Charleston, SC. These sites are paired with sites currently monitored by Charleston Waterkeeper (a non-profit community interest group) for coliform bacteria and other water quality indicators. This study aims to characterize the relationship between chemical contaminants and bacterial levels among sites, between seasons, and year to year comparisons. Preliminary results suggest that silicone bands are effective samplers for multiple classes of chemical contaminants. Analysis of chemical and biological data revealed a seasonal correlation between PAH50 levels and bacterial levels, with both contaminants more prevalent in the warmer months. Additional spatial and temporal analysis will be employed to relate contaminant data to land-use activities and identify potential strategies for environmental management.

4.10.P-We-121 Exploring the Impact of Chlorine Oxidation and Temperature Dependent Reaction Rates on the Atmospheric Lifetimes and Concentrations of Volatile Methyl Siloxanes in CESM

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Volatile Methyl Siloxanes (VMS) are a class of organosilicon chemicals widely used in personal care products (PCP). Due to their high volatility and inclusion in PCP, large quantities of VMS are emitted into the atmosphere particularly near densely populated areas. These emissions have attracted considerable attention due to the relatively long lifetimes of VMS, their ability to form secondary organic aerosols, and their potential to serve as atmospheric tracer compounds. As a result, several previous studies have used atmospheric chemical-transport models to predict the concentrations of VMS and their oxidized by-products. However, these models have had limitations such as simplified chemical mechanisms and narrow geographical domains. To address these limitations, we herein present the development of and outcomes from a modification of the Community Earth System Model (CESM2.2) which incorporates 3 VMS compounds (D4, D5, and D6) and their first-generation oxidation products. In particular, we discuss the calculation of global, country-specific, per capita emissions rates for these compounds, the creation of global emissions files using these per capita rates, and the modification of the chemical mechanism to include temperature dependence and chlorine oxidation which have not been captured in previous models. We present results from model runs including the impact of chlorine and temperature dependence on VMS concentrations and lifetimes both globally and in specific areas where these factors have outsized effects. Finally, we present comparisons between modeled concentrations and measurements of VMS from previously conducted field measurements in order to assess the accuracy of the model and the calculated per capita emissions rates.

4.10.P-We-122 A Novel Cyclosiloxane Extraction and Analysis Method in Human Serum

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Siloxanes are a group of substances characterized by a chain of alternating silicon (Si) and oxygen (O) atoms. In cyclosiloxanes, the Si and O atoms are bonded and form a ring. The cyclosiloxanes most often found in environmental matrices are: hexamethylcyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6). These are used in the manufacture of silicones, in combination or alone in sealants, plastics, cosmetics, medical devices, hygiene products, food contact materials, etc. Siloxanes are categorized by the EPA as persistent, bioaccumulative, and toxic (PBT) compounds that are linked to reproductive and hepatic system disorders. According to studies conducted by NIH in 2016 and 2020, cyclosiloxanes can overcome cellular barriers causing irreversible damage to the epidermis, absorption into the body, and migration to other tissue. Additionally, D4 has been classified as an endocrine disruptor by EU. Although studies have indicated that cyclosiloxanes are present in air, water, foodstuffs, breast milk, adipose tissue, skin samples, urine, and plasma samples; few studies have produced an effective method to analyze cyclosiloxanes in human blood serum. Therefore, we are building a novel and rapid method to extract cyclosiloxanes in human blood serum by liquid-liquid extraction and analyze them with Gas Chromatography-Triple Quadrupole Mass Spectrometry coupled with an isotope dilution method. This study is a collaboration with the National Institute of Standards and Technology (NIST) to validate the method by measuring the cyclosiloxane levels in NIST human serum standard reference materials (SRMs) for the first time. This method has shown minimal laboratory background levels, and excellent recoveries and precision for both the carbon labelled surrogate recovery rates (80-120%) and the native D3, D4, D5 and D6 in laboratory control samples (80-110%). Our preliminary data showed that D3 through D6 were found in SRM1958 at the level of tens to hundreds of ppb, while only D4, D5, and D6 were detected in SRM1957. In pilot testing using archived samples collected in 2010s (=44), we found that D4, D5, and D6 were detected in 98, 41, and 7 percent of samples tested, respectively. Once this method is finalized, we will use it to quantify the levels of cyclosiloxanes in NIST SRM materials, determine the extent of cyclosiloxane exposure in California, and assess temporal trends in exposure.

4.10.P-We-123 Enhanced Evaluation of Long-Range Transport of Cyclic Volatile Methylsiloxanes

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The accurate evaluation of a chemical's long-range transport potential (LRTP) is necessary when assessing its potential impacts on human health and the environment. This is particularly pertinent in remote areas where direct chemical emissions resulting from anthropogenic activities are not typically expected. Recently, an advanced multimedia mathematical model known as the Emissions Fractions Approach (EFA) has been introduced. This model the existing OECD Pov and LRTP Screening Tool (the Tool), providing a more precise evaluation of a chemical's LRTP. This approach was applied across a range of chemical categories, including decamethylcyclopentasiloxane (D5). Despite the high characteristic travel distance (CTD) predicted for D5 by the Tool, the EFA results suggested that D5 would not accumulate in remote surface media to a concentration that could cause harm to human and the environment? . We have further optimized the EFA model by integrating more accurate partition coefficients and degradation half-lives in ocean water, as well as considering realistic emission scenarios for cyclic volatile methylsiloxanes (cVMS), which include octamethylcyclotetrasiloxane (D4), dodecamethylcyclohexasiloxane (D6) as well as D5. Our enhanced assessment revealed that the fractions of remote transfer (ϕ2) and accumulation (ϕ3) were significantly lower—more than two orders of magnitude—than the proposed threshold values. combined with chemical specific parameters for cVMS, leads to the conclusion that these substances would not accumulate or have adverse effects in remote environments.

4.10.P-We-124 Monitoring Emerging Contaminants in Soil and Household Dust Samples in the Miami-Dade, Florida Region

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Chemical environmental exposure can occur outdoors or indoors in several ways, such as through soil and dust ingestion, inhalation or dermal contact. The objective of this study was to determine and evaluate the distribution of organic contaminants in soil and household dust samples in Miami-Dade using multivariate analysis. We use a non-targeted analysis technique (NTA) that employs liquid chromatography and high-resolution mass spectrometry (LC-HRMS) for the screening and identification of emerging contaminants in environmental and biological matrices. NTA allows us to analyze samples extensively without prior knowledge of their chemical composition, facilitating the identification of a wide range of previously unknown and undetectable chemical compounds by traditional targeted analysis methods. In this study, we collected 197 soil and 208 dust samples from several areas in Miami - Dade - FL between May 2022 and February 2024. Soil samples were dried at 37 °C for 24 h, sieved through a 100-mesh sieve and added to stainless steel chambers with florisil, graphited carbon black (GCB), and internal standard. The dust samples followed a similar procedure but did not go through the drying process. The extraction was performed by ASE (Accelerated Solvent Extraction) at 1,500 psi, heating for 5 minutes at 1,000°C, and an extraction phase of 30 mL of methanol and acetonitrile (50/50 v/v). The extracts were collected, evaporated under nitrogen, and resuspended in 1 mL of methanol. Analyzes were performed using LC-MS/HRMS/Orbitrap in positive and negative scan (100.0 to 800.0 m/z) and full scan and dd-MS2 mode. Further data processing was performed with Compound Discoverer v3.3 software, which compares components between samples, establishes molecular formulas, identifies potential structures, and search compounds using available online databases and libraries. All anthropogenic pollutants were prioritized using filters. Missing values are replaced by 1/5 of the smallest peak area value to avoid statistical errors. Data were normalized by mean, Pareto scale, and logarithmic transformation. Principal component analysis (PCA) was plotted to visualize sample clustering and trends, whereas Partial least squares discriminant analysis separated the groups and identified distinct characteristics. Our findings will provide insights into the occurrence, spatial distribution, and risks of environmental pollutants, supporting risk assessments and safety guidelines

4.10.P-We-125 Evaluating Heavy Metals Exposure in the Stool and Indoor Dust Samples of Children

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Evaluating Heavy Metals Exposure in the Stool and Indoor Dust Samples of Children

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Environmental pollution is known to cause harm to humans, in particular, children are the most vulnerable population due to their susceptibility to harm, adverse effects on their developmental stages and lifelong consequences. Accurate estimation of soil and dust exposure among children is critical to understand the risks. Exposure to heavy metals has been associated with various chronic illnesses development such as malignancies and neurotoxic effects, and metals like Lead (Pb), Arsenic (As), Chromium (Cr) are known to be harmful persistent chemicals. To estimate the soil and dust exposures among 6 months to 6 years old children, this study enlisted 140 children in Reno-Sparks area of Nevada, United States. Both environmental (outdoor soil and indoor dust) and biological (urine and stool) samples were collected and analyzed using inductively coupled plasma mass-spectrometry (ICPMS), with the aim of quantifying inorganic contaminant concentrations. Urinary metal concentrations of selected toxic metal ions are 0.130 µg/L for Cadmium (Cd), 0.226 µg/L for Pb, 0.021 µg/L for Cr, and 7.21 µg/L for As. Fecal concentrations of those metal ions were 4.56 ng/g for Cd, 9.04 ng/g for Pb, 18.3 ng/g for Cr, and 19.2 µg/g for As. Average Cd, Pb, Cr, and As concentrations found in indoor dust samples were 0.027 ng/g, 0.018 ng/g, 0.005 ng/g, and 0.002 ng/g, respectively, the concentrations in outdoor soil samples were 0.024 ng/g, 0.039 ng/g, 0.000 ng/g, and 0.003 ng/g, respectively. Pearson correlations were conducted to investigate potential inequalities in the spatial distribution and concentrations. There are statistically significant, positive relationships between Cd concentrations ($r = 0.46$, $p < 0.05$), and V concentrations ($r = 0.57$, $p < 0.01$) measured in stool and indoor dust samples. The correlations suggest that children's exposures to indoor dust might be the primary source of toxic metal exposures. Further researches are needed to elucidate the factors influencing these heavy metals bioavailability, absorption, and excretion in the children population, as well as to identify their potential sources of exposure beyond household dust.

4.10.P-We-126 Development and Validation of Analytical Method for Determination of Nitrogen/Protein Content of Pumpkin Seed Extract by Dumas Nitrogen Analyser

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The analytical method was developed and validated for determination of nitrogen content by Dumas nitrogen analyzer in pumpkin seed extract. The method development includes mainly different oxygen rate and dosing quantity, which ensure proper combustion of sample. This is essential to accurate determination of nitrogen content by dumas method. Nowadays, Dumas combustion method is becoming increasingly popular due to its fast analysis, non-use of toxic chemicals and accurate and low-level detection level to replace the Kjeldahl analysis (wet chemical method). The nitrogen content in sample is determined in order to subsequently determine the protein content by using the protein factor. The pumpkin seed extract samples were prepared in three different concentrations in water and analyzed using Rice flour as analytical reference standard. The method was validated following international guideline

SANTE/2020/12830, Rev.1 (February 24, 2021) to support pre-clinical toxicology study for the dose verification. The calibration curve with correlation coefficient >0.99, recovery range 90-110% with relative standard deviation (RSD) 0.9–1.1%. Thus, the method is categorized quick, sensitive, precise, and accurate for the analysis of nitrogen/protein content of pumpkin seed extract in water.

4.10.P-We-128 Assessment of Trace Metal Exposure from Ingestion of Play Surface Materials

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Playground fill material is widely used for safety purposes, but presents a pathway for the exposure of children to toxic trace metals through dermal contact, inhalation, and ingestion. Fill material may vary in metal content, depending upon source, composition, and age. We assessed ingestion as a route of trace metal exposure to local children by collaborating with La Crosse, WI community volunteer "citizen scientists", who aided in collection of fill materials near their homes and schools. Samples were collected using clean techniques from 32 local playgrounds and parks, representing wood chip, rubber chip, soil, and sand fills. The majority of the samples (27) were either wood or rubber chip, and their results are presented here. To simulate ingestion, 2-gram samples were added to solutions of de-ionized water (control) and pH 1.5 hydrochloric acid (simulated gastric solution) and extracted at body temperature for two hours under gentle agitation. Extracts were then filtered and analyzed for Pb, Mn, Zn, Cu, As, Cr, and Cd by ICP spectroscopy. Results revealed that Pb in rubber chip extracts at pH 1.5 averaged 66.4+/-24 ppb (mean+/-sd), while wood chips were only 5.6+/-4.2 ppb, a nearly 12-fold enrichment. For Mn in pH 1.5 extract solutions, the opposite trend was observed; rubber chip extracts averaged 185+/-121 ppb, while wood chips were 1754+/-1037 ppb. While these results do not accurately represent actual ingestion by children, it provides a framework for assessment of the relative risk of the fill materials. The enrichment of Pb in rubber chip extracts is of particular importance, as there is no safe lower exposure limit. Our results suggest that the City of La Crosse may want to consider replacement of rubber chip surfaces with natural alternatives that would lower the relative exposure of Pb to children. Future work includes the sampling of additional playground sites and fill material types for comparative purposes.

4.10.P-We-129 Abiotic Factors Influence on the Stability of Antimycin-A in Water

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Antimycin-A (ANT-A) has been used to remove nuisance aquatic species to restore native fish populations in high mountain water streams and scaled fish from catfish aquaculture ponds. The liquid ANT-A formulation historically used in fish restorations contains ANT-A (active ingredient) and multiple inert ingredients. Since the initial liquid ANT-A formulation use, the abiotic factors influencing chemical degradation in water have not been analytically verified due to poor analytical instrument capabilities. Therefore, ANT-A was neutralized following stream application to minimize downstream non-target affects beyond the restoration site. Previous ANT-A stability and degradation experiments have shown that water pH and sunlight are associated with degradation. Alkalinity, water hardness, and temperature were determined to have little to no effect on ANT-A degradation. Initial experiments relied on fish and yeast toxicity bioassays to indicate the presence (e.g., degradation) of ANT-A and chemical bioavailability. Studies reporting a loss of toxicity assumed ANT-A degradation, but this remains uncertain due to the lack of chemical analytical verification in exposure water. Currently, analytical instrumental methods have improved. Bernardy et al published an ANT-A water method using solid phase extraction (SPE) alongside HPLC coupled with mass spectrometry (MS) for the quantification of ANT-A at concentrations as low as 8 ng/L, which is sufficient to analytically verify ANT-A water concentrations at levels used during fish restorations. The following presentation will give an overview of ANT-A water degradation across multiple abiotic factors to inform future fish restorations.

4.10.P-We-131 Derivation of Dilution Factors for 1st Tier Exposure Assessment in Asian Region Using a Chemical Exposure Analysis Model with High Spatio-Temporal Resolution

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The environmental impact of down-the-drain chemicals in consumer products has received increasing attention in recent years. For the comprehensive environmental risk assessment of chemicals contained in consumer products, we derived dilution factors (DF) to be used in the 1st-tier exposure assessment. There are two exposure scenarios for chemicals discharged from households to rivers: wastewater treatment plants (WWTP) discharge and direct discharge. However, existing reports on DF derivation do not distinguish these two scenarios. Since untreated domestic wastewater may affect the river ecosystems in the countries where the sewage penetration is not 100%, we derived DFs separately for WWTP discharge and direct discharge, by conducting the analysis at a resolution of 1 x 1 km. The river flow analysis was performed for Southeast Asian countries using Global-SHANEL, based on AIST-SHANEL (national institute of Advanced Industrial Science and Technology- Standardized Hydrology-based Assessment tool for chemical Exposure Load), which has been used to derive DFs in Japan's Chemical Substances Control Law. Global-SHANEL can conduct unsteady analyses based on arbitrary meteorological conditions, which is a considerable advantage in evaluating chemical risks in low precipitation scenario. As a result, we obtained DFs in 101 rivers in 9 countries. Parameters such as precipitation, population density, and sewage penetration can affect DFs, but national averages or representative values of these parameters did not correlate well with DFs, indicating that analysis using a high-resolution model and considering the local river flows and discharges would be useful for deriving DFs. Using the obtained DFs, the exposure assessment of linear alkylbenzene sulfonate (LAS) was conducted considering two different scenarios, direct discharge and WWTP discharge. Although the DFs for the direct discharge scenario were larger than those for WWTP discharge, the predicted environmental concentrations of LAS in the direct discharge scenario were higher in some countries. It was shown that in some cases, risk assessment based only on WWTP discharge was not sufficient. This study provides the basic information for the initial risk assessment of chemicals reflecting the local high concentration area. Comprehensive exposure assessment can be achieved by combining the 1st tier assessment using DFs, and the 2nd tier assessment using computational models, such as Global-SHANEL.

4.10.P-We-132 Development of Adverse Outcome Pathways (AOPs) for Chemical-Induced Autoimmune Response

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Over the years, the incidence of human autoimmune diseases has increased. Epidemiological data and animal studies have enhanced our understanding of the pathophysiology behind various autoimmune diseases. A hallmark of autoimmunity is the production of biomarkers known as autoantibodies and there are methods to monitor for their presence in patient serum (e.g., enzyme-linked immunoassay-based methods). To date, it is unclear how environmental factors contribute to the production of these biomarkers or are linked to the increasing incidence of autoimmune diseases. We aim to shed light on how chemical exposure can perturb normal immune system function. To understand the effects of chemical exposure on antigen presenting cells (APCs) we conducted a literature review on the biology of APCs, the expressed surface molecules on APCs and other key events of the signaling pathways. The findings suggest that exposure to environmental contaminants may activate pathways associated with the autoimmune response. For example, evidence from occupational human exposure studies, animal models, and *in vitro* experiments with APCs suggest that early molecular events of silica toxicity may contribute to the development of several autoimmune diseases such as systemic sclerosis, rheumatoid arthritis and systemic lupus erythematosus. Currently, there are no test guidelines for new approach methodologies (NAMs) that assess chemical-induced autoimmunity. Therefore, we believe investigating the early molecular events and identifying key initiating events can inform future NAMs focused on autoimmunity. By developing adverse outcome pathways (AOPs) for autoimmune responses to chemical exposure, it may be possible to understand how APCs process abnormal antigens and trigger the production of autoantibodies that target specific organ systems. Our research will examine different chemical mode of actions (MOAs) that result in APC dysfunction and the eventual production of autoantibodies to support the development of AOPs.

4.10.P-We-134 Airborne Gunshot Residue Outdoor Exposure Assessment

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Military and law enforcement officers are routinely exposed to a complex mixture of gunshot residue (GSR) from discharging firearms during training scenarios. GSR is a complex mixture released into the air as a result of incomplete combustion and micro fractionation from the inside of the barrel. Both inorganic (e.g. Pb, Ba, Sb, Cu) and organic (e.g., Nitrotoluenes, Diphenylamine, Ethyl centralite) components are released in multiple size fractions including the established health relevant size fraction particulate matter <2.5 microns ($PM_{2.5}$). $PM_{2.5}$ from various sources have been associated with cardiorespiratory diseases, however few studies have investigated the chemical composition of GSR $PM_{2.5}$ in an outdoor scenario. This project explores daily differences between pistol (n = 6), rifle (n = 6), and combined (n = 3) firearm training scenarios for the University of Mississippi Police Department's outdoor trainings. A $PM_{2.5}$ personal sampler was worn by officers at each training to collect released GSR (n = 3-5 samples per training). Real-time $PM_{2.5}$ samplers were used for three of the sampling days to explore exposure differences between the instructor and shooter. Meteorological parameters

(e.g., temperature, humidity, wind direction) were collected for each sampling scenario to assess impact on chemical concentrations. Filter samples underwent non-destructive analysis for black carbon (sootscan transmissometer) and inorganics (XRF), and were then extracted via standard methods for organic analysis (GC-MS). Air concentrations of black carbon and inorganics were blank corrected to field blanks. Black carbon concentrations were significantly different between sampling days (two-way ANOVA, $p \leq 0.05$). Pistol trainings had a significantly higher concentration of Pb compared to rifle trainings, while Cu was significantly higher in the rifle trainings (Kruskal-Wallis, $p \leq 0.05$). The real-time $PM_{2.5}$ measurements found significantly higher concentration of $PM_{2.5}$ for the shooters when compared to the instructor across all firearm scenarios (two-way ANOVA, $p \leq 0.05$). Organic analysis is currently underway for organic GSR compounds (e.g. Diphenylamine, Ethyl centralite). The impact of meteorological metrics will be investigated using a general linear model upon completion of organic analysis. The completion of this project will assess the outdoor chemical exposure within the $PM_{2.5}$ size fraction and determine effects of meteorological factors on concentrations.

4.10.P-We-136 Biota Ingestion Rate Updates to EPA's Preliminary Remediation Goal (PRG) and Dose Compliance Concentration (DCC) Calculators for Use in Risk Assessment at Radioactively Contaminated Superfund Sites

***Karessa Manning**, Oak Ridge National Laboratory, United States*

The Preliminary Remediation Goal (PRG) and Dose Compliance Concentration (DCC) online tools are used in risk assessment characterization for developing risk and dose based cleanup levels for radioactively contaminated air, biota, soil, and water. These tools utilize ingestion rates derived from the Exposure Factors Handbook (EFH) specifically for home-produced biota consumed by subsistence residents and farmers. Traditionally, default ingestion rates are calculated based on the mean fresh weight (FW) with an alternative option for mean cooked/prepared weight (CPW). The National Oil and Hazardous Substances Pollution Contingency Plan (NCP) characterizes Reasonable Maximum Exposure (RME) using the 95th and 50th percentiles. These statistical measures, including the mean, are presented for both FW and CPW. Recent proposals suggest setting default ingestion rates at the 95th percentile to better estimate risk when dealing with biota that significantly contributes to the diet of a particular receptor, such as a subsistence farmer or resident. For broader risk assessments or when developing risk or dose-based concentrations that reflect the dietary patterns of the general local population consuming typical homegrown foods, the 50th percentiles or the mean values are recommended. The proposed biota ingestion rates now take into account the typical preparation methods for different biota (e.g., beef vs. berries), choosing between FW and CPW as appropriate. Additionally, the PRG and DCC calculators are being updated to include enhanced soil-to-plant transfer factors for tropical and newly added arid climate zones, aligning with the latest findings from the International Atomic Energy Agency (IAEA) TECDOC report IAEA-TECDOC-1979, "Soil-Plant Transfer of Radionuclides in Non-temperate Environments." These updates ensure that the tools remain at the forefront of safety and environmental protection standards.

4.10.P-We-137 Integrating Tribal Biota into EPA's Preliminary Remediation Goal (PRG) and Dose Compliance Concentration (DCC) Online Tools for use in Risk Assessment at Radioactively Contaminated Superfund Sites

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The Preliminary Remediation Goal (PRG) and Dose Compliance Concentration (DCC) online tools are used in risk assessment characterization for developing risk and dose based cleanup levels for radioactively contaminated air, biota, soil, and water. Current biota models in these tools focus on evaluating the risk and dose to subsistence farmers and residents consuming radioactively contaminated biota, covering 25 types of produce and 14 farm animal/animal products. Acknowledging the importance of addressing exposure pathways to tribal communities from ingestion of biota, the EPA has launched data collection efforts. This initiative aims to compile information on additional animal products and produce commonly consumed by subsistence hunter/gatherer/forager tribes. Currently, the EPA is evaluating 30 new animal products and 70 new produce items for potential integration into the PRG and DCC calculators. This poster aims to spotlight available data, pinpoint existing data gaps, and delineate the challenges linked to incorporating these new biota. Moreover, it emphasizes the significance of considering other land use routes in risk characterization, such as exposure from use in tribal ceremonies.

4.10.P-We-138 Ecotoxicity of Water-Soluble Synthetic Film (III)

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Water soluble synthetic films and polymers (WSSPs) are used in industrial, food, household, and biomedical products, but their presence in the aquatic environment and their impact on aquatic organisms is still under investigation. PVOH is a typical WSSP used in liquid detergent packaging. To date, we have investigated two water-soluble films (Hi-Selon™, manufactured by Mitsubishi Chemical Corporation) based on polyvinyl alcohol (PVOH) and additives using Daphnia magna and Medaka. Acute and chronic toxicity as well as combined effects with hydrophobic compounds and metals have been reported. No acute or chronic toxicity was found when exposed to PVOH alone in the Daphnia test. However, in the case of combined exposure to PVOH and a metal or hydrophobic substance, PVOH was suggested to mitigate the effects of each. The water-soluble polymer PVOH is a synthetic polymer produced by hydrolysis of polyvinyl acetate and is widely used in the laundry cycle as detergent pods. The biodegradability of PVOH has been studied by many researchers, but its ecotoxicity in the ocean remains unclear and its elucidation is still desired. However, to date, the only globally harmonized ecotoxicity test method for biodegradable plastics has been published in ISO 5430*) with few application examples, and its validation as an assessment method has not been fully verified. Therefore, we report the ecotoxicity of Hi-Selon™ in luminescent bacterial tests and in acute toxicity tests on the marine crustacean Artemia based on ISO 5430.

4.10.P-We-139 Per- and Polyfluoroalkyl Substances (PFAS) in Urban Watersheds Across California

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The Stream Pollution Trends Monitoring program (SPoT) is a statewide program initiated in 2008 under the Surface Water Ambient Monitoring Program of the California State Water Resources Control Board. The program has analyzed several organic contaminant classes in sediments collected annually from up to 100 sites across nine Regional Water Quality Control Boards in California. Data from SPoT are used to identify trends in sediment toxicity and contaminants and their relationships with land use. Per-and polyfluoroalkyl substances (PFAS) monitoring was added to the analyte list in 2023 to evaluate spatial and temporal trends at sites influenced by urban land use. PFAS are a group of thousands of synthetic organofluorine compounds which are highly persistent and bioaccumulative and linked to several toxic effects in humans and wildlife. These contaminants have widespread industrial, commercial, and household usage and are ubiquitous in the environment. Sediments were analyzed from 40 sites representing seven out of nine regions in California. A suite of 40 PFAS including perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs) and replacement PFAS were measured in sediment samples. Total PFAS were calculated by summing reported concentrations of all 40 PFAS and assigning zeroes for measurements that were below the limit of quantitation. Percent contributions to total PFAS burdens were calculated for individual compounds. The highest detection frequency across samples was observed for perfluorooctanesulfonic acid (PFOS, 88%) followed by perfluorododecanoic acid (PFDoDA, 70%) and perfluorodecanoic acid (PFDA, 55%). Sum PFAS ranged from 0-14 ng/g dry sediment weight. Samples with higher sums were often found to be dominated by PFOS. Notably, 6:2 fluorotelomer sulfonate, a replacement PFAS, had high percent contributions, exceeding PFOS concentrations at five of the 10 sites where it was detected. Long-chain PFCAs (≥ C8) were reported more frequently in urban sediments than short-chain (< C8). These data provide valuable information on sediment contaminant profiles across California watersheds indicating presence of complex PFAS mixtures that need to be further monitored. Ongoing work will identify trends at regional and statewide levels and assist in prioritizing monitoring strategies.

4.10.P-We-140 Non-Targeted and Total Fluorine Assessment of Per-and Polyfluoroalkyl Substances (PFAS) in Previously Detected Florida Surface Water Hotspots

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Per-and polyfluoroalkyl substances (PFAS) are a group of persistent chemicals that have been associated with an array of adverse human and environmental health effects. In addition to a growing list of health concerns, these chemicals are also ubiquitously present, have an innate ability to be highly mobile once released into the environment, and have an unmatched resistance to degradation once formed. As such, PFAS have been detected in a wide variety of matrices, including soil, biota, and drinking water; however, perhaps one of the matrices most closely related to the release of PFAS in Florida is surface water. Thus, the primary objective of this study was to focus on the detection and quantification of PFAS in previously detected surface water hotspots within the state of Florida (United States) using a non-targeted approach. In this study, surface water samples were collected (via trained citizen scientists), with a large variety of sample site characteristics from each of the hotspots found in Florida. The surface water samples were extracted using solid phase extraction and a non-targeted analysis for PFAS was performed. This was achieved by using ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS, with a high-resolution mass spectrometer). A total fluorine analysis using combustion ion chromatography was paired with this study to compare the previously published targeted work, along with the newly non-targeted workflow. This study provided more detailed information to the overall PFAS landscape of highly impacted areas. Furthermore, this data allowed for the comparison of diverse surface water PFAS profiles geographically within the sampling region, the state of Florida, and to other US states. We hope that this comprehensive analysis will provide more attention toward solutions, overall community awareness, and ideally, statewide regulations aimed at mitigating these harmful chemicals in our surface water.

4.10.P-We-141 Development and Application of Silicone Band Passive Sampler Rate Constants for Per- and Polyfluoroalkyl Substances (PFAS) for Use in Coastal Ecosystems

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Per- and polyfluoroalkyl substances, or PFAS, are a group of anthropogenic compounds containing carbon-fluorine chains. PFAS are extremely stable compounds used in a wide variety of consumer and industrial applications and consequently have been detected virtually everywhere. To date, measuring PFAS in estuarine and coastal ecosystems has been fairly limited. Sampling in these ecosystems can be challenging due to factors such as tidal flux and matrix interferences at increased salinities. However, silicone band passive sampler devices can serve as an inexpensive and easy tool for environmental monitoring. NOAA's National Centers for Coastal Ocean Science in Charleston, SC, USA, has been working with silicone band passive samplers since 2020 to detect and quantify polycyclic aromatic hydrocarbons and current-use pesticides in marine and estuarine environments, and more recently has been applying this technology to detect PFAS in these same ecosystems. In the first phase of our research, laboratory-based exposures will be conducted over a 21-d period with different salinities (2, 10, 20, and 30 ppt) to establish uptake stage (linear, curvilinear or equilibrium) for seven PFAS (perfluorohexane sulfonic acid (PFHxS), perfluorohexanoic acid (PFHxA), perfluorobutanoic acid (PFBA), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and two fluorotelomer sulfonic acids (6:2 FTS, 8:2 FTS)). After which a water sampling rate constant (R_s) or sampler partitioning coefficient (K_{pw}) will be determined. Next, silicone band passive samplers will be deployed at 20 sites around Charleston Harbor, on a seasonal basis (winter, spring, and summer), for 21 days. Silicone bands will be extracted for PFAS and water-based concentrations will be determined for each site using the laboratory derived R_s or K_{pw} values for each analyte. Seasonal trends will be explored and discussed for all sites. Preliminary laboratory tests have indicated that both PFOS and PFOA bind to silicone bands that were deployed in estuarine water for a period of 21 days. These data will be used to increase awareness of PFAS in the coastal zone and allow for a more simplified technique for environmental sampling of PFAS.

4.10.P-We-142 Mass Balance Estimates of Perfluorooctane Sulfonic Acid (PFOS) and Perfluorooctanoic Acid (PFOA) in a Simulated Saltmarsh Ecosystem

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Per- and polyfluoroalkyl substances (PFAS) are a group of compounds synthesized for industrial and consumer products. These chemicals are widely known to be persistent in the environment and can cause adverse effects on human health and ecosystems as they bioaccumulate in organisms. Preliminary findings suggest different behaviors of PFAS based on carbon chain length and functional group substitutions, thus ushering a need to fill knowledge gaps regarding their fate and behaviors in estuarine environments. A mesocosm based exposure is one approach that allows for the estimation of mass balance calculations. At NOAA's National Centers for Coastal Ocean Science, a 28 day mesocosm test was performed to study the fate and effects of two PFAS (perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS)) in simulated estuarine environments. Mesocosm systems (n = 24) were located in a greenhouse in Charleston, SC, USA where they were exposed to natural light and temperature conditions with a semi-diurnal tide. Systems contained seawater, sediment trays, marsh grass (*Spartina alterniflora*) and estuarine organisms including *Cyprinodon variegatus* (sheepshead minnow), *Tritia obsoleta* (mud snail), *Palaemonetes pugio* (grass shrimp), *Mercenaria mercenaria* (hard clam), and *Leptocheirus plumulosus* (amphipod). There were six treatments with four replicates per treatment: PFOS low 0.55 mg/L, PFOS high 5.5 mg/L, PFOA low 7 mg/L PFOA high 70 mg/L, PFOS/PFOA mixture 0.55 and 7 mg/L and control. Environmental compartments were sampled for chemical analysis throughout the experiment to track movement of PFOS and PFOA which allowed for the calculation of a mass balance. Preliminary results indicate that both PFOS and PFOA partitioned rapidly from the water to the sediment, however, PFOS dosed in the water moved more readily into the sediments and biota when compared to PFOA (bioconcentration factor in fish at low dose, PFOS:228, PFOA:3). Differences in chemical uptake between PFAS and species were observed, e.g., PFOS body burden was greatest in fish, whereas amphipods contained the highest levels of PFOA. Providing a quantitative measure of how these pollutants move through environmental compartments, we can better understand PFAS accumulation in estuarine environments and employ effective management strategies to mitigate the impacts of these chemicals.

4.10.P-We-143 Identifying Novel Alternatives to PFAS-Containing Aqueous Film-Forming Foams: Summary of Multi-Taxa Toxicity Tests with Birds, Reptiles, Fish, and Aquatic Invertebrates

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Amid global concern regarding the persistence, bioaccumulation, and human and ecological impacts of per- and polyfluoroalkyl substances (PFAS), there is an urgent need to develop and validate PFAS-free products across their diverse usage. Historically, aqueous film-forming foams (AFFF) used in firefighting contained PFAS compounds, with recent legislation mandating the phase out of legacy AFFF and development of novel fluorine-free foams (F3s). To

support the selection and implementation of appropriate F3s that minimize impacts on the environment, the present study aimed to determine the comparative ecotoxicity of a suite of seven F3s and one legacy PFAS-containing AFFF. Acute and chronic toxicity tests were conducted across multiple taxa, including birds (bobwhite quail, *Colinus virginianus*), reptiles (brown anole, *Anolis sagrei*), aquatic invertebrates (midge larvae, *Chironomus dilutus*), and fish (fathead minnows, *Pimephales promelas*), with effects on survival, growth, reproduction, and development considered. No and Lowest Observed Effect Concentrations (NOECs and LOECs), effective and lethal concentration (EC and LC) values were calculated and compared to EPA Alternatives Assessment Criteria where appropriate. For aquatic toxicity tests, the PFAS-containing AFFF (Buckeye) typically exhibited lower toxicity compared to the majority of tested F3s, with two formulations, Avio Green and Angus JetFoam, exhibiting the greatest toxicity. For the avian studies, acute dietary tests indicated toxicity at or above the limit of ~1500 mg/kg for all tested F3s and the legacy AFFF. For chronic tests on bobwhite quail, results were more complex. Few significant impacts were observed on bobwhite quail reproduction (i.e., number of eggs per hen and hatching success) after 60 d of exposure; however, effects on chick growth, lipid content, and biometrics were observed for several foams after parental exposure. Acute and chronic exposure of reptiles indicated that *A. sagrei* were not sensitive to either F3s or a legacy AFFF, with analysis of sublethal endpoints ongoing. This presentation will provide a comprehensive multi-taxon ranking of the toxicity of novel F3s that will ultimately facilitate the selection of appropriate PFAS-free replacement firefighting products.

4.10.P-We-144 Are Brood X Periodical Cicadas (*Magicicada* spp.) A Source of PFAS to the Terrestrial Environment?

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Periodical cicadas (*Magicicada* spp.) evolved synchronous emergence and predator satiation strategies that maximize their utilization as a food source during the brief time they are present in the above-ground environment. Given the large mass of cicada tissues consumed by some predators, cicadas could provide a meaningful and relevant contaminant transfer event for per- and polyfluoroalkyl substances (PFAS), even given the brief duration of their emergence, if cicada larvae are accumulating PFAS during their 17-year development period within the soil. This study evaluated archived Brood X periodical cicadas collected in 2021 from a site with previously characterized widespread PFAS contamination draining an active air force base (AFB), and from a site with no known direct PFAS sources, Big Gunpowder Falls State Park (BGF). Whole cicadas were analyzed for a suite of PFAS compounds based on EPA method 1633 and results are reported as ng/g wet weight (mean ± standard error). The only PFAS detected in cicadas from BGF was PFOS (3.5 ± 1.4 ng/g) while cicadas from AFB contained both PFHxS (4.0 ± 1.4 ng/g) and PFOS (7.4 ± 1.5 ng/g), resulting in a sum PFAS that was more than three times greater at AFB. Data on PFAS levels in terrestrial invertebrates are generally lacking in the literature and no data on PFAS in periodical cicadas have yet been reported. While these values were lower than those reported for some terrestrial invertebrates in other studies, these results suggest that periodical cicada emergence from contaminated landscapes may still be enhancing PFAS transfer to predators.

4.10.P-We-145 Compatibility of Serum and Plasma in Legacy and New/Replacement Per- and Polyfluoroalkyl Substances Measurement Using Automated Ultra High-Performance Liquid Chromatography Tandem Mass Spectrometry

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Per- and polyfluoroalkyl substances (PFAS) have been associated with a number of adverse health outcomes, leading to concern about their widespread human exposure. Although measurement of PFAS in human serum is widely used to evaluate the exposure, the demand for PFAS to be measured in plasma samples is increasing due to the unavailability of serum. To better understand the distribution of PFAS in serum versus plasma, 91 paired samples collected in California were analyzed using a newly developed online solid-phase extraction (SPE) ultra high-performance liquid chromatography tandem mass spectrometry (UHPLC/MS/MS) method. All 91 pairs were analyzed for 12 legacy PFAS while 36 pairs were also analyzed for an additional 30 new/replacement PFAS, including perfluorocarboxylic acids (PFCA), perfluorosulfonic acids (PFSA), fluorotelomers, polyfluorinated phosphate esters, perfluoroalkylphosphonic acids, and ether based perfluoroalkyl acids, etc. Twenty PFAS were detected in both serum and plasma matrices with comparable concentration range and detection frequency. Eleven PFAS with detection frequencies higher than 30% showed the coefficient of determination between serum vs. plasma concentrations ranging from 0.709 to 0.985. Our results suggest that researchers can use either matrix to assess PFAS exposure within reasonable analytical errors. Legacy PFAS remain dominant, but the short-chain PFAS such as perfluorobutane sulfonic acid (PFBS) show increased detection frequency compared to previous biomonitoring studies in California. In addition, some new/replacement PFAS such as perfluoropentane sulfonic acid (PFPeS), perfluoroheptane sulfonic acid (PFHpS), perfluoro-4-ethylcyclohexane sulfonic acid (PFECHS) and 9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (F53B) were detected in more than 30% of samples, although their concentrations were still measured in the ultra-trace/trace levels. The increase in the detection frequency of these compounds may reflect shifts in industry use and therefore in human exposure. *The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.*

4.10.P-We-146 Assessment of Legacy and Emerging Per- and Polyfluoroalkyl Substances (PFAS) in AC and Engine Filters in Vehicles

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Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic compounds used widely in manufacturing, consumer products, and pharmaceuticals. Their ubiquitous presence in nature poses concerns toward their potential environmental and human health related risks. Previous studies have shown that PFAS can accumulate in dust, which can lead to increased indoor exposure to both humans and animals. In vehicles, air is recycled through cabin AC filters, which prevents dust and microparticles from being released through the vents and into the passenger compartment. This experiment monitored 92 PFAS in both new (n = 5) and used (n = 10) cabin AC filters, and new (n = 7) and used (n = 10) engine filters via ultra-high pressure liquid chromatography – tandem mass spectrometry. Cabin AC filters were found to contain the highest mean ∑PFAS concentration (105 ppb) compared to engine filters (12 ppb). For cabin AC filters, the prominent species were polyfluorinated dialkylated phosphate esters (diPAPs), which accounted for 52% of ∑11PFAS, and in engine filters, the profile was dominated by perfluoroalkyl carboxylic acids (PFCAs), which represented 34% ∑10PFAS. We propose that like home AC filters, both car based filters could serve as novel matrices (both circulating in the passenger compartment and within the car's engine), allowing for the further assessment of PFAS exposure in lesser studied everyday environments (e.g., cars).

4.10.P-We-147 Enhancing Compound Identification and Exposure Assessment with a Novel Software Application for Managing Libraries

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As targeted screening and non-targeted identification workflows advance, empirical high-resolution mass spectra remain the benchmark for confident compound identifications/annotations. Spectra exist in many formats, both openly available (e.g., MassBank or MoNA) and commercial (various MS vendors) in nature. Combining spectra from the various sources into a single screening analysis is crucial to expand the identifiable chemical space of screening workflows. However, integrating these disparate formats into a single consolidated format is cumbersome and error-prone, if not impossible due to commercial format restrictions. To expand the identifiable chemical space of screening workflows and enhance exposure assessment, we present a novel software application that was built to centrally manage mass spectral library data. Data can be interconverted between formats without reliance on a single format. A chemical structure standardization and chemical substance classification pipeline was established as the backbone of the software to form the compound-centric data model to consolidate and organize data from multiple sources. Compound, chemical structure, method, and spectral data are stored from multiple common database and library formats during an import process. Consolidated data can be exported in one of several common industry standard database and library formats to support data analysis and compound identification/annotation workflows. As a proof-of-concept, ~4500 spectral files (as .txt) from MassBank EU were imported using the default import process. The standardization and classification pipeline consolidated the data, resulting in an addition of 640 chemical substances to the library manager. Additionally, an internal library with 1430 compounds and ~3800 spectra was imported and merged with the original import of MassBank EU data. Duplicate compounds were removed by the classification procedure to merge data together. The result was a list of 1846 compounds organized in a compound-centric fashion with ~245 compounds existing in both sets. Exporting this new list to the same internal library format doubled the available spectra for screening and identification in a downstream data analysis application. This library manager application and data integration workflow has been applied to metabolomics studies, PFAS library generation, and environmental screening studies to enhance identification and improve library management for data analysis.

4.10.P-We-149 Collection and Curation of Externally Shareable High Resolution Mass Spectrometry Spectral Libraries to Aid in Identification of Environmental Contaminants

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Laboratories are increasingly utilizing high resolution mass spectrometry (HRMS) as a tool to detect and identify environmental contaminants and possible exposure to them in clinical samples. Experimental production spectra, collected with HRMS are compared to spectral libraries for analyte identification. Commercial libraries frequently contain only the most commonly tested compounds and are typically vendor-specific, i.e., a library purchased for one instrument platform may not be compatible with another instrument platform from a different vendor. An ideal spectral library to support environmental toxicology and related human exposure would cover a wide array of chemicals, including existing and emerging toxins, drugs, and analytes of concern. Additionally, this chemical library would be readily available to all laboratories and frequently updated to address new and emerging analytes. This library is suitable for both environmental and clinical samples, bridging the gap between environmental concerns and impact on public health. In this work, we leverage our HRMS capabilities to create spectral libraries that can be shared externally to assist other laboratories in the detection of new, or unknown analytes. Reference standards of over 500 toxins and drugs were purchased and analyzed on HRMS instruments to collect the fragmentation spectra at multiple collision energies. This is performed across multiple instrument platforms for availability in four formats to cover different vendors and library searching software (Agilent-, Thermo-, Sciex-, and NIST-compatible). To confirm applicability of these spectra for compound identification needs, a rigorous quality review process was developed. Library entries are curated for more accurate matching, and replicate injections are performed to test library match score consistency for each analyte at each collision energy on each platform. All data is internally reviewed before public release to confirm high quality and accuracy, with library entries failing to meet required standards removed. Through these efforts, CDC is supporting nationwide detection and surveillance of existing and emerging environmental concerns and the ability to determine environmental concerns that may impact public health. *Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, or the U.S. Department of Health and Human Services.*

4.10.P-We-150 Toxicity Assessment of Energetics Using Benchmark Dose Modeling Software

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Dose-response data development is critical to understanding substance toxicity to protect the health of individuals who are exposed to potentially toxic substances. Traditional toxicity thresholds are based on the highest dose at which no adverse effect is observed (NOAEL) or the lowest dose at which an adverse effect is observed (LOAEL), but these values are limited. Modeling approaches have been developed to refine this method. One such tool is the Benchmark Dose Software (BMDS) provided by the United States Environmental Protection Agency, which models the dose at which a substance reaches a toxicity threshold for a selected endpoint, the benchmark dose (BMD), based on an empirically generated dose-response curve. The BMD is predicated on selection of a threshold of toxicity (benchmark response, BMR),

which is based on data type (e.g., continuous vs. dichotomous) and severity of the modeled endpoint (e.g., mild vs. severe effect). The most common BMR is a 10% effect level (BMD) for sublethal effects. The dose-response data are fitted to numerous mathematical models to identify the model that most accurately represents the data. Toxicity assessment applications frequently use a one-sided lower 95% confidence bound for the BMD estimate, referred to as the BMDL, to account for uncertainty in the model. We have used this tool to assess the toxicity of energetic material. Based on 90-day dosing studies, the BMD and BMDL have been calculated for various energetics. The BMD and BMDL seminiferous tubule formation for 3-nitro-1,2,4-triazol-5-one (NTO) are 70 and 40 mg/kg-day. The BMD and BMDL for extramedullary hematopoiesis with 2,4-dinitroanisole (DNAN) are 4.08 and 2.3 mg/kg-day. The BMD and BMDL for hemoglobin concentration with 1,1-diamino-2,2-dinitroethene (FOX-7) are 12 and 10 mg/kg-day in female rats. The BMDLs for nervous system injury and reproductive effects with hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are 1.3 and 0.23 mg/k-day. Lastly, in a 14-day repeated dosing study with 2,4,6-trinitro-3-bromoanisole (TNBA), the BMD and BMDL for splenomegaly in female rats are 59 and 41 mg/kg-day. The BMDS-generated values offer a fine-tuned approach for risk assessment and subsequent decision-making for the use of energetics by military personnel.

4.10.P-We-152 Chemical Similarity and Read-Across: With Great Power Comes Great Responsibility

***Kai Benjamin Paul**, and Jamie Marshall, Blue Frog Scientific, United Kingdom*

Read-across is a popular resource in the toolbox of many stakeholders for chemical prioritization and endpoint generation. The premise is, structurally similar chemicals inevitably have similar extrinsic properties, e.g., dissolution, aquatic and/or genetic toxicities. Or that similar structures lead to a clear pattern in extrinsic properties e.g., longer alkyl chain length and increasing log Kow. This concept enables some QSARs to be trained. However, it has become clear that not all read-across is built equal and that not all similar chemical structures elicit similar effects. Often overlooked within read-across are the specific differences in sub-structures such as substituent placement and slight differences in functional groups which can radically change biological activity. It is the interplay here that impacts e.g., 3-dimensional conformation, charge shared across the chemical and the likelihood for dissociation/reactivity to occur. Metabolism and pH dependencies can also be overlooked. It is of utmost importance to communicate these in read-across argumentation as such data may inform modelling approaches. Using experimental data we introduce these concepts and go deeper into a read-across case between chemicals with an amine central moiety. We discuss the pH dependent toxicity in aquatic environments, highlighting how addition of a single methyl, or hyroxyl group removal, on an otherwise identical structure can impact toxicity by orders-of-magnitude, despite the same mechanism of action (MOA). We then explore mammalian toxicity between the two similar chemicals and demonstrate how an added ethyl substituent leads to toxicity levels orders-of-magnitude different, driven by metabolism and a disparity in MOA. We demonstrate how one chemical will lead to aberrant phospholipids and sphingolipids, driven by conserved biological pathways for monoethanolamine (MEA). The effects have implications for developmental and neurological toxicity such as demyelination. We also briefly discuss MOA and species differences related to choline perturbation of the chemical and discuss how these were missed by some modelling approaches. The research highlights factors that can be overlooked in modelling and read-across approaches and though we have been given the keys to use read-across we can easily take a wrong turn and nothing should be taken in isolation. With great power comes great responsibility for correct implementation, progress and safety.

4.10.P-We-153 Effect of Feature Set Reduction on the External Prediction Accuracy of Toxicity Models

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Many researchers estimate toxicity using read across based methods in which the analogs are selected in terms of molecular similarity via a general fingerprint. This approach assumes that each descriptor has an equal effect on the toxicity endpoint. The goal of this study is to investigate whether feature reduction methods (e.g., genetic algorithm and permutation importance) can improve the external prediction accuracy by limiting the descriptor set to features which are relevant to the toxicity endpoint. Feature reduction methods use an iterative approach to find the subset of descriptors which maximizes the training set cross validation performance. The effect of feature reduction will be evaluated for several different QSAR methods (e.g., random forest and kNN) and molecular descriptor sets (WebTEST, PaDEL, RDKit, and ToxPrints). Results will be presented for several different toxicity datasets (e.g., acute aquatic toxicity, acute mammalian toxicity, skin sensitization, and mutagenicity) to determine the best overall modeling options.

4.10.V General: Chemistry and Exposure Assessment

4.10.P-We-151 Development and Application of Structural Activity Groups for Chemical Classification and Clustering as a Non-animal Method for Safety Assessment

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The Research Institute for Fragrance Materials (RIFM) has developed a robust, reliable, and reproducible method of classifying chemicals known as a Structural Activity Group (SAG) in an effort to institutionalize knowledge gained from manually assessing thousands of chemical pairings of fragrance ingredients as a non-animal method for safety assessment. This method should improve accuracy, consistency, transparency, and explainability while at the same time reducing the need for expert judgment and any resulting bias. The SAG of a material is created via a top-down approach through the use of standardized decision trees. These decision trees provide a series of Indicator Phrases (IPs) which are unambiguous text expressions. The IPs are based on (1) the heteroatom present (e.g., hydrocarbon, O-containing, etc.), (2) key organic functional group(s) (e.g., alcohols, acids, esters, etc.), and (3) structural features within the hydrocarbon skeleton (e.g., straight chain, branched chain, cyclic, etc.). Each SAG is structured and presented as a string of IPs separated by backslashes and contain all of the distinct chemically and toxicologically significant structural features of a substance needed to classify and organize it among a diverse chemical inventory (e.g., linalool has the SAG of oxygen_containing/alcohols/tertiary/branched_chain/unsaturated/multiene/vinylene_vinyl/alpha_beta_unsaturated/allylic_alcohol/c6toc13). This approach has been applied to over 6000 discrete fragrance and fragrance-like organic chemicals and has been shown to perform well for various functionalities observed in this chemical space. Additionally, the decision trees are adaptable and can be expanded to account for functionalities not present in fragrance materials. SAGs allow easy, transparent, and repeatable separation of an inventory of thousands of chemicals into small groups of chemicals that share the same IPs. Similar groups of chemicals can then be identified when the SAGs share all but one or a few of the same IPs. This differentiates SAGs from SMILES, as SMILES define a single chemical and not a group. Furthermore, the IPs used in the SAGs can be encoded and used with machine learning techniques. This can allow for the development of predictive toxicology applications to refine and strengthen safety assessments.

4.11.A.T Identifying and Linking Environmental Exposure to Biological Effects

4.11.A.T-01 Structure-Dependent Toxicity of Perfluoroalkyl Substances using Quantitative Adverse Outcome Pathway with Bayesian Network: Safe-by-Design Approach

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Recent toxicological research on per- and polyfluoroalkyl substances (PFAS) underscores their potential systemic toxicity. Despite the critical need for developing safer PFAS, gaps remain in understanding how the structural properties of PFAS, such as carbon chain lengths and functional groups, influence toxicity. A Bayesian Network (BN) is a model that presents probabilities of the dependencies between any two variables in a graphical form, offering capabilities for diagnosis, prediction, and causal inference. Therefore, this study evaluates the toxicity of 12 PFAS (carbon chain lengths with C4-C14 and functional groups with carboxylates and sulfonates) within the adverse outcome pathway (AOP) framework employing BN model. We first developed a general cell stress AOP by identifying common key events (KEs) from existing AOPs in the AOP Wiki, supplemented by literature reviews. We then established in vitro-based integrated testing strategies (ITS) for assessing these KEs. The effects of PFAS on these KEs were evaluated using six human cell lines representative of systemic organs: liver (HepG2), lung (Beas-2B), brain (SH-SY5Y), immune system (Jurkat), and developmental stages (embryonic stem cells and neural stem cells) and quantified with BN model. In NSCs, both the carboxylates and sulfonates groups showed increasing toxicity with chain length, particularly affecting cell viability. Notably, the sulfonates demonstrated a greater impact on cell viability compared to the carboxylates. Additionally, PFOS, PFNA, PFDA, PFUnDA, and PFTeDA, which were detected in high concentrations in recent study, showed significant alteration across more than 4 KEs and cell death indicating potential developmental neurotoxicity. Overall, this study provides insights beyond mere cytotoxic effects, incorporating changes in molecular markers within toxicity pathways via the AOP framework. This approach enhances the reliability and interpretability of the results, paving the way for the application of the Safe-by-Design concept in developing safer PFAS. *This work was supported by Korea Environmental Industry & Technology Institute through "Core Technology Development Project for Environmental Diseases Prevention and Management" (2021003310005) and through Technology Development Project for Safety Management of Household Chemical Products (RS-2023-00215309), funded by Korea Ministry of Environment.*

4.11.A.T-02 Exploring PFAS Exposures In Utero: Streamlining Non-Targeted Analysis of PFAS in Neonatal Dried Blood Spots

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Non-targeted analysis (NTA) of contaminants in biological matrices can provide a comprehensive assessment of environmental influences on health. PFAS are an emerging public health concern that require non-targeted analysis approaches to detect the 10,000+ compounds that comprise this class. Only a limited number of these compounds have been evaluated to date and our understanding of their impact on children's health is limited. To evaluate exposures during a critical window of vulnerability on development, we conducted PFAS NTA in dried blood spots (DBS) collected from newborns within 48 h of birth. The objective of this study was i) to develop a non-targeted analysis workflow to annotate MS/MS matching of PFAS classes using fragmentation rules, in-silico libraries based on structure fragment relationships, dot product matching against spectral libraries, and fragment screening and ii) to explore novel, previously unknown or unexpected PFAS exposures in 360 neonatal DBS archived through the California Linkage Study of Early-Onset Cancers (CALSEC). Neonatal DBS and paired card blanks were analyzed using liquid chromatography high resolution mass spectrometry. FluoroMatch (v5.0) was adapted to perform NTA on these biospecimens with the software automating file conversion, peak picking, blank filtering, homologous series detection, annotation, confidence reporting, and interactive data-visualization. Analysis revealed 18 PFAS-like compounds in neonatal DBS; 11 of these compounds did not overlap with the targeted panel of 30 PFAS reported by the CDC. These annotations included unsaturated perfluorocarboxylic acids (PFCAs), H-substituted PFCAs, fluorotelomer perfluorosulfonic acids, and ether containing PFCAs and PFSAs. An additional 94 PFAS-like compounds were found in the card blanks, highlighting the need for paired card blanks in DBS studies to account for background contamination. These findings demonstrate the utility of neonatal DBS biospecimens to assess early life PFAS exposures. The availability of paired genomic data and disease diagnoses (including cancers) through CALSEC will allow future evaluation of chronic disease risk in relation to PFAS exposures.

4.11.A.T-03 Histopathology and Transcriptomic Results in Zebrafish (*Danio rerio*) Male Livers from the P and F1 Generations of a PFOS Multi-Generational Exposure

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Decades of use of poly- and perfluoroalkyl substances (PFAS) for a variety of commercial and industrial applications has resulted in widespread and persistent contamination of both aquatic and soil matrices. Acute and/or chronic PFAS exposure can have numerous deleterious effects on humans and other vertebrates including, but not limited to, development, reproduction, and growth. Furthermore, research in terrestrial and aquatic vertebrates has shown the liver to be a target organ of PFAS accumulation with a potential of resulting hepatotoxicity. To further explore the connection between chronic, sub-lethal PFAS exposure and negative impacts on the

liver, we used liver tissues from a three-generation exposure of zebrafish (*Danio rerio*) to sub-lethal concentrations of the PFAS perfluorooctane sulfonic acid (PFOS) to perform liver histopathology and transcriptomic analysis (using RNA sequencing) to determine the extent of potential adverse effects on livers of 180 day old male fish from the first two exposed generations (P & F1). Histopathological analysis showed that livers from fish exposed to 100 µg/L PFOS had significant incidences of lipid-type hepatocellular vacuolation in both the P and F1 generations compared to controls with the severity and frequency of vacuolation greater in the F1 generation. Transcriptomic analysis showed that gene expression was overall more perturbed in the F1 generation than the P generation when compared to respective controls with the F1 100 µg/L livers having the greatest number of differentially expressed transcripts overall. Across all generations, pathways associated with lipid metabolism, including cholesterol biosynthesis and the peroxisome proliferator-activated receptors (PPAR) pathway, were found to be significantly affected by exposure to PFOS. These results provide further evidence for the liver as a target organ for adverse effects due to PFOS exposure, and indicate continuous, transgenerational exposure to sub-lethal PFOS concentrations may result in increasing impacts on lipid metabolism and hepatoxicity with each generation. Finally, we present a draft adverse outcome pathway (AOP) that connects the molecular initiating event (MIE) of PFOS binding PPAR isoforms resulting in disrupted nuclear signaling to the key events of disrupted lipid metabolism and finally to the adverse outcome (AO) of liver steatosis.

4.11.A.T-04 Non-Targeted Metabolomics for Discovering Robust Human Biomarkers

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Wastewater analysis provides an effective means to assess drug usage, diseases, and overall wellness within a population. Wastewater metabolomics is an ideal tool for monitoring as it is a true representation of the population which has been proven recently through COVID-19 surveillance. Non-targeted metabolomics is an unbiased, comprehensive analysis of measurable metabolites produced by humans. Biomarkers are measurable characteristics that can be used as an indicator of certain properties or conditions and human metabolites are an example of biomarkers. Human metabolites as biomarkers have a wide range of applications such as assessment of human health, monitoring community health trends, estimating population-level drug use, evaluation of public health interventions, and early warning systems for disease outbreaks. Thus, it is crucial to have a population-level biomarker that can be used to normalize responses based upon population size. A suitable normalization biomarker should be consistently present in all wastewater samples, and variation by site should mostly reflect the size of the population and not due to other social factors. To find suitable normalization biomarkers, untreated wastewater was collected from 6 different wastewater catchments corresponding to specific municipalities of different sizes within Durham region (Ontario, Canada): Corbett, Port Darlington, Harmony, Bayly, Uxbridge, and Port Perry. Samples were collected 2 days a week for 45 days. Aqueous biomarkers were extracted using a modified Bligh-Dyer liquid-liquid extraction method and analyzed using non-targeted data-dependent acquisition (DDA) acquisition on a LC-QTOF (Agilent 1260 LC and 6545 QTOF). The obtained data from the instrument was analyzed using Profinder software (Agilent), the statistical analysis was performed using Mass Profiler Professional (MPP, Agilent). We will present our suggestions for stable human biomarkers in wastewater that can be used for population normalization to aid in drug and health surveillance and discuss further applications and approaches.

4.11.A.T-05 Perfluorinated Alkyl Substances Impact Breast Cancer Cell Proliferation

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PFAS (Per-and-Poly Fluorinated Alkyl Substances) are a family of synthetic industrial chemicals with variable length, fluorinated carbon chains attached to an acid group. PFAS are used in fire retardants, waterproofing solutions, and other industrial applications. Historically, the long-fluorinated chain compounds were considered non-bioactive, hence their wide distribution in commercial products. Unfortunately, recent data has shown this is far from the truth. Concerns about measured PFAS body burdens in humans and the potential for endocrine disruption have motivated many toxicity studies that indicate deleterious effects from exposure. PFAS's role in cancer progression is also a major health concern; we address that concern with a set of cell proliferation assays using cell lines, including MCF-7 cells cultured in the presence or absence of PFAS. We address two questions: First, does PFAS impact growth in a dose-dependent manner? To answer this, we exposed cells to a series of PFAS concentrations that include and exceed values similar to those measured in biosolid samples across Arizona. Second, we seek to interrogate differences between legacy PFAS species (species with eight or more fluorinated carbons) and newer short-chain PFAS species (ostensibly purpose-built to be safer). In these experiments, we use PFOA (perfluorooctanoic acid; eight-carbon chain) as our model legacy PFAS and PFPA (perfluoropentanoic acid; five-carbon alternative) as our model next-generation PFAS. Preliminary results suggest these PFAS species cause increased growth in MCF-7 cells compared to unexposed control. Further description of this phenomenon could contribute to the field by informing regulators and scientists of novel LOEL (lowest observable effect level) and NOEL (no observable effect level) concentrations and unravel a mechanistic understanding of PFAS effects on cell growth.

4.11.A.T-06 Nontarget Prioritization Using Standard Toxicity Assays: A Spatial and Temporal Study of Stream Sediments Across California

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Since 2008, the Stream Pollution Trends (SPoT) program of the State Water Resources Control Board's (SWRCB) Surface Water Ambient Monitoring Program has monitored up to 100 sites across California for sediment contamination and toxicity, providing crucial information for impaired waterbody listings. To assess the health and condition of CA waterbodies, SPoT is testing the application of nontarget analysis (NTA), an *a priori* method capable of detecting and identifying known and unknown contaminants beyond a traditional targeted capacity. In a pilot effort, sediment samples were collected in 2021 from a subset of SPoT sites and analyzed using NTA. A second round of analyses were conducted in 2023 on selected sediment samples in collaboration with the SWRCB's Division of Water Quality. This spatial and temporal variation offers the opportunity to highlight the value of NTA in monitoring complex environmental systems. Alongside this spatial and temporal complexity, nontarget analyses regularly generate 10,000+ features per sample. With complete feature elucidation not yet feasible due to the manual validation of matches, methods are needed to direct efforts and prioritize features of concern. To prioritize nontarget features with the greatest impact on waterbodies, nontarget peak heights from sediment samples were correlated with standard toxicity markers, including *Hyalella azteca* (HA) growth and mortality and *Chironomus dilutus* (CD) mortality. Nontarget features were detected using both liquid and gas chromatography with high-resolution mass spectroscopy (LC-HRMS and GC-HRMS). Prioritizing 2021's nontarget features using HA and CD endpoints resulted in a ten times reduction in features, with the reduced feature space containing a mix of toxic contaminants (ex: tributyl phosphate, galaxolide, bifenthrin, fipronil) and natural products. To examine sublethal responses to the complex chemical mixtures extracted from SPoT samples, receptor activity assays were included in 2023. Ongoing work is focused on collecting receptor activity data, analyzing 2023 NTA results, and integrating the spatial and temporal results to further nontarget feature prioritization. Overall, these results highlight how the prioritization of nontarget features using aquatic invertebrate toxicity endpoints can focus the feature space, but additional endpoints, such as in vitro bioassays, can be incorporated to further prioritize contaminants based on more sensitive biomarker information.

4.11.B.T Identifying and Linking Environmental Exposure to Biological Effects

4.11.B.T-01 Selecting Appropriate Biological Matrices for Exposomics: Insights from A Systematic Model-based Investigation

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Targeted and non-targeted analytical methods enable the identification of human exposomes through monitoring chemical substances in various biological matrices (mostly biological fluids, e.g., blood, urine, mother milk, semen, saliva, or sweat). This is a prerequisite for linking human exposomes with adverse health outcomes at target organs or the whole body. However, selecting appropriate biological matrices always remains contentious due to concerns about whether the levels of contamination in non-invasive matrices (e.g., mother milk) adequately reflect those in the systemic circulation (e.g., serum and plasma) and, by extension, in the target organ (e.g., follicular antrum in the ovarian follicle). This presentation will explore this issue through a model-based investigation into the mechanisms and factors that influence whether contamination levels in various biological fluids align. Specifically, we develop a computation model that accounts for both thermodynamics and kinetics in chemical partitioning between blood and invasive and non-invasive biological fluids, whereby we quantify the impacts of two effects, i.e., "volume change" and "kinetic delay," on chemical distribution between biological fluids. For illustration, we collect measurements from peer-reviewed publications on the chemical distribution of 300+ chemicals in paired samples of blood and follicular fluid, blood and mother milk, and blood and urine. A statistical analysis indicates that the collected data align well with our proposed theoretical framework. Overall, this presentation will offer a practical guide for selecting biological matrices and optimizing analytical methods in exposome analysis, helping avoid potential pitfalls and biases when identifying and linking environmental exposures to biological effects.

4.11.B.T-02 Organic Contaminants Detected at Illegal Cannabis Grow Operations on Federal Land

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Illegal cannabis growing operations on public land continue to be prolific in California, despite the legalization of cannabis in 2016. Evidence of unregistered pesticides have been found at these sites and the extent of their environmental impact is not yet fully understood. Three grow sites last active 10 months to 2 years prior, were surveyed in 2023; soil, water, and sediment were screened for over 240 pesticides (insecticides, fungicides, herbicides, and rodenticides) and nontargeted analysis (NTA) was performed to evaluate other organic contaminants. Multiple fungicides and insecticides were quantified in the soil collected from each site, including imidacloprid, malathion, bifenthrin, cyfluthrin, permethrin, triadimefon, myclobutanil and fluopyram. No targeted pesticides were detected in the water or sediment. NTA identified phthalate and phosphate plasticizers in soil, sediment, and water at all sites. Cannabidiol (CBD), cannabinol (CBN) and delta-9 tetrahydrocannabinol (d9-THC) were detected in all soils collected from grow plots, near empty fertilizer and pesticide bottles and sprayers, and near living quarters at all sites. Additional pesticides, anthelmintics and plant growth regulators were identified using NTA in addition to several pharmaceuticals, tire-wear related compounds, and personal care products. In addition to the environmental impact associated with anthropogenic activities at these remote sites, the use of unregulated fertilizers and pesticides could be a source of chronic exposure of associated contaminants to local wildlife. This survey provides an initial look at the complexity of organic contaminants remaining at these sites and sets the stage for future work investigating the longer-term fate of these contaminants onsite.

4.11.B.T-03 Accumulation and Fate of Organic Contaminants in Soils Amended with Fertilizing Residual Materials (FRMs)

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Fertilizing residual materials (FRMs) from industrial or municipal origins are commonly used to enhance the properties of soil and improve plant growth. However, the application of FRMs to agricultural soils can lead to the accumulation of various organic contaminants, including bisphenols, plasticizers, personal care products, *etc.* These contaminants can pose potential risks to both environmental and human health due to their persistence and bioaccumulation. Some contaminants may be degraded/metabolized in soils/plants into compounds of unknown toxicity. To better understand the accumulation behavior and fate of these contaminants, the current study aimed to develop an analytical method for the simultaneous targeted analysis of fourteen bisphenols and fourteen plasticizers, as well as the non-targeted analysis of contaminants and their degradation products in soils amended with different fertilizers. The method was then applied to soil samples collected from a 2-year controlled field experiment set up in Quebec, Canada. More specifically, soil amended with municipal biosolids, deinking residues, ash, and urea were collected at four time points (prior to the application, 7 and 56 days post-application,

and harvest). The samples were then freeze-dried and homogenized. The extraction method was first optimized for the extraction solvent, and acetonitrile with 1% formic acid was selected because it yielded the highest extraction efficiencies for the targeted compounds. The samples were then extracted by the optimized method and analyzed by LC-QToF-MS. The levels of the targeted analytes were determined, and their accumulation was observed from day 7 in soils amended with municipal biosolids and deinking residues. Several phthalates (DEP, DiBP, DBP, DiNP, DOP, DPHP) and bisphenols (D-8, D-90, TGSA) were not detected in soil prior to amendment with fertilizers, but their levels significantly increased after the amendment. However, DnOP and DEHP, two commonly used plasticizers, were detected (at approximately 10 ng g^{-1}) in soils prior to amendment with fertilizers, and the levels further increased after the application of fertilizers. Some degradation products of bisphenols and phthalates were tentatively identified through the non-targeted workflow, highlighting the potential uptake and accumulation of these compounds by the crops grown in the amended soils. This is the first study on the fates of contaminants in soils amended with deinking residues.

4.11.B.T-04 Assessing Potential Risks to Insectivorous Birds from Per- and Polyfluoroalkyl Substances on Department of Defense Sites: Exposure Dynamics and Metabolomic Impacts

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The use of aqueous film-forming foams (AFFF) in fire suppression and training activities on Department of Defense (DoD) sites has led to contamination of the environment with per- and polyfluoroalkyl substances (PFAS). Previous modeling studies of threatened and endangered species on DoD sites identified potential risks of PFAS exposure to sensitive life stages of avian insectivores. However, significant uncertainty exists in the importance of dietary preferences (i.e., aquatic or terrestrial prey), variability in PFAS in abiotic media, and the role of physiological parameters in driving risk to avian receptors. Consequently, the present study aimed to elucidate the exposure of two insectivorous bird species, the house wren, *Troglodytes aedon*, and the tree swallow, *Tachycineta bicolor*, to PFAS and co-contaminants on four DoD sites. The diet of wrens is dominated by terrestrial prey items, whereas tree swallows feed primarily on emergent aquatic invertebrates, facilitating a comparison of PFAS exposure across feeding habits. Invertebrate prey items, abiotic media samples (soil, sediment, and surface water), and nestlings were collected and analyzed for PFAS, with polycyclic aromatic hydrocarbons (PAHs) also measured in abiotic samples. In addition, untargeted metabolomics were performed on wren and swallow liver samples to identify potential metabolic impacts of PFAS exposure. Data obtained for year one indicated differences in PFAS concentrations of aquatic and terrestrial prey items, with emergent aquatic invertebrates typically having higher concentrations relative to terrestrial prey. For nestling samples, proximity of nest boxes to an AFFF-contaminated water body appeared to be a major driver in PFAS exposure. Abiotic media samples showed significant spatial variability, with large differences in total PFAS concentrations among sublocations of individual sites. Metabolomic analysis indicated few differences in swallow metabolic profiles between PFAS-contaminated and reference sites; however, differences among PFAS-contaminated sites were recorded. For wrens, metabolomic analyses indicated significant downregulation of metabolites relating to steroid hormone and fatty acid biosynthesis at a single site relative to two others. These findings will be incorporated into spatially explicit exposure models to reduce the uncertainty in assessing the ecological risk of PFAS exposure to avian receptors in the natural environment.

4.11.B.T-05 Event Driven Taxonomy (EDT)-Based HRMS Screening Library: Identifying Suspect and Non-Target AhR-Active Contaminants in Sediment

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Aryl hydrocarbon receptor (AhR) activity has been often detected in aquatic sediment worldwide, particularly in urban waterways, calling for rapid and effective methods to identify the main toxicants responsible for this response. Effect-directed analysis has been shown effective for identifying causative agents for a certain bioactivity, but its application was limited by tedious cycles of fractionation and bioassay. According to the event-driven taxonomy (EDT) concept, a rapid screening strategy was developed for effectively identifying both suspect (known unknown) and non-target (unknown unknown) AhR-active contaminants in sediment extracts using two high resolution mass spectrometry (HRMS) libraries, namely event driver (ED) and event driver ion (EDION) libraries, respectively. The ED library was assembled with by data curation with AhR-active compounds in the ToxCast, while EDION library was complemented by predictive deep learning models. Sediment extracts were analyzed using a Thermo Scientific Orbitrap Exploris 240 LC-MS/MS without cycles of fractionation and bioassay, and AhR-active contaminants were detected using the AhR-ED and AhR-EDION libraries embedded in the Compound Discoverer template. Compared with the commonly used ChemSpider database, more suspect AhR-active compounds in sediment mixture were identified by the ED library along with lower incidence of false positives in tentative candidates. Furthermore, the implementation of EDION library identified additional AhR-active compounds beyond the suspect list, and the highest increment in chemical classes was industrial intermediates and transformation products that lack of a priori knowledge. The improvement of identifying toxicants with limited bioactivity and HRMS information highlighted the effectiveness of the developed strategy in expanding the pool of "unknown unknowns" in AhR active contaminants. Collectively, the construction of EDT-based HRMS libraries provided an effective and rapid solution for identifying bioactive contaminants in complex chemical mixtures.

4.11.B.T-06 Microbial Biotransformation of 6:2 Disubstituted Polyfluoroalkyl Phosphate in Human Fecal In Vitro Suspensions

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Polyfluoroalkyl phosphate esters (PAPs) have diverse commercial applications and are ubiquitous in modern society. This raises concern as PAPs have been shown to be biotransformed into fluorotelomer alcohols (FTOHs) and perfluorinated carboxylic acids (PFCAs) with known negative environmental and human health impacts. Our previous research has shown that human intestinal microbes, found in fecal samples, are capable of biotransforming PAPs. In this study we investigated sex-based differences within intestinal microbe 6:2 diPAP biotransformation. *In vitro* testing was completed by incubating the fecal samples in an anaerobic chamber at 37°C with 6:2 diPAP (concentrations ranging from 1-5 ppm) up to 2 hours. The reactions were then terminated and the samples prepared for GC- and LC-MS

analysis. Metabolites of interest included two hydrolysis products, the 6:2 monoPAP and 6:2 fluorotelomer alcohol (6:2 FTOH), and five oxidative metabolites formed from 6:2 FTOH under both oxic and anoxic conditions. Fecal samples also underwent 16sRNA sequencing to compare the relative abundance of the various microbial phyla in males vs females. Our data revealed differences in the rate of biotransformation, however the differences were not sex related. This differentiated response shows the unequal additive impacts from the gut microbiome on host detoxification and/or activation of xenobiotics. This study is important for equitable and informed risk assessment of the continued and future use of PAPs in consumer products.

4.11.P-Mo Identifying and Linking Environmental Exposure to Biological Effects

4.11.P-Mo-081 Storm Impact on PFAS (Per- and Polyfluoroalkyl Substances) Distribution in Lower Atlantic City Reservoir and Connected Streams in New Jersey

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PFAS is a class of persistent environmental contaminants that has toxic effects on human and animal health. PFAS enters the environment via both point and non-point sources. Since drinking water is the primary source of PFAS to humans, continuous monitoring of drinking water sources is vital to provide safe water to the residents. Atlantic City Reservoir provides drinking water to the residents of Atlantic City, which is a heavily urbanized and polluted region in New Jersey and storm events may further increase the risk of contamination via storm runoff. In this study, we quantify concentrations of 14 PFAS including long and short chain carboxylic acids, sulfonic acids, and an ammonium salt of a carboxylic acid in Lower Atlantic City Reservoir and three connected streams before and after heavy storms. We estimate riverine mass fluxes of PFAS in stream inflow and outflow of the reservoir to evaluate the role of rivers on total standing load of PFAS in the reservoir. We further compare pre- and post-storm PFAS concentrations and mass fluxes to evaluate the contribution of storm runoff to the total PFAS distribution in Atlantic City Reservoir and connected streams. Since identification of sources is critical to regulate PFAS inputs to the environment, we evaluate correlations of PFAS distribution and land use that streams flow through before entering the reservoir. It will delineate the possible sources contributing to the levels of PFAS in Atlantic City Reservoir and support managing point sources located near the reservoir including Atlantic City International Airport.

4.11.P-Mo-082 From Fin to Fork: PFAS in Florida Atlantic Estuarine Fishes

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Per- and polyfluoroalkyl substances (PFASs) are considered forever chemicals due to their environmental persistence and are detected in all environmental media (air, water, soil), wildlife, food items and humans. Dietary ingestion is the predicted dominant pathway among the general population, with the consumption of fish and shellfish being a primary source of PFAS for those without exposure to contaminated drinking water or through occupational exposures. The objectives of this study were to collect fish in Florida estuaries to quantify PFAS in edible tissues, identify hotspots, and assess the need for fish consumption advisories (FCA). Recreationally important estuarine fishes were targeted for collection from four estuaries along the Atlantic coast of Florida: the St. Johns River Estuary (SJRE), Indian River Lagoon (IRL), St.

Lucie River Estuary (SLRE) and Lake Worth Lagoon (LWL). Fillets ($n = 265$) from 16 species were processed and analyzed for a suite of 40 PFAS following previously published methods. Twenty PFAS were not detected (ND) in any fish collected in this study. The sum of 24 PFAS ($\sum_{24}$PFAS) concentrations in the edible tissues of fish ranged from ND to 51,500 ng/kg. Overall, fish collected from the IRL ($\bar{x}$ = 6,790 ng/kg; range: 2.5 – 51,500 ng/kg) had the highest concentrations of $\sum$PFAS$_{24}$ followed by SJRE ($\bar{x}$ = 2,300 ng/kg; range: 112 – 9,500 ng/kg), LWL ($\bar{x}$ = 1,040 ng/kg; range: ND – 8,540 ng/kg), and SLRE ($\bar{x}$ = 880 ng/kg; range: ND – 5900 ng/kg). The acceptable daily dose (ADD) of perfluorooctanesulfonic acid (PFOS) was calculated for harvestable Striped Mullet, White Mullet, Red Drum, Sheepshead, Yellowfin Mojarra, and Common Snook. The ADDs were derived for subchronic exposures (3 meals/week) and chronic exposures (7 meals/week) for comparison with the final noncancer reference dose (RfD 1.0e-7 mg/kg/d) published by the U.S. EPA. For many demographics, consuming both 3 and 7 meals per week (subchronic and chronic exposures) of Red Drum, Striped Mullet, and Common Snook exceeded the noncancer RfD. There were regional differences in the ADDs for each species. For instance, the ADD for adult chronic exposures of PFOS in Red Drum collected from the IRL was 65 times higher than the RfD, whereas the ADD for this species collected in the LWL was 7.5 times higher than RfD. These data will aid public health administrators in assessing the need for regional FCAs for some species in Florida waters.

4.11.P-Mo-083 Expanding Per- and Polyfluoroalkyl Substances Coverage in Nontargeted Analysis Using Data-Independent Analysis with Q-RAI and IonDecon

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FluoroMatch Flow and Visualizer are open-source tools that simplify suspect and nontarget screening of PFAS compounds. FluoroMatch automates file conversion, chromatographic peak picking, blank feature filtering, PFAS annotation based on precursor and fragment masses, and annotation ranking. The software library contains 15,643 PFAS fragmentation patterns based on rules derived from standards and literature, and the software automates a process to add more compounds. FluoroMatch Flow and Visualizer are freely available from innovativeomics.com/software. FluoroMatch Flow directly processes vendor files and includes a systematic scoring framework to communicate confidence for every feature, alongside reporting confidence levels via the Schymanski schema. IonDecon filters All Ions data to only those fragments correlating with precursor ions. This software can be used to deconvolute any All Ions files and generates an open source Data Dependent Acquisition (DDA) formatted file, which can be used in any downstream nontargeted analysis workflow. We received samples through the NIST PFAS Interlaboratory Studies and wanted to use the data to evaluate the capabilities for FluoroMatch IonDecon to process various DIA file types like All Ions fragmentation and Quadrupole Resolved All Ions (Q-RAI). Three methanolic solution containing a mixture of per- and polyfluororalkyl substances were supplied by NIST for an interlaboratory study. Sample were injected without any further sample preparation and without any dilution. Samples were injected four times for iterative exclusion information-data dependent analysis (iterative MS/MS), then in All Ions acquisition mode and finally with Q-RAI acquisition mode. The instrument was an ultra-high-performance liquid chromatography (UHPLC) system connected to a quadrupole time-of-flight mass spectrometer (Q-TOF MS). Blanks were acquired every other injection for blank filtering. PFAS were detected in negative electrospray ionization mode. Data was acquired from m/z 50-1500, with MS/MS collision energy set to 0and 40 eV. Once the MS and MS/MS data has been collected, FluoroMatch Modular was used for annotation and FluoroMatch Visualizer was used to provide users with customizable graphs, variables, and tables to help with data interpretation.

4.11.P-Mo-084 Developmental Toxicity Screening of Per- and Polyfluoroalkyl Substances (PFAS) Using a Larval Zebrafish Assay

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Per- and polyfluoroalkyl substances (PFAS) are found in many consumer and industrial products. While there is evidence that some PFAS, notably perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), cause developmental toxicity in mammals, the developmental toxicity potential of the vast majority of PFAS is unknown. This project uses zebrafish larvae to fill this knowledge gap and paint a clearer picture of the effect of PFAS on vertebrate development. To assess this, a medium-throughput larval zebrafish assay was used to conduct a concentration-response study with 182 unique chemicals in the EPA PFAS chemical library. Embryos were collected the day of fertilization (0 days post fertilization [dpf]) and exposed to either dimethyl sulfoxide (vehicle, 0.4% *v/v*) or one of the PFAS ($n = 6$ embryos/chemical/concentration; ≤ 100 µM; 8 concentrations per chemical). At 6 dpf, two independent, blinded observers graded developmental endpoints for each larva (mortality, hatching, swim bladder inflation, edema, abnormal spine/tail, and cranial-facial abnormalities). Concentration-response data were processed using the ToxCast Data Analysis Pipeline (tcpl) R-package (tcpl v.3.2.0) by aggregating endpoint counts to percentages. Those percentages were then fit to five bounded models (constant, hill, gain-loss, and exponential 4-5), with the winning model selected by the lowest Akaike Information Criteria score, a statistical calculation used to compare model quality. A baseline level (BMR) was calculated from the control data, and the point where that BMR intersected the winning model resulted in benchmark concentration (BMC) values. Of the 182 PFAS tested, 55 (30%) produced developmental toxicity, commonly seen as mortality, edema, or swim bladder non-inflation. Perfluorooctanesulfonamide (PFOSA) was the most potent developmental toxicant (BMC = 0.26 µM), followed by *N*-methylperfluorooctanesulfonamide (BMC = 0.44 µM), and ((perfluorooctyl)ethyl)phosphonic acid (BMC = 0.58 µM). PFOS was also developmentally toxic, with a BMC of 7.5 µM. Therefore, both mammals and zebrafish have exhibited toxicity after developmental exposure to PFOS. However, the developmental toxicity of sulfonamide-containing PFAS, namely PFOSA, is largely unexplored in mammals, indicating a knowledge gap surrounding PFAS developmental toxicity. These effects in zebrafish may, therefore, inform the toxicity profile of these chemicals in mammals. *This abstract does not reflect USEPA policy.*

4.11.P-Mo-087 Rapid Assessment Bioaccumulation Screening: Utilizing Surface Water to Assess Bioaccumulation & Health Outcomes for Emerging Per- & Polyfluoroalkyl Substances Mixtures that Lack Analytical Standards

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Emerging per- and polyfluoroalkyl substances (PFAS) identified in environmental matrices often lack commercially available standards. Analytical grade standards are necessary for quantitative research approaches for assessing health outcomes, bioaccumulation potential, and dose-response toxicological endpoints. However, the sheer number of emerging PFAS being identified, combined with challenges of chemical synthesis, impedes the ability to prioritize and synthesize PFAS for studies. To overcome the lack of individual analytical standards, we propose an *in vivo* design to investigate the bioaccumulation potentials and adverse health effects of environmentally relevant mixtures of emerging PFAS which in turn will facilitate chemical prioritization. Specifically, bioaccumulation is a key metric that can help prioritize PFAS for synthesis as increased bioaccumulation *in vivo* may indicate higher potential for toxic effects due to greater uptake and longer residence time in tissues. This study used weak-anion exchange to concentrate hundreds of liters of industrially impacted surface water containing a complex mixture of novel PFAS from a New Jersey PFAS manufacturer. Male CD-1 mice were dosed once daily by oral gavage for 7 days with concentrated surface water extract in 2% Tween 80 solution at 100X, 300X, 500X and 1000X (n = 6/group) of the original surface concentration, as well as a vehicle control (DI water and Tween 80). After a 24-hour depuration, serum, liver, and brain were collected for analysis using high resolution mass spectrometry (HRMS) nontarget analysis (NTA) to characterize emerging PFAS and obtain pseudo bioaccumulation factors ($\mathrm{BAF}_{\mathrm{psuedo}}$). Both legacy and emerging PFAS were identified in the surface water extract. Twelve emerging PFAS were subsequently detected in liver, with notable dose-dependent increases in mouse tissues. To determine whether exposure to the concentrated surface water resulted in adverse health outcomes, sensitive endpoints of PFAS toxicity, including body and tissue weight, thyroid hormone assessment, hepatic nuclear receptor target gene expression, and serum/liver metabolomics were assessed. Health effects were noted on multiple endpoints at all doses examined. The RABS study format allows the investigation of PFAS mixtures for which

commercial standards are not available, and this study demonstrates the feasibility of using this design to measure bioaccumulation and health effects of complex environmental mixtures.

4.11.P-Mo-088 From Trend to Discovery: Temporal Analysis of Contaminants in Watersheds Using High Resolution Mass Spectrometry

*Karl Oetjen and **Kendra Adams**, SCIEX*

The vast array of emerging organic contaminants and their diverse properties make complete characterization using traditional mass spectrometry methods challenging, thus requiring new analytical approaches with broader molecular coverage. Nontarget screening using high-resolution mass spectrometry (HRMS) is crucial for discovering unknown compounds in environmental samples without prior information—a significant challenge considering that the United States Toxic Substances Control Act lists about 85,000 chemicals. A recent study in Europe demonstrated the power of combining targeted, suspect, and nontarget screening using HRMS in monitoring the Rhine River. This approach identified 90 targeted compounds and 10 major spill events of previously undetected compounds, leading to corrective measures to prevent future pollution. However, this method has not yet been implemented in the United States, and its effectiveness as a monitoring tool in smaller suburban watersheds remains unproven. This presentation will communicate the temporal trends in a small stream (that functions as a popular recreational waterway) using data reduction techniques via enviMass software. Unknown spectral features with distinct temporal trends were identified based on intensity patterns and a comprehensive inventory of chemicals that may impact receiving waters and human health were identified.

4.11.P-Mo-089 Associations Between Exposure to OPEs and Rheumatoid Arthritis Risk Among Adults in NHANES, 2011-2018

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Rheumatoid arthritis (RA) has an intricate etiology that includes environmental factors as well as genetics. Organophosphate esters (OPEs) are frequently used as chemical additives in many personal care products and household items. However, there has been limited research on their potential effects on rheumatoid arthritis (RA). The specific associations between OPEs and RA remain largely unexplored. The objective of this study intended to investigate any potential associations between adult rheumatoid arthritis risk and exposure to OPEs. We investigated data from the National Health and Nutrition Examination Survey (NHANES) 2011–2018 among participants who were over 20 years old. In two models, multivariable logistic regression was utilized to investigate the relationship between exposure to OPEs and RA. Furthermore, subgroup analyses that were stratified by age, gender, and dose exposure response were evaluated. Generalized additive models and smooth curve fits were used to characterize the nonlinear relationship between RA and OPEs. In conclusion, 5490 individuals (RA: 319, Non-RA: 5171) were analyzed. Higher quantiles (Q4) of DPHP and DBUP showed a higher prevalence of RA than the lowest quartile. It was evident from our findings that adult RA prevalence is higher in those who have been exposed to OPEs. Interestingly, these correlations seem to be stronger among women, the elderly, those with higher BMIs, and those who have diabetes. The dose-response curve for BCEP shows a U-shaped relationship with the probability of RA. The dose-response curve for BCPP shows an inverted U-shaped relationship with the probability of RA. The dose-response curve for DBUP demonstrates an upward sloping trend. The dose-response curve for DPHP indicates a positive association with the probability of RA. The dose-response curve for BDCPP shows a complex relationship with a peak at lower concentrations followed by a decrease. The Weighted Quantile Sum (WQS) regression analysis was conducted to assess the contribution of various exposures to the overall WQS index. The WQS index concluded that DPHP and DBUP are the primary exposures driving the RA.

.11.P-Mo-090 INQUIRE - Improving Indoor Air Quality and Health: Identification of Chemical and Biological Determinants, Their Sources, and Strategies to Promote Healthier Homes in Europe

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Air pollution is a driver for early death risk, and air quality standards for a range of pollutants in outdoor air have thus been established. However, the indoor environment may be more relevant for exposure as Europeans spend up to 90% of their time indoors, and concentrations of many air pollutants are higher indoors than in urban outdoor air. Existing indoor air quality (IAQ) guidelines are limited, and the evidence-based consensus is that the determinants of IAQ are more numerous and complex than current guidelines account for. To address this, we are deploying a comprehensive R&I program, including nonintrusive monitoring in residential indoor environments. We are gathering personal data, IAQ sensor data, air samples for volatile and semi-volatile organic compounds (indoors + outdoors), dust samples, and urine samples in 8 diverse European countries. In each country, 25 families and homes are selected, prioritizing homes with young children. This will yield a total of 200 family/home pairs across Europe. To provide a global context, parallel sampling will be conducted in Australia. The study will also carry out supplemental sampling to enable identification of sources of the chemical and biological determinants by screening three source categories: (1) The outdoor environment, (2) The built structure, and (3) Furnishings, consumer products, and personal behaviours. The collected samples will be subject to suspect and nontarget screening for volatile and semi-volatile organic chemicals and microbiome, and target analysis of chemicals and allergens. Also, comprehensive toxicological effect-studies, including in-vitro effect screening and assessment of respiratory toxicity, endocrine disruption, and impaired immunity; and in-vivo validation of effects of local and systemic toxicity will be carried out. AOPs and virtual EDA will be applied to identify risk drivers of identified toxicities and key events of these. Following up on the initial phase, we will revisit homes to implement strategies for improving IAQ based on air purifier and ventilation systems, behavioural changes, renovations, and use of consumer products. With the findings from this study, we will fill knowledge gaps on the identity, sources, exposure and health risks of hazardous chemical and biological determinants in residential indoor environments and provide effective strategies to reduce the risk for residents. All data generated in the study will be made available as FAIR data.

4.11.P-Mo-091 Effects of Pharmaceutical Exposure on Wild Fish Health: A Survey of Red Drum Across Florida Estuaries

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Fish health is key to maintaining fisheries and the ecosystem services they provide, yet it is not typically accounted for in fisheries management. Blood analyses can reflect fish condition by showing evidence of toxicity, physiological stress, and tissue damage. Pharmaceutically active compounds and their metabolites have been recognized as an important class of emerging contaminants in aquatic environments because they have been found at concentrations that may have the potential to elicit adverse effects in fish. In Florida, there is increased evidence of the presence and risk of pharmaceuticals in coastal areas. Because of the potential of adverse sublethal effects, we hypothesized that pharmaceutical exposure would lead to deteriorated health in exposed wild fish. Red Drum are an excellent indicator species for pharmaceutical toxicity due to its presence along the entire Florida coast and various behaviors subjecting them to localized environmental conditions, including benthic foraging in sediment, low mobility, and high site fidelity. In this study, nine different estuaries located along Florida's coasts were sampled for Red Drum (*Sciaenops ocellatus*), an economically important sport fish. The blood of 101 different fish were assessed through hematological and biochemical analyses as well as for 94 common pharmaceuticals (e.g., antibiotics, psychoactive, and cardiovascular medication). We focus on establishing blood analyte reference intervals, evaluating hematological and biochemical health analytes, and understanding the risk of pharmaceuticals to wild fish. Preliminary results show Red drum condition across the state is under average, and biochemical parameters reveal stress in the populations. Biochemical health assemblages across populations are similar, except for 2 populations with high quantities of pharmaceuticals having distinct health assemblages. We found correlations between pharmaceutical exposure and certain blood biomarkers. There is a marginal relationship between liver function and damage with increasing quantities of pharmaceuticals.

4.11.P-Mo-092 Assessing the Impact of Increased Levonorgestrel Exposure on Surface Water Pathogen Detection

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Levonorgestrel-based contraceptives represent significant advancements in reproductive health technology. Increased accessibility, such as recent over-the-counter (OTC) approval by the FDA on July 13, 2023, for a daily oral product with no age restrictions, represents even greater strides. However, this accessibility could increase pharmaceutical residues in surface waters. Recent studies have shown that high levels of endocrine disruptors and synthetic hormones found in contraceptives such as ethynylestradiol can alter gene expression in surface water pathogens which has the potential to impact their detection. This study investigates the impact of levonorgestrel, a synthetic progestin on the detection of *Escherichia coli*and *Pseudomonas* spp. in efforts to confirm the hypothesis that increased levonorgestrel concentrations interfere with the detection of these microbes. Through controlled laboratory experiments, bacterium cultures are exposed to a gradient of levonorgestrel concentrations ranging from 0.1 ng/L to 1000 ng/L.

The interference of levonorgestrel with bacterium detection is assessed using Polymerase Chain Reaction (PCR) for DNA amplification efficiency and enzyme activity assays for microbial viability. This approach helps to determine the concentration thresholds at which levonorgestrel begins to interfere with bacterium detection and identification, providing vital insights into the compound's ecological and public health implications. The findings aim to inform future water quality monitoring and management strategies, emphasizing the need for updated guidelines to address the presence of pharmaceuticals and pathogen detection in water systems.

4.11.P-Mo-093 Advancing Harm Reduction Strategies in Ontario: Analysis of Opioid Consumption through Wastewater-Based Epidemiology in the Durham Region

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The opioid crisis, intensified by the COVID-19 pandemic, remains a severe public health challenge in Canada, with Ontario observing a steady increase in opioid-related harms for more than a decade, including a significant increase in the Durham Region with 129 deaths in 2021, seven times the number in 2013. This epidemic, therefore, demands informed and targeted interventions, and acquiring accurate data on opioid usage is critical for implementing effective harm-reduction strategies. Traditional monitoring methods of opioid consumption, such as clinical overdose data, are often expensive, cumbersome, and subject to potential biases. As an alternative, this study explores the application of wastewater-based epidemiology (WBE), a novel approach to assess drug consumption by analyzing municipal wastewater for drugs and their metabolites. We employ WBE to monitor opioid consumption patterns within the Region of Durham, leveraging its capability to provide non-invasive, comprehensive, and real-time data. The selected opioids for this study include morphine, codeine, oxycodone, fentanyl, hydromorphone, methadone, and heroin, as these represent the compounds most closely associated with opioid-related mortalities within Ontario. Our methodology involves collecting 24-hour composite wastewater samples from six regional treatment plants, analyzed through liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF). This approach not only allows for accurate quantification of opioids but also enables the identification of spatial and temporal patterns in opioid usage. The anticipated outcomes of this research include an enhanced understanding and management of the opioid crisis in the Durham Region. By integrating WBE with traditional monitoring methods, we expect to provide more detailed and timely insights into opioid consumption trends, contributing to more effective public health strategies and harm reduction efforts.

.12.P-Th Legacy and Emerging Pollutants in the Environment: Current Trends in the Developing World

4.12.P-Th-047 Potentially Toxic Elements Contamination and Risk Assessment in Paddy Soil-Rice System in a Semi-Deciduous Forest Zone of Ghana

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Food security is a crucial issue for sustainable global development. Potentially toxic element including As, Cd, Cr, Cu, Hg, Pb, Ni and Zn contamination in crops, particularly rice, threatens food security and affects human health leading to morbidity and mortality. This research therefore ascertained the concentrations, bioaccumulation, translocation, and associated human health risk of potentially toxic elements through consumption of rice across paddy fields in the semi-deciduous forest zone of Ghana including Nobewam, Sokwe, Asotwe and Ejura. A total of 205 composite soil and 205 rice samples were collected and analyzed for metals and metalloids by x-ray florescence (XRF) and inductively coupled plasma mass spectrometry (ICP-MS). To ensure precision and accuracy replicate analyses were conducted along with certified reference materials (NIST 1575a for rice and NIST 2711a for soil). The mean elemental concentrations in rice grain samples decreased in the order Cd > Zn > Ni > Cu > Hg > Pb > As > Cr while the mean elemental concentrations in paddy soil decreased in the order Cr > Zn > Ni > Cu > As > Cd > Hg > Pb. The mean concentrations of As, Cd, Ni, Pb and Hg in rice grain exceeded the WHO/FAO permissible limit. Pollution indices including contaminated factor (CF), geoaccumulation index (Igeo), enrichment factor (EF) and potential ecological risk (PERI) indicated that the soils in the study areas were highly contaminated with Cd and considerably contaminated with As, especially in Nobewam. Bioaccumulation and translocation factors showed that accumulation of potentially toxic elements into rice in the study areas were low except Cd which recorded very high accumulation and considerable accumulation for Zn, Cr and As. Ongoing work include determining in vitro bioaccessibility (IVBA) for a subset of the rice samples. The IVBA data along with the concentrations of the elements will be used to determine the overall carcinogenic and non-carcinogenic risks associated with ingestion of rice from each paddy rice field. Finally, recommendations on limiting exposure to potentially contaminated soils and rice will be recommended and additional research to ascertain the implications for human health and methodology reduce the elemental uptake and translocation from soil to rice grain in these localities.

4.13.A.T Mercury Bioaccumulation and Effects on Wildlife: Ecological Pathways, Cycling, and Risk

4.13.A.T-01 Spatial and Temporal Patterns in Soil Mercury Reservoirs of the United States

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Soils store globally significant quantities of mercury and have been enriched from their natural state as a result of human activities. The timescales over which soil mercury is released to the atmosphere and downstream aquatic systems can vary from less than a year to greater than centuries, extending the surface lifetime of anthropogenic mercury releases. Interpreting environmental responses to changing global and domestic mercury emissions therefore requires improved understanding of how mercury cycles in soils. Here, we present recent work examining the temporal and spatial patterns of mercury in soils from the contiguous United States. Temporal changes in soil mercury were examined in a forest floor record spanning 50 years. Across organic horizons mercury concentrations were found to decrease alongside declines in modeled mercury deposition. Mercury concentrations were highest at the beginning of the record (1969) and the most pronounced decreases were observed in litter layer concentrations. Spatial patterns were examined using a country-wide soil survey of ~4,800 sites. Mercury content was found to vary by landcover type and was greatest in forested regions and croplands. Total mercury stocks in the top 100 cm of the conterminous United States were found to be 158 ± 2 Gg (mean ± SD), the majority of which (62%–95%) was unexplained by contributions from parent material. We conclude with insights from ongoing mercury stable isotope analyses of U.S. soils to assess qualitative differences in temporal and spatial mercury trends.

4.13.A.T-02 National Assessment of Methylmercury in Adult Amphibians

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Mercury (Hg) is a toxic contaminant that has been mobilized and distributed worldwide and is a threat to many wildlife species. Amphibians are facing unprecedented global declines due to many different stressors including contaminants. While the biphasic life history of many amphibians creates a potential nexus for methylmercury (MeHg) exposure in aquatic habitats and subsequent health effects, the broad-scale distribution of MeHg in amphibians remains understudied. We used nonlethal sampling to assess MeHg bioaccumulation in over 3000 juvenile and adult amphibians during 2017–2021. We sampled 26 populations (14 species) across 11 US states, including several imperiled species that could not have been sampled by traditional lethal methods. We also examined whether life history traits or total Hg in sediment or dragonflies could be used as indicators of MeHg bioaccumulation in amphibians. Methylmercury contamination was widespread, with a 33-fold difference in concentrations across sites. Variation among years and clustered subsites was less than variation across sites. Life history characteristics such as size, sex, and whether the amphibian was a frog, toad, newt, or other salamander were the factors most strongly associated with bioaccumulation. Total Hg in dragonflies was a reliable indicator of bioaccumulation of MeHg in amphibians, whereas total Hg in sediment was not. Our study, the largest broad-scale assessment of MeHg bioaccumulation in amphibians, highlights methodological advances that allow for nonlethal sampling of rare species and reveals immense variation among species, life histories, and sites. Our findings can help identify sensitive populations and provide environmentally relevant concentrations for future studies to better quantify the potential threats of MeHg to amphibians.

4.13.A.T-03 Linking Patterns of Atmospheric Mercury Deposition with Bioaccumulation in Aquatic Ecosystems at a National Scale

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Despite decadal declines in atmospheric mercury emissions and deposition, clear linkages with similarly trending biotic concentrations in the environment remain elusive. We evaluated interannual temporal patterns in biosentinel dragonfly larvae mercury concentrations from more than 100 sites in protected lands (e.g. national parks, national wildlife refuges, and national forests) across the US, and also assessed the relationships between watershed level atmospheric deposition and dragonfly larvae mercury concentrations from more than 700 paired site-year measurements across the US. Preliminary findings suggested high variability in site-specific temporal trends of dragonfly mercury concentrations, with some sites decreasing, some increasing, and some with no directional trends. Additionally, at the site-scale there were no relationships between paired wet Hg deposition or precipitation Hg concentrations and mercury concentrations in dragonfly larvae, suggesting a decoupling between delivery of atmospheric inorganic mercury to a waterbody and the amount of mercury accumulating through the food web. A substantial proportion of this variability can be explained by site-specific biogeochemical characteristics that regulate mercury bioavailability and methylmercury production. However, when accounting for variability associated with individual sampling locations, we found that at a national scale, annual average dragonfly mercury concentrations declined by approximately 16% between 2011 and 2020, and were highly correlated with average annual Hg concentrations in precipitation, which similarly declined by 19% over the same time frame. Collectively, our preliminary findings indicate that despite considerable site-level variation in relationships between Hg deposition and bioaccumulation, when integrated at a national scale, temporal patterns in mercury bioaccumulation are consistent with patterns in the amount of mercury delivered in precipitation.

4.13.A.T-04 Comparing Mercury Sources and Bioaccumulation Dynamics Across Lake Huron and Lake Ontario

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In the Great Lakes, mercury (Hg) is a persistent concern for human and wildlife health due to heightened methylmercury (MeHg) food web bioaccumulation. Although the Great Lakes have some of the lowest aqueous MeHg concentrations in the world (~6 pg L^{-1}), they display one of the highest bioaccumulation rates, reflected by fish advisories across the lakes. Despite declines in atmospheric Hg emissions, assessments of fish tissue have observed either unchanged or increasing Hg concentrations in game fish, although the exact drivers of the variable bioaccumulation are unknown. Inherently, each lake receives different Hg source inputs and exhibits unique water quality characteristics (e.g., trophic status, microbial activity, dissolved organic carbon content), motivating the need for tailored bioaccumulation assessments for each lake. In this study, we compare source portfolios and benthic and pelagic bioaccumulation patterns in Lake Huron (LH) and Lake Ontario (LO) to understand how differences in water quality and land use in the surrounding watersheds impact bioaccumulation. Hg stable isotopes revealed that LH sediments were predominantly influenced by atmospheric Hg inputs in offshore areas and nearshore zones contained mixed watershed and industry Hg contributions. In contrast, LO sediments had comparatively elevated Hg concentrations and were dominated by industrial Hg sources across the lake. Considering the lower food web, LH mussels and seston were on average 11.3±1.8 ng g^{-1} and 20.5±11.0 ng g^{-1}, respectively, but we found that nearshore seston had 3.3 times higher MeHg than offshore collections. Industrial contamination in nearshore LH sediments were not reflected in the pelagic food web, possibly due to reduced bioavailability or methylation of Hg in shallow embayments. We hypothesize that LO seston will be similarly influenced by nearshore and offshore ecosystem gradients, however biota may be overall higher in comparison due to the elevated proportions of legacy Hg in LO sediments. Our observations identify regions vulnerable to elevated Hg bioaccumulation (e.g., nearshore areas) within LH and LO, essential information for determining effective long-term management and remediation strategies for Hg in Great Lakes' food webs.

4.13.A.T-05 Sources of Bioavailable Mercury Along the Southern Shoreline of Lake Ontario

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In aquatic systems, mercury cycles readily and may become mobilized into the food chain by disturbances, such as flooding or erosion, among others. Despite a decreasing trend in fish mercury burden since the early 2000s due to policies restricting emissions, some species are experiencing increasing burdens once again in Lake Ontario. We suggest that nearshore disturbances, namely extensive cattail wetlands from water level regulation, and *Cladophora* mat growth due to the onset of invasive dreissenid mussels and the nearshore shunt, have created sites of elevated mercury bioavailability. In the summer of 2023 cattail wetland detritus, *Cladophora*, and sediment were collected from the southern Lake Ontario shoreline to confirm wetlands and *Cladophora* biomass as sites of elevated total and methylmercury. Total and methylmercury concentrations in cattail detritus were compared between a set of restored and unrestored wetlands. Additionally, location within the wetland was compared between the terrestrial interface and the wetland middle. Three different types of *Cladophora* were compared: (1) living, sloughed *Cladophora* in littoral zone, (2) dried on the shoreline, and (3) living attached to substrate, such as a pier, or rock. Sandy sediments were collected as a natural "control" treatment comparison. Concentrations of the *hgcA* gene, which is responsible for microbial mercury methylation, alongside measurements of the microbial community composition via16S rRNA gene sequencing across these materials allows for a comparison of how much and which microbes within each material methylate mercury. Understanding the consequences of these nearshore disturbances will help inform fish contaminant monitoring efforts.

4.13.A.T-06 Building a National-Level Mercury Biomonitoring Program with People and Parks and the Dragonfly Mercury Project

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One of the most recognized citizen science projects across the U.S. National Park Service, the Dragonfly Mercury Project (DMP) is a decade-long, nationwide assessment that uses dragonfly larvae as indicators of mercury risk in national parks. Mercury is a neurotoxin that harms human and wildlife health. The DMP engages public (particularly youth) through active field sampling and data collection to support providing actionable information about mercury in freshwater ecosystems to reduce risk to public health. It also informs policy and resource management, and advances science and public understanding of mercury pollution. Spanning more than 700 water bodies across 48 states, mercury concentrations in over two-thirds of sites indicate that predatory fish may exceed EPA benchmarks for human health. In addition to quantifying risk, long-term records also contribute to spatial and temporal models of mercury across the U.S., feeding into our understanding of the effectiveness of both domestic and global mercury emission reduction policies and informing how to mitigate mercury risk. Developing educational opportunities and informing policy, the DMP demonstrates how participatory science can lead to more effective research outcomes by integrating scientific expertise with local knowledge and community engagement. The program prospers on an integrated design, or a "network of networks" where participant groups, including resource managers, scientists, schools, and local community members are linked together to make the study possible. Park engagement continues to spark the roughly 7,000 public participants contributing greater than 20,000 volunteer hours to the study. Data integrity is accomplished through standardized sampling procedures coupled with a centralized laboratory that employs robust quality assurance measures and high-quality data documentation. The diversified funding model amplifies the program's resiliency, while the participatory science component leverages new and existing partnerships and reduces the costs of mercury monitoring. By fostering interdisciplinary collaboration and making science more accessible and engaging, programs like the DMP can have a positive impact on multiple audiences, including the next generation of environmental stewards.

4.13.B.T Mercury Bioaccumulation and Effects on Wildlife: Ecological Pathways, Cycling, and Risk

4.13.B.T-01 Marine Resource Use Increases Mercury Exposure in Alaskan Wolves

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Although all wolves (*Canis lupus*) express a high degree of dietary plasticity, the coastal wolves in Southeast Alaska, USA (*Canis lupus ligoni*) have demonstrated an extremely diverse diet dominated by ungulates but including marine resources opportunistically throughout their range. Recent studies have shown a drastic dietary shift towards sea otters (*Enhydra lutris*) in the region near Glacier Bay National Park and Preserve (GBNPP), specifically for the wolves inhabiting Pleasant Island and the Gustavus Forelands. Sea otter populations were decimated by the fur trade prior to their protection in 1911. Following their reintroduction to Southeast Alaska in the 1960s, sea otters have since increased in numbers, especially near GBNPP. This recovery has allowed the ranges of sea otters and wolves to overlap, resulting in a shift in wolf diet from terrestrial herbivores (ungulates such as deer and moose) to a marine predator (sea otters) in certain locations. As monomethylmercury is produced and biomagnifies mainly in aquatic food webs, we expected to find higher concentrations of total mercury in wolves with strong connectivity to marine food webs. To determine baseline contamination and identify the trophic transfer of mercury, we measured total mercury concentrations and the stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values) in fur from wolves and sea otters, as well as in the tissues of intertidal fishes and invertebrates from Pleasant Island and the Gustavus forelands. We also compared $\delta^{13}C$ and $\delta^{15}N$ values and total mercury concentrations of Pleasant Island/Gustavus wolves ($n = 26$) with data from wolves ($n = 301$) from other locations in Southeast Alaska, Denali National Park, and Yukon-Charley Rivers National Preserve. Our results indicate substantial variation in fur total mercury concentrations (0.05 to 17.1 ppm), $\delta^{13}C$ (-26.4 to -13.9‰), and $\delta^{15}N$ (4.5 to 14.0‰) values across regions. Both $\delta^{13}C$ and $\delta^{15}N$ values were correlated with fur total mercury concentrations demonstrating that dietary reliance on marine resources is a major driver of mercury exposure for wolves. Due to their predation of sea otters, wolves from Pleasant Island had the highest total mercury concentrations, which may ultimately affect their health.

4.13.B.T-02 Implications of Coastal Glacial Retreat for Mercury Export and Accumulation in Near Shore Food Webs

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Coastal ecosystems in high latitude regions are especially vulnerable to the adverse impacts of climate change as a result of disproportionately high rates of warming driven by polar amplification. This is particularly evident in Alaska, where increasing temperatures are causing rapid retreat of coastal glaciers, which are known to release elements such as mercury (Hg) into downgradient aquatic environments. Subsequent microbial transformation processes that occur in aquatic systems can convert Hg to methylmercury (MeHg), a highly neurotoxic and bioaccumulative form of Hg present in fish muscle. MeHg also biomagnifies and can reach potentially toxic levels in the muscle of high trophic level and/or long-lived fish species. Because microbial production of MeHg proceeds at a rate that is dependent on biochemical conditions (which are also altered by climate-driven factors), climate change may increase the degree of MeHg production and uptake into marine food webs, with potential implications for both ecological and human health. We previously found a positive correlation between percent glacial coverage and total Hg (tHg) concentrations in mussels (*Mytilus trossulus*) collected from various watersheds in the Gulf of Alaska (GoA), which supports a number of recreationally and commercially important fisheries species. These species also serve as a primary food source for remote subsistence communities, which are already considered to be especially vulnerable to dietary MeHg exposure. Therefore, the goal of the present study was to further investigate the potentially important links between coastal glacial retreat and tHg burdens in long-lived piscivorous fish from the GoA. The black rockfish (Sebastes melanops) was selected as a model for the present study, due to its high site fidelity within near shore habitats, long lifespan, relatively high trophic position, and consumption by humans. We analyzed tHg in muscle samples collected between 2018 and 2023 from four distinct regions in the GoA, each characterized by varying degrees of glacial influence. Results show important temporal and spatial differences in tHg concentrations in black rockfish muscle, with higher concentrations generally present in fish collected from areas with a higher degree of glacial coverage. These findings provide additional support for a link between climate-driven changes in glacial retreat and Hg cycling in coastal food webs.

4.13.B.T-03 Examining Controls on Mercury Methylation and Bioaccumulation Within Everglades National Park

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Mercury (Hg) contamination has been a persistent concern in the Florida Everglades for over three decades due to elevated atmospheric deposition and the system’s propensity for methylation and rapid bioaccumulation. Given declines in atmospheric Hg concentrations in the conterminous United States and efforts to mitigate nutrient release to the greater Everglades ecosystem, it is vital to assess how Hg dynamics respond on temporal and spatial scales. This study used a multimedia approach to explore Hg delivery, methylation, and bioaccumulation across air, water, fish, and invasive Burmese pythons across Everglades National Park. Hg concentrations across matrices showed air, water, and biota from the system were inextricably linked. Temporal patterns in water and fish were driven primarily by hydrologic and climatic changes in the park and no evidence of a decline in atmospheric Hg deposition from 2008-2023 was observed. In the Shark River Slough (SRS), elevated dissolved organic carbon and sulfate were consistently delivered from upgradient canals, which promoted Hg methylation. Within the SRS a strong positive correlation was observed between methylmercury (MeHg) concentrations in surface water and resident fish. In addition, similar patterns between sulfate and MeHg were observed in waters from coastal sites due to saltwater intrusion. Within distinct geographic regions of the Everglades (SRS, Marsh, Coastal), the geochemical controls on MeHg dynamics differed and highlighted regions susceptible to higher MeHg bioaccumulation, particularly in regions influenced by upgradient canals and saltwater intrusion. Data further demonstrated pythons collected from SRS had the highest concentrations observed within the Everglades, reaching levels 60 times higher than the recommended Hg consumption limit. Eastern regions and marsh sites within the park had lower Hg concentrations in water, fish, and pythons when compared to SRS. This study demonstrates the strong influence that dissolved organic carbon and sulfate loads have on spatial and temporal distributions of MeHg across the Everglades. Importantly, improved water quality and delivery are two key restoration targets of the nearly 30-year Everglades restoration program, which if achieved, this study suggests would lead to reduced MeHg production and exposure.

4.13.B.T-04 Dietary Transfer of Mercury from Native and Non-native Apple Snails and Daily Mercury Intake Estimates for Everglade Snail Kites in South-Central Florida, USA

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The Everglade snail kite (*Rostrahamus sociabilis)* is a federally endangered raptor with a dietary preference for apple snails (*Pomacea* sp.). Historically, Everglade snail kites preyed on native Florida apple snails (*P. paludosa*), but due to widespread increases in non-native apple snails, Everglade snail kites have shifted their diets to include the non-native snail (such as *P. maculata*). Concentrations of mercury (Hg) in native and non-native apple snails and snail kites have not been extensively studied. Therefore, we measured concentrations of total Hg (THg) (n = 94) and methyl Hg (MeHg) (n = 44) in apple snails and THg in nestling Everglade snail kite feathers (n = 111) across south-central Florida. Mean (+/- 1SD) THg and MeHg concentrations for native and non-native apple snails were 121 +/- 59 ng/g dry weight (dw) and 71 +/- 44 ng/g dw, respectively. Mean THg concentration in nestling Everglade snail kite feathers was 319 +/- 285 ng/g dw. Nestling Everglade snail kite feather THg concentrations increased with apple snail Hg concentrations for shared sampling sites (THg: $R^2 = 0.84$, MeHg: $R^2 = 0.90$). We estimated the potential daily intake of Hg for adult Everglade snail kites foraging on apple snails within their average prey size selection range (30-65 mm shell length) and above their average prey size selection range (> 65 mm shell length). Using apple snail Hg concentrations from the present study and previously published bioenergetic data for three classes of adult Everglade snail kites (pre-zygotic, laying, and non-breeding), we found laying female Everglade snail kites to have the highest potential daily Hg intakes. However, all potential daily Hg intakes for kites were below thresholds associated with adverse effects, suggesting that Everglade snail kites are at low Hg risk when foraging on native or non-native snails within or above their average prey size selection range.

4.13.B.T-05 Examining Hg Sources and Hydrologic Factors Impacting Hg Bioaccumulation in Invertebrates of Great Salt Lake, Utah

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Though Great Salt Lake (GSL) is hypersaline and does not support fish, GSL invertebrates, including brine shrimp, brine flies, and spiders, are key prey items for millions of resident and migratory bird species. However, elevated methylmercury (MeHg) has been documented in GSL waters, particularly in the Deep Brine Layer (DBL) where values can exceed 30 ng/L. These elevated MeHg concentrations in the water column have been attributed to elevated concentrations observed in invertebrates and several waterfowl species, resulting in consumption advisories. MeHg bioaccumulation within GSL poses a serious exposure risk to both waterfowl and humans, but sources of Hg bioaccumulating in GSL food webs and the impacts of hydrologic changes (such as the presence/absence of the DBL and lake level fluctuations) on MeHg bioaccumulation are understudied. Here, we leverage Hg concentration and stable isotope measurements on brine fly, brine shrimp, and Western Spotted Orb Weaver spiders to examine temporal changes in Hg delivery and processing within GSL. Mercury (Hg) concentrations in brine flies are inversely correlated with lake level and, by extension, salinity. This may reflect less biodilution during periods of higher salinity where brine fly and brine shrimp population densities are lower, and/or may reflect enhanced bioaccumulation due to stunted growth at higher salinities due to osmoregulatory stress. The presence or absence of the DBL did not have a significant impact on Hg bioaccumulation in brine shrimp or brine flies. Hg stable isotope signatures (Δ^{199}Hg and δ^{202}Hg) in brine shrimp were seasonally dependent and reflect greater photochemical processing in late summer and fall months, likely due to increasing water clarity and light penetration. Examination of the Δ^{200}Hg tracer further indicates that the majority of Hg accumulating in GSL invertebrates is of atmospheric origin, and correspondence of Hg isotope values in co-located brine fly and spider samples confirm the occurrence of aquatic to terrestrial transfer of MeHg at GSL. This study highlights physical, chemical, and biological factors impacting Hg bioaccumulation in lower trophic level biota from GSL, which will help us assess Hg cycling within the system in response to climate and water use driven changes to lake level.

4.13.B.T-06 Rapid Biological Uptake of Water-Column Methylmercury During Destratification of an Arid Land Reservoir

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Thermal stratification and oxygen depletion in lakes and reservoirs facilitates methylmercury (MeHg) production in sub-oxic portions of the water column and play a key role in determining MeHg availability within, and downstream, of some reservoirs. Studying the processes by which this MeHg is incorporated into food webs is critical to understanding the resulting environmental and health risks, as well as identifying potential mitigation strategies. We examined the magnitude and timing of Hg uptake into a reservoir food web relative to stratification conditions by collecting surface water, epilimnetic zooplankton, and two species of fishes (Bluegill, *Lepomis macrochirus*; and Smallmouth Bass, *Micropterus dolomieu*) biweekly over a two-year period. Preliminary findings indicate that filter-passing MeHg concentrations in surface waters increased up to 110% during destratification and that this pulse of MeHg was rapidly incorporated into zooplankton, increasing zooplankton MeHg concentrations by up to 250%.

Zooplankton MeHg concentrations were well correlated with filter-passing MeHg during periods when the reservoir was mixed ($R^2 = 0.95$, $p < 0.001$) or destratifying ($R^2 = 0.57$, $p < 0.001$), but not when the reservoir was stratified ($R^2 = 0.21$, $p = 0.12$). The influence of destratification on fish THg concentrations varied between species and size classes of each species. Concentrations in young of year of both species and adult Bluegill increased 20-70% following destratification, although these increases lagged 4-8 weeks behind increases in water and zooplankton. Mercury concentrations in adult bass were not affected by reservoir stratification status. These data highlight the important role destratification can play in facilitating the uptake of MeHg into reservoir food webs as well as the speed with which these pulses of MeHg can be incorporated into food webs.

4.13.P-Mo Mercury Bioaccumulation and Effects on Wildlife: Ecological Pathways, Cycling, and Risk

4.13.P-Mo-094 Cormorants and Mink as Mercury Sentinel Animals in an Interior Aquatic Ecosystem

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Emissions of global pollutants such as heavy metals are a known One Health issue. In aquatic ecosystems the heavy metal mercury (Hg) exists in its methylated form (MeHg), and it biomagnifies and bioaccumulate up the food web. Two apex-predators, mink (*Neovison vison*) and cormorant (*Phalacrocorax carbo sinensis*) in Innlandet county, Norway, were used as sentinel animals as they consume the same fish as humans. The goal for this study was to investigate if various body measures (weight, chest girth, length, or wingspan) were associated with [THg], and could be used as a measure for bioaccumulation. From 2022 to 2024 hunted and trapped cormorants (N = 52) and mink (N = 56) were collected from local hunters. Necropsies were performed and we sampled liver, fur, and muscle for Hg analyses. Statistical analyses showed no significant associations between [THg] and the different body measures for either species. For cormorants we measured mean ± SD (range) of 12.21± 14.38 (1.92 – 74.8) µg/g for liver [THg], which was higher than the liver [THg] for mink which was had 4.33 ± 5.34 (0.31 – 28.94) µg/g. These findings suggest that body measures do not explain the [THg] on its own and there might be other variables driving the [THg] bioaccumulation. This can include potential [THg] adverse effects in animals with relative high exposure or spatial distribution with localized mercury “hot spots”. We recommend further research into spatial distribution to possibly identify other drivers.

4.13.P-Mo-095 Northern Sea Otters (*Enhydra Lutris Kenyoni*) as Indicators of Changing Mercury Dynamics in Kachemak Bay, Alaska

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Mercury (Hg) is a naturally occurring heavy metal that has adverse health effects at high concentrations in various species. In Alaska, climate change is expected to affect the transport and environmental fate of Hg, which could pose significant risks to wildlife and the coastal communities that rely on healthy natural resources. Top trophic level predators in aquatic ecosystems, such as marine mammals, are more vulnerable to higher concentrations of Hg due to bioaccumulation and biomagnification processes. Northern sea otters (*Enhydra lutris kenyoni*) are a keystone species for the health and diversity of nearshore ecosystems. Their small home range and local foraging makes otters an ideal sentinel species for identifying contaminant and pathogen presence in the local environment and identifying potential spatio-temporal trends. This study sought to (1) quantify total Hg concentrations ([THg]) in hair (n = 31), muscle (n=30), liver (n = 31), kidney (n = 32), brain (n = 23), and whole blood (n = 18), from deceased stranded Northern sea otters from Kachemak Bay, Alaska. Archived samples from 2004-2022 were analyzed to determine how Hg is being stored throughout the body and assess if variation in [THg] is associated with individual intrinsic factors (e.g., sex, age-class). Preliminary results indicate that hair (1.40 ± 0.01, µg/g dry weight) and kidney (1.61 ± 0.03, µg/g dry weight) samples have higher [THg] on average than whole blood (0.05 ± 0.00009, µg/g wet weight) and muscle (1.06 ± 0.03, µg/g dry weight) samples. These data will be the basis for long-term monitoring of Hg, which will benefit local and Alaska Native communities in Kachemak Bay. Additionally, this project will serve to better aid management agencies that monitor these sea otter populations who are susceptible to decline due to contaminant exposure.

4.13.P-Mo-096 Spatial and Temporal Trends of Mercury in Landlocked Arctic Char in the Canadian Arctic. Unraveling the Effects of Climate Warming and Local sources

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Mercury (Hg) is a contaminant of high concern in the Arctic due to elevated levels in top predators and in humans. Remote Arctic lakes are valuable sites for studies of the temporal trends and biogeochemical cycling of Hg, encompassing atmospheric deposition pathways, transformation to methyl Hg (MeHg), and biomagnification in aquatic food webs. Landlocked Arctic char are the only top predators in most high Arctic lakes and serve as a unique sentinel species for changes of inputs of Hg and for impacts of climate change. Long term trends (14-27 collection years) of Hg concentrations in Arctic char are available for 5 lakes on Cornwallis Is. (Resolute, Char, North, Small, Amituk) and for Lake Hazen (northern Ellesmere Is) with earliest samples from 1989 (Amituk). Despite similar atmospheric inputs there are significant lake-to-lake differences in length and δ^{15}N-adjusted Hg concentrations among lakes on Cornwallis Is. Highest mean concentrations are in char from Amituk (1.0 µg/g; 2005-2022) a very remote lake, while Small had the lowest concentrations (mean of 0.10 µg/g). The best explanatory variables for lake-to-lake differences on Cornwallis Is. were dissolved and particulate organic carbon concentrations. While declines in gaseous elemental Hg (GEM) have been observed at the Alert monitoring station in the high Arctic since the mid-2000s, trends in char Hg (δ^{15}N adjusted) over the same period varied from increasing (North +4.5%/yr), to no significant change (< +/-1% /yr; Char, Small, Resolute) to significant declines (Amituk (-4.5%/yr, Hazen (-5.3%/yr). Proximity of the lakes to the airport, roads, municipal waste burning, and diesel electrical power generation (Small, Resolute, Char) did not correlate with Hg concentrations in char muscle but those sources may explain the lack of long term decline compared to the remote lakes. In Lake Hazen, a significant relationship between char Hg and GEM was found. No single climate variable was related to char Hg across all populations, however, snow fall and accumulation were positively correlated with declining Hg in Amituk and Hazen char. In Hazen, char Hg was also correlated with increasing ice free days which may be due to food web shifts resulting in greater pelagic feeding and greater glacier runoff. Significantly increasing length-at-age was found for char in all lakes except Hazen, suggest faster growth rates possibly influenced by greater primary productivity due to warming temperatures.

4.13.P-Mo-097 Spatial Variability in Mercury Concentrations in Fishes and Crabs in the Matagorda Bay System (Texas, USA) with a Focus on the Alcoa/Point Comfort Superfund Site

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Located on the Texas coast, the Matagorda Bay system has several important commercial and recreational fisheries including red drum (*Sciaenops ocellatus*), black drum (*Pogonias cromis*), spotted seatrout (*Cynoscion nebulosus*), southern flounder (*Paralichthys lethostigma*), and blue crab (*Callinectes sapidus*). There is limited information on the spatial variability in mercury (Hg) concentrations for these species within the bay system, especially in the Alcoa/Point Comfort Superfund site in eastern Lavaca Bay. Following the release of Hg into the bay in the 1960s and 70s, the bay area around Alcoa was closed to fishing in 1988 and established as a Superfund site which undergoes annual biomonitoring of Hg concentrations in red drum and juvenile blue crab. A large-scale study is needed to investigate Hg concentrations in other species in the Superfund site and compare them to other areas of the bay system. Using a Direct Mercury Analyzer, this study investigated the total mercury (THg) concentrations in red drum, black drum, spotted seatrout, southern flounder, hardhead catfish (*Ariopsis felis*), and blue crab caught in the Superfund site and compared the values to conspecifics caught in western Lavaca Bay, Tres Palacios Bay, and southern Matagorda Bay (n = 3-50 per species per site). For all species, mean wet weight THg concentrations were between 1.2 and 9.2-times greater in the Superfund site compared to other areas of the bay system. In each area, red drum, black drum, and hardhead catfish had the greatest mean THg wet weight concentrations. 56% of red drum, 54% of the black drum, and 36% of hardhead catfish caught in the Superfund site had a muscle tissue Hg concentration that exceeded the adverse biological effects threshold level of 0.5 µg/g wet weight. In comparison, 2% of red drum caught in Port Lavaca, and 6% of red drum and 40% of hardhead catfish caught in southern Matagorda Bay exceeded the threshold level. In the Superfund site, 32% of red drum, 34% of black drum, and 10% of hardhead catfish exceeded the state Hg human health-based standard of 0.7 µg/g wet weight, while only 2% of red drum exceeded the advisory level in western Lavaca Bay, and in southern Matagorda Bay, 4% of red drum and 18% of hardhead catfish exceeded the advisory level. Overall, THg concentrations are greater in fish and shellfish from the Superfund Site and continued biomonitoring of several fish and shellfish species is advised.

4.13.P-Mo-098 Temporal Analysis of Mercury Concentrations in Five Seabird Species of Northwest Greenland

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Mercury (Hg) contamination poses a significant threat to Arctic ecosystems, with anthropogenic sources contributing to elevated concentrations in terrestrial and aquatic environments. Seabirds, acting as sentinels of marine pollution, provide critical insights into Hg dynamics due to their widespread distribution and high trophic position. Our study assesses temporal trends in Hg concentrations of five seabird species in northwest Greenland from 2010 to 2023, spanning periods before and after the implementation of the Minamata Convention. Blood samples were collected and analyzed for total Hg, as well as carbon and nitrogen stable isotopes which were used to infer dietary patterns. Results reveal significant inter-species differences in Hg concentrations, with murres exhibiting the highest levels. We did not observe consistent temporal trends across species. Variability within species underscores the complex interplay of factors influencing Hg bioavailability. These findings highlight the importance of continued monitoring and research to assess the effectiveness of conservation measures and mitigate the impacts of Hg contamination on Arctic wildlife.

4.13.P-Mo-099 Mercury and Methylmercury Isotopes Reveal Internal Cycling and Detoxification in Dolphins from the Indian River Lagoon, Florida

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Bottlenose dolphins, considered sentinel species in coastal and estuarine systems due to their trophic position, life span, spatial fidelity, and ability to bioaccumulate contaminants, exhibit highly elevated mercury (Hg) levels in the Indian River Lagoon (IRL), a large estuary on the northeast coast of Florida. The IRL watershed, known for having some of the most diverse fauna among North American aquatic ecosystems, has also incurred significant stresses from rapid urbanization, loss of shoreline wetlands and water quality degradation. Although there are no known point source releases of Hg, IRL game fish and especially dolphins bioaccumulate Hg to high levels. Little is known about Hg exposure pathways to dolphins, and to address the information gap, we conducted an assessment of waters, sediments, fish, and dolphins from several reaches of the IRL. In addition, we further examined internal detoxification of Hg within dolphins by analyzing paired muscle, liver, and kidney samples. Total Hg (HgT) in dolphin muscle ranged from 0.8-60 ppm wet weight and spanned a large range in percent methylmercury (MeHg; 3-100%; avg. = 73%). These highly elevated Hg concentrations were independent of spatial trends in HgT in prey and sport fish from IRL. Liver and kidney HgT concentrations were significantly higher than in muscle tissue ($p < 0.0001$), while the percent MeHg in liver (1-13%) and kidney (3-32%) was much lower than in muscle tissue. Determination of the HgT stable isotopic composition of fish and dolphin muscle tissues elucidate reaction processes affecting Hg signatures. δ^{202}Hg values in dolphins were dissimilar to sediments and fish measured within IRL. Further assessment of the δ^{202}Hg in dolphin muscle, liver, and kidney showed a strong correlation with the percent inorganic Hg, similar to studies of other marine mammals and birds, providing evidence of similar internal detoxification processes. To further examine the MeHg detoxification process, a subset of dolphin tissues were analyzed using high energy-resolution X-ray absorption near-edge structure (HR-XANES) spectroscopy. HR-XANES analysis showed MeHg was demethylated step-wise to two inorganic Hg species (Hg-tetraselenolate and nanoparticulate Hg selenide), which was greatest in the liver > kidney > muscle and fractionate δ^{202}Hg. The complementary use of Hg stable isotopes and X-ray speciation highlight the need to consider the metabolism of Hg to assess risk and link to environmental sources of Hg.

4.13.P-Mo-100 Mercury and Selenium Concentrations in Greater Amberjack, Great Barracuda, and Cobia in Texas Waters: Risk Assessment and the Need for a Mercury Advisory

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The Texas Department of State Health Services (TDSHS) can issue a Hg advisory for recreationally caught fish when the mean muscle Hg concentration exceeds the 0.7 µg/g wet weight human health-based standard. Greater amberjack (*Seriola dumerili*), great barracuda (*Sphyraena barracuda*), and cobia (*Rachycentron canadum*) are three commonly caught species which currently do not have a Hg advisory. Because these species are long-living and high trophic level predators, it is expected that they will have a high Hg concentration; as a result, a health advisory may be needed. In addition, Hg advisories regarding fish consumption are issued based on the concentration of Hg in muscle tissue, however, selenium (Se) has a protective effect against Hg toxicity, and it has been argued that the Se:Hg molar ratio and Se health benefit value (HBV_{Se}) should also be used in risk assessment. This study measured the Hg and Se concentration and calculated the Se:Hg molar ratio and HBV_{Se} in muscle tissue of greater amberjack (n = 69), great barracuda (n = 66), and cobia (n = 61) caught off the Texas coast between 2016 and 2023. Samples were analyzed for Hg using a Direct Mercury Analyzer and Se using microwave acid digestion and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis. Mean Hg and Se concentrations (µg/g wet weight) were greatest in amberjack (1.10, 0.967), followed by barracuda (0.966, 0.711), and cobia (0.887, 0.683). There was a positive relationship between the muscle Hg concentration and body length in all species. 87.0% of amberjack samples, 65.2% of barracuda samples, and 60.7% of cobia samples exceeded the Texas human health-based standard. The mean Se:Hg molar ratio was >1:1 in all species suggesting that Se may have a protective effect against Hg toxicity, however, one barracuda and nine cobia had ratios <1:1. For all species there was an inverse relationship between Se:Hg molar ratio and body length. All amberjacks had a positive HBV_{Se} indicating there may be health benefits from consuming this species; however, one barracuda and eight cobias had a negative HBV_{Se} indicating there may be health risks from consuming them. It will be difficult to use the Se:Hg molar ratios and HBV_{Se}s as additional seafood safety criterion for these species due to the variability in the values. Due to the high Hg concentrations and large percentage of individuals that exceeded the Texas human health-based standard, TDSHS should issue a Hg advisory for these species.

4.13.P-Mo-102 Mercury Bioaccumulation, Interactions with Cortisol on Endocrine and Immune Biomarkers, and Maternal Transfer in Elephant Seals

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Adult female northern elephant seals have an extreme life history strategy, traveling long distances to the remote North Pacific Ocean where they forage on deep-ocean fish and squid, and then hauling out on coastal California beaches twice per year where they fast during breeding and molting. We assessed the interactive effects of mercury and cortisol (main mammalian stress hormone) on animal health by quantifying blood biomarkers (thyroid hormones, reproductive hormones, and immune markers) in relation to mercury (skeletal muscle and blood mercury) and serum cortisol concentrations. Mercury and cortisol concentrations had synergistic or additive relationships with multiple endocrine and immune biomarkers, thus providing new evidence on the complex mechanisms that exist between these factors in free-ranging animals. As one example, the patterns observed in the concentrations of the thyroid hormone thyroxine suggested interactive effects of mercury and cortisol, where the direction of the relationship with each factor changed depending on the concentration of the other. Mercury is also transferred by adult females to pups during gestation and lactation, and maternal transfer of mercury was strongly correlated with maternal foraging locations and diving depths. Mercury concentrations were highest in pups of the deepest diving, and farthest offshore females. Moreover, pup lanugo (hair grown *in utero*) mercury concentrations were strongly repeatable among successive pups of individual females, demonstrating relative consistency in both maternal foraging strategies and pup mercury exposure. Mercury concentrations in lanugo were among the highest observed worldwide for young pinnipeds (geometric mean 23.01 mg/g dw, range 8.03–63.09 mg/g dw; n = 373); thus, some pups may be at risk for sub-lethal adverse health effects. Deleterious effects on animals' abilities to maintain homeostasis (thyroid hormones), fight off pathogens and disease (innate and adaptive immune system), and successfully reproduce (endocrine system) can have significant individual and population level consequences. Our results suggest that mercury within mesopelagic North Pacific food webs may also pose an elevated risk to deep-ocean foraging predators and their offspring.

4.13.P-Mo-103 Mercury Concentrations in Biota from the Alcoa/Point Comfort Superfund Site (Lavaca Bay, Texas)

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Between 1966 and 1979, the Aluminum Company of America (Alcoa) released mercury (Hg) into Lavaca Bay, resulting in the closure of the bay next to the facility to fishing in 1988. The contaminated area is a Superfund site and Hg concentrations in red drum (*Sciaenops ocellatus*)

and juvenile blue crab (*Callinectes sapidus*) have been monitored annually since 1996. Other important commercial and recreational fish and shellfish species, as well as their prey and predators, have not been examined. Using a Direct Mercury Analyzer, this study measured the total Hg (THg) concentration in marsh grass (*Spartina alterniflora*), bivalve mollusks [hooked mussel (*Ischadium recurvum*), eastern oyster (*Crassostrea virginica*)], crustaceans [grass shrimp (*Palaemonetes sp.*), white shrimp (*Litopenaeus setiferus*), fiddler crab (*Uca longisignalis*), blue crab], teleost fishes [red drum, black drum (*Pogonias cromis*), spotted seatrout (*Cynoscion nebulosus*), southern flounder (*Paralichthys lethostigma*), Gulf menhaden (*Brevoortia patronus*), Atlantic croaker (*Micropogonias undulatus*), striped mullet (*Mugil cephalus*)], and cartilaginous fishes [bull shark (*Carcharhinus leucas*), scalloped hammerhead shark (*Sphyrna lewini*), cownose ray (*Rhinoptera bonasus*)] collected from the Superfund site in 2022 and 2023 (n = 5-30 per species). In addition**,** carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) stable isotope ratios were measured to examine food web ecology. Mean THg concentrations (µg/g dry weight) were greatest in sharks, red drum, black drum, and blue crab (2.56 – 2.84), and lowest in grass shrimp (0.112) and marsh grass (0.0038). Overall, higher trophic level organisms had greater THg concentrations than lower trophic level organisms, indicating that Hg is biomagnifying in the Superfund site. Mercury concentrations in the examined species are greater in the Superfund site compared to other areas of Lavaca Bay and Matagorda Bay. Future research needs to determine environmental Hg concentrations and exposure pathways.

4.14.P-Tu Methods for Assessing Environmental Fate and Effects of Difficult-to-Test Substances

4.14.P-Tu-145 Considerations for Accurate Sampling, Extraction, and Analysis of Cyclic Volatile Methylsiloxanes (cVMS) in Snow

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The potential for cyclic volatile methyl siloxanes (cVMS) to enter remote ecosystems via long-range atmospheric transport and back deposition has been long debated. In this context, snow is an important compartment to study as deposition via snowfall has been suggested as a possible driver for the concentrations of cVMS in the terrestrial and marine environment in cold regions such as the Arctic. Snow is expected to have a larger potential than rain for scavenging cVMS from air given i) larger surface area, ii) lower vapor pressure at low temperatures, and iii) higher concentrations of cVMS in arctic air in winter compared to summer. The latter is a consequence of decreased degradation of cVMS via OH radicals during the polar night. cVMS are volatile, hydrophobic and undergo degradation processes in water, properties which makes it highly challenging to analyze these compounds in snow. Given the high volatility of cVMS, it is likely that a large proportion of any cVMS scavenged by snow will be revolatilized before or during snowmelt. However, for cVMS to be able to enter the terrestrial and/or the marine environment, cVMS still need to be present in the snow during snowmelt. We address this in this study by including both fresh snow and late-season snow samples. This to allow for better documentation of the potential link: atmospheric cVMS - snow scavenging - snow melt - recipient compartment. To achieve this goal, a new technique has been developed for the accurate collection, storage and processing of snow samples which was tested in the area surrounding Ny-Ålesund, Svalbard, Norway: In the field, a clean ~35L stainless steel tank was filled with snow, representing one sample. The tank was then resealed and brought back to Ny-Ålesund. Here, the tank was pressurized with pure N_2, and the snow left to melt with assistance of heating cables on the exterior of the tank. The meltwater was pushed through a metal cylinder containing XAD2 sorbent which collects any cVMS present in the snow or the tank's headspace. The XAD2 cylinders were brought back to the lab for cVMS analysis. This presentation highlights the challenges encountered during implementation of collections and the considerations made to ensure accurate results. Despite implementation of strict measures in the field and in the lab to minimize cVMS contamination from personnel or local sources, the use of field blanks was critical in capturing the challenges encountered during the work.

4.14.P-Tu-146 Beyond PFOS and PFOA: Screening PFASs with Diverse and Challenging Characteristics for Sublethal Toxicity to a Sensitive Aquatic Insect, Chironomus dilutus

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Of the many kinds of per- and poly-fluoroalkyl substances (PFASs) detected in the environment, ecotoxicity data are only available for a few structural groups. We have previously reported 7-d sublethal effect concentrations for several PFASs in three freshwater invertebrates (cladoceran [*Ceriodaphnia dubia*], amphipod [*Hyalella azteca*], and midge [*Chironomus dilutus*]). In most cases, EC50s for perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs) and fluorotelomer sulfonic acids (FTSAs) were at or above 1 mg/L, with toxicity decreasing with decreasing chain length. However, midge were markedly more sensitive to PFOS and PFNS, with EC50s (0.01-0.02 mg/L) approaching environmental concentrations. This high sensitivity of midge to certain PFSAs raises the question of what other PFAS might also show higher hazard, and what structural characteristics might be associated with that hazard. In this presentation, we discuss toxicity testing of a wide variety of PFAS structural types, including fluorotelomer alkyl betaines, perfluoroalkyl biphosphinic acids, perfluoroalkyl phosphonic acids, perfluoroethylcyclohexane sulfonate, and more. In contrast to the compounds we tested previously, which were typically high purity, low cost, soluble and stable in exposure waters, non-biotransformative, and amenable to quantification with existing methods, some of these other PFASs presented methodological challenges, which we discuss. Preliminary results indicate that PFASs whose structures deviate even slightly from the mid-length PFSAs (e.g. longer or shorter chains, partially fluorinated chains, cyclic structure, different head groups) are much less toxic to midge. *This abstract does not necessarily reflect the views or policies of the United States Environmental Protection Agency.*

4.14.P-Tu-147 Blood Biomonitoring of Maternal Serum Using an Expanded List of Per- and Polyfluoroalkyl Substances (PFAS)

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Per- and polyfluoroalkyl substances (PFAS) are persistent organic pollutants that pose potential harm to human health. Assessing PFAS risk is complicated by the high number of known compounds, diverse exposure pathways that are poorly described, and lack of *in vivo* hazard data. Knowledge of human blood levels for emerging PFAS is very limited as they are not routinely monitored, limiting our ability to assess exposure. With *in vitro* toxicity and toxicokinetic data increasingly available, blood concentrations, used to infer exposure, could be used in conjunction with *in vitro* points-of-departure (PODs) to calculate bioactivity to exposure ratios (BERs). Chemicals with exposures that approach the bioactivity-based POD signal potential human health risk and may be of interest for further evaluation. To facilitate a better understanding of human PFAS exposure, we developed a highly sensitive, targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to detect and quantify 70 PFAS from multiple classes in human blood. Our method was demonstrated using 105 archived maternal serum samples from general population volunteers participating in the National Children's Study. The LC-MS/MS method incorporates a simple protein precipitation with quantitative analysis on a Sciex 7500 triple quadrupole mass spectrometer operated in negative ion mode with scheduled multiple reaction monitoring (sMRM). Chromatographic separation was achieved using gradient elution with a polar reverse phase column. Estimated limits of detection (LOD) range from 10 pg/mL to 1 ng/mL. Preliminary results showed that legacy PFAS perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), and perfluorohexanoic acid (PFHxA) were detected in all samples. In addition to other commonly occurring PFAS, we observed emerging chemicals including 7H-perfluoroheptanoic acid (7H-PFHpA), 9H-perfluorononanoic acid (9H-PFNA), perfluoro-3,6-dioxa-4-methyl-7-octene-1-sulfonic acid (PFESA BP1), and 7H-perfluoro-4-methyl-3,6-dioxaoctanesulfonic acid (PFESA BP2) at low abundances in multiple samples. The occurrence of emerging PFAS in a small survey of the general population highlights the need for blood biomonitoring with an expanded PFAS target analyte list to aid in PFAS hazard and exposure assessment. *The views expressed in this abstract are those of the author(s) and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.*

4.14.P-Tu-148 Sorption of Novel Per- and Polyfluoroalkyl Substances (PFAS) Evaluated Using High Resolution Mass Spectrometry

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Sorption mechanisms of per- and polyfluoroalkyl substances (PFAS) are complex and depend on both soil and PFAS properties. This study evaluates sorption behavior of 32 targeted and 50 non-targeted PFAS, across 23 soils using high resolution mass spectrometry (HRMS), and reports sorption of 51 of these PFAS for the first time. Sorption coefficients (K_d, L/kg) were measured in batch reactors containing soil, 1mM $CaCl_2$, and spiked with PFAS standards (targeted PFAS) or

commercial product (non-targeted PFAS). Results were evaluated for trends in soil properties, PFAS chain length and functional group, and influence of alkyl CH_2 spacers. As previously published, sorption increased with increasing chain length for PFAS with chain length >5-6 (i.e., $(CF_3(CF_2)_n$ where $n > 4$-5), which is consistent with increasing hydrophobic interactions with increasing fluoroalkyl chain length. Sorption of shorter PFAS (chain length <5-6) did not exhibit chain-length dependent trends. For example, in the majority of 23 soils tested PFCA sorption exhibited the following K_d trend: PFBA > PFPeA < PFHxA. Additionally, individual K_{oc} values ($K_{oc} = K_d/f_{oc}$) for PFBA, PFPeA, and PFHxA decrease with increasing f_{oc} whereas f_{oc} - normalization of other PFCAs leads to relatively constant values for all soils tested as expected when hydrophobic interactions with organic carbon are the dominant sorption mechanism. Results suggest that other molecular interactions (e.g., electrostatic) govern sorption of short-chain PFAS with soils. Regardless of mechanism, sorption of these PFAS in all soils with f_{oc} 2.5% was only 0.6 0.3 L/kg, so they are low-sorbing and highly mobile. Sorption of n:2 FTSs was lower, in many cases by 10x, than their fully fluorinated counterparts (e.g., 6:2 FTS vs. PFOS), suggesting that reduced fluorination can lower sorption of anionic PFAS. Zwitterionic PFAS with a terminal negative charge exhibited higher mobility compared to those with a non-terminal negative charge, indicating the influence of charge position on sorption. Substituted PFAS (e.g. chlorine/hydrogen/sulfur substituted PFAS) that are not routinely monitored, exhibited similar sorption characteristics (means and medians within ~0.5 log unit) to highly regulated and toxic PFAS. Given the limited understanding of fate and transport mechanisms for the majority of PFAS we evaluated, this study provides valuable insights for site characterization, prioritization, and selection of remedial approaches.

4.14.P-Tu-149 Standardized Methods Used to Access Environmental Safety of Polyvinyl Alcohol

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PVOH is a nonionic polymer commercially used in many different applications including textiles, adhesives, binders, and polymerization aids. Nonmodified PVOH polymers (CAS# 25213-24-5) ranging in degree of hydrolysis (DH) from 85% to 90% with viscosity ranging from 7 to 23 mPa s are one of the types of PVOH resins used to make detergent films. The purpose of this research was to use polyvinyl alcohol (PVOH) 18-88 (viscosity =18 mPas, DH =88%) as a case study to evaluate the environmental fate, ecotoxicity, and overall safety profile of water-soluble, nonmodified PVOH polymers used in detergent films. Ingredients that are disposed of down the drain will move from a consumer's home into the sewer system, be transported via wastewater, and enter a domestic wastewater treatment plant (WWTP). It is therefore critical to understand the environmental fate of PVOH 18-88 during wastewater treatment to quantify removal and evaluate the dominant receiving compartment(s). A variety of tests following OECD guidelines and modifying these guidelines for applications to polymers were conducted including OECD 301, 302B, and 303A. The data show that the water-soluble PVOH materials were fully mineralized in respirometric tests by both activated sludge and river water inoculum and underwent high removal under simulated wastewater treatment conditions (>90%) with no adsorption evident. The OECD 303A study quantitatively verified that surface water is the dominant receiving compartment for PVOH 18-88 post wastewater treatment. This data was combined with industry volume estimates to predict environmental concentrations and compared to ecotoxicity data. Acute algae, invertebrate, and fish embryo (fish embryo acute toxicity test

[FET]) ecotoxicity studies quantified the 50% lethal/effect concentration (L/EC50) for PVOH 18-88 at >1000 mg/L. This research shows that the water soluble PVOH materials are not persistent, are highly removed in wastewater treatment, continue to degrade in surface water, and are not ecotoxic at the levels expected in the environment.

4.14.P-Tu-150 Development and Evaluation of Novel Passive Samplers using Green Electrospinning Techniques

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Passive samplers for environmental monitoring have increased in use over the past few decades. Common, commercially available passive samplers include low-density polyethylene (LDPE) polyoxymethylene (POM), polydimethylsiloxane (PDMS) and ethylvinyl acetate (EVA). While these polymers have many advantages including availability, consistency in production quality, and relatively low expense, they are not easily altered or manipulated. In contrast, laboratory-synthesized passive samplers can be designed for specific purposes and media as well as newer classes of conventional and emerging contaminants. In order to develop novel passive samplers for the purpose of increasing performance in the rate of equilibration and selectivity, we are investigating electrospinning techniques. Electrospinning allows for the production of micro to nanofibers where the morphology and size can be altered by manipulating variables such as relative humidity, solvent matrix, voltage, etc. This presentation will describe the experiments and approach to develop novel passive samplers using electrospinning. For example, the commercially available polymers LDPE, POM, PDMS and EVA are challenging to electrospin due to the difficulty in dissolving these materials into solution. While procedures exist for POM and EVA, they require harsh solvents and/or expensive equipment. To circumvent these challenges, we are approaching development with the intent of using green chemistry techniques. When a product is synthesized, we will perform rigorous laboratory and field testing to compare the novel passive sampler with the commercially available options. This evaluation will determine the method limitations of each type of passive sampler produced to optimize utility in environmental media. If successful, this work will identify an approach for synthesizing novel passive samplers using electrospinning and result in the expanded usefulness of passive sampling.

4.14.P-Tu-151 Reduced Uncertainty in Solid-Water Distribution Coefficients for Per- and Polyfluoroalkyl Substances Through Exclusion of Non-equilibrium and Unsaturated Conditions

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Adsorption onto soil and sediment is one of the fundamental processes that can affect the fate and transport of per- and polyfluoroalkyl substances (PFAS) in the environment. PFAS adsorption is often characterized with the solid-water distribution coefficient (K_d) and the organic carbon-water partitioning coefficient (K_{oc}). While these metrics can affect fate and transport modeling and the relative concentrations of PFAS in environmental samples, their reported values range widely in currently available studies. For example, the Interstate Technology and Regulatory Council (ITRC) compiled PFAS K_{oc} values from over 40 studies and found that the range reported for perfluorooctanoic acid (PFOA) spanned nearly 5 orders of magnitude. By controlling for confounding factors in these available studies, estimates of K_{oc}

(and, by extension, K_d) could be better constrained. Here, we systematically reviewed and compiled K_d and K_{oc} data for soils and sediments from the ITRC reference list and additional studies that met the following criteria: (1) system is in equilibrium (or nearly so); (2) air-water partitioning is negligible; and (3) study design and analytical methods facilitate robust measurement of organic carbon content and PFAS concentrations. We found 12 lab-based experimental studies representing 106 experimental conditions that met all of these criteria. Using this compilation to report log K_d and log K_{oc} estimates for several prevalent PFAS, we found that both parameters were significantly lower and more constrained than the ITRC compilation and other reports that did not rigorously control for equilibrium and air-water partitioning. We determined the relative importance of organic carbon content, soil type, and solution chemistry as predictors of log K_d for the dataset, finding that the effects vary systematically by PFAS chain length and head group. Overall, this work provides new and well-constrained estimates of K_d and K_{oc} for several prevalent PFAS that will be useful in evaluating PFAS transport in aquifers, surface waters, soils, and sediments.

4.14.P-Tu-153 Critical Review of In Vitro Dosing Methods for Petroleum UVCB Substances

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Alternative approaches to traditional ecotoxicity tests, which include *in vitro* testing, are being promoted to support regulatory chemical assessments. *In vitro* test methods can be used in multiple contexts, from prioritization and screening to supporting chemical grouping and read-across. Eventually, it is anticipated that *in vitro* tests will be used in place of whole organism *in vivo* testing. An important challenge of most *in vitro* testing methods is how to establish, maintain and confirm defined test substance concentrations throughout the test. This is particularly challenging for petroleum UVCB substances (Unknown, Variable composition, Complex reaction products, or Biological origin) that typically contain a large number and variety of hydrophobic and (semi)volatile hydrocarbon constituents that are very prone to evaporative and sorptive losses. The ability to deliver and maintain stable exposure of petroleum substances in *in vitro* test systems is challenged by several factors, including: high surface area to volume ratios of multi cell well plates, which increases the likelihood of sorption to plate walls; the inability to seal some test vessels (e.g., volatile constituents can escape open test vessels and may contaminate neighbouring cell wells); poor solubility of hydrophobic constituents in biological media and presence of lipids and proteins in biological media may differentially bind individual constituents. Here we present the findings from a critical systematic review on the state of science of *in vitro* methods, their challenges, and their applicability in (eco)toxicological assessments of petroleum UVCBs. The outputs will be used to identify relevant *in vitro* tests and dosing methods and adaptations best suited for petroleum substances in future research and regulatory testing efforts.

4.14.T Methods for Assessing Environmental Fate and Effects of Difficult-to-Test Substances

4.14.T-02 Challenges and Insights for Assessing Environmental Fate of Poorly Soluble Polymers

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Polymers play a pivotal role in modern materials and products, yet the characterization of their environmental fate profile poses specific challenges compared to discrete/non-polymeric substances. Poorly soluble and insoluble polymers may be considered difficult-to-test substances and thus require adaption of testing approaches. In this presentation, we delve into methodologies for evaluating the environmental fate of poorly soluble polymers in aqueous systems through coating the polymers on silica gel as a mechanism to increase their bioavailability. We report on two different approaches and one unique challenge that has been encountered. Firstly, interest in the fate of poorly soluble and insoluble silicone-containing polymers has increased greatly. However, the silica gel commonly used in our studies is hydrophilic and may react with the silicone-containing polymers. Hydrophobic silica gels are available and could be used, but they generally have smaller particle sizes than hydrophilic gels. Thus, we investigated the influence of different particle size silica gels on the extent of biodegradation of a material. Multiple silica gels stretching across 3 orders of magnitude of particle size were obtained and coated with anthraquinone, a poorly soluble, discrete, organic material. No significant effect of particle size was observed in this experiment. Secondly, we investigated the effects of loading rate on the silica gel to understand it's impact on bioavailability. Higher loading rates may lead to the formation of thick films on the silica gel surface, which could reduce bioavailability. Conversely, lower loading rates also may result in lowered bioavailability if the materials tightly bind to the gel and are not re-solubilized. Several polymeric materials were coated at loading rates from 5% to 30%. For at least one material, a lowered loading rate (10%) decreased the lag phase of the study, while not affecting the total biodegradation observed. Lastly, we report on general challenges encountered when attempting to coat film-forming polymers on silica gel. These materials have a tendency to rise to the surface as the solvent is evaporated off, impacting homogeneity. Alternatively approaches and their effectiveness will be discussed.

4.14.T-03 Precursor Per- and Polyfluoroalkyl Substances: Biotransformation and Poor Exposure Stability May Confound Toxicological Measurements with Sensitive Model Species

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The toxicity of precursor per- and polyfluoroalkyl substances (PFAS) and their transformation products in toxicological assays are not well studied. To that point, this study focuses on N-Alkyl perfluorooctane sulfonamides (N-AlkFOSAs) as well as alkylated and non-alkylated perfluorooctane sulfonamidoacetic acids (FOSAAs), examining their uptake, transformation, and toxicity to larval *Chironomus dilutus*. Seven-day sublethal toxicity testing performed here has shown strong toxic effects for N-methyl and N-ethyl perfluorosulfonamides (N-EtFOSA, N-MeFOSA) and minor toxic effects for the N-methyl and N-ethyl sulfonamidoacetic acids (N-EtFOSAA, N-MeFOSAA) at the highest obtainable exposure concentrations for these chemicals given solubility limitations and strong adsorptive losses. Solubility and adsorption issues enhanced the difficulty of testing these chemicals with an observed 90% loss over 24-hour static renewals in the test solutions. In-house toxicity tests have also demonstrated that both perfluorooctanesulfonic acid (PFOS) and perfluorooctane sulfonamide (PFOSA) are highly toxic to *C. dilutus*. *In vivo* transformation of N-AlkFOSAs and FOSAAs into PFOS or PFOSA may confound exposure-response interpretation and requires thorough analytical characterization. To interrogate the impact of biotransformation on these results, separate uptake studies were conducted at non-toxic exposures of each parent PFAS individually to elucidate biotransformation processes and estimate product formation at toxic exposure concentrations where tissue analysis is not practically feasible. Preliminary results with N-EtFOSA and N-EtFOSAA show biotransformation to PFOSA but not subsequent oxidation to PFOS. Estimated tissue concentrations of PFOSA produced *in vivo* during toxicity tests are high enough to convolute exposure-response interpretation of the parent chemical, with nearly all the toxicity observed in the N-EtFOSA test attributable to conversion to PFOSA. In-vivo transformation, low water solubility, adsorptive losses, and variable exposure concentrations underscore the challenge these PFAS pose to toxicity assays. *This abstract does not necessarily reflect the policies and views of U.S. Environmental Protection Agency, DOE, or ORAU/ORISE.*

4.14.T-04 Ex-Situ Passive Samplers to Evaluate Bioavailability of Per-/Polyfluoroalkyl Substances (PFASs) in Marine Sediments

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Per- and polyfluoroalkyl substances (PFASs) are a pervasive threat to aquatic ecosystems and human health. Sediment contamination is a critical pathway of exposure for benthic organisms, potentially impacting higher trophic levels. Determining the bioavailability of sediment-associated PFASs is challenging due to complex matrix interactions and depends on the understanding of highly variable sediment characteristics, water quality, and sorption. Passive sampling devices (PSDs) are a promising approach to assess the bioavailability of residual contamination in soils and sediments by selecting for bioavailable PFASs from the sediment-porewater matrix, mimicking passive biological uptake. In this work, we evaluate synthetic materials as *ex-situ* PSDs in sediment-water systems, including polyacrylamide-coated solid-phase microextraction (SPME) fibers, hydrophobic-lipophilic balance–weak-anion exchange (HLB-WAX) SPME blades, and a surface-modified graphene monolith. Our objective is to identify an optimal material to selectively sequester bioavailable PFASs from sediment-seawater slurries. We compare the effectiveness of these PSDs in capturing a wide range of PFASs and measure time to equilibration and partitioning coefficients (K_ds) under conditions relevant to marine benthic exposures. We will also evaluate the accuracy of *ex-situ* PSDs for predicting tissue burdens in marine polychaetes exposed to similar fortified marine sediments. Initial findings have shown the achievement of rapid equilibrium of SPME fibers within 48 hours in water for all studied 15 PFASs, and the derivation of K_d values with positive chain-length trends.

These materials will effectively accumulate short and long-chain PFASs and give insight into the changes in the bioavailable fraction occurring with changing sediment characteristics, which influences the biological uptake of PFASs from sediments by benthic biota. Thus, assessing PFAS bioavailability underscores the need for comprehensive approaches to PFAS risk assessment in aquatic environments. This research contributes to a broader understanding of PFAS fate and transport in aquatic systems, facilitating informed decision-making for the protection of ecosystem health and human well-being.

4.14.T-05 Solid-phase Reactivity-Directed Extraction (SPREx): A Novel Approach for the Detection of Toxic Electrophiles Produced in Water Treatment Applications

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Chemical oxidants such as chlorine and ozone are increasingly used to eliminate harmful pathogens and micropollutants during (waste)water treatment processes. Unfortunately, a wide variety of toxic oxidation byproducts are also produced through the reaction of these oxidants with organic matter. Organic electrophiles are an important class of oxidation byproducts as well as environmental toxicants due to their link to adverse health outcomes such as skin diseases, neurodegenerative diseases, and various cancers. Despite their importance, however, their detection presents significant challenges. Many organic electrophiles are highly polar and/or volatile, so they do not survive traditional preconcentration and extraction methods for environmental samples. In addition, many organic electrophiles such as carbonyls are not readily ionizable in liquid chromatography-high resolution mass spectrometry (LC-HRMS), making them undetectable if proper derivatization reagents are not utilized prior to analysis. To address these challenges, a novel analytical platform Solid-Phase Reactivity-directed Extraction (SPREx) was developed. In SPREx, nucleophile probes were immobilized onto solid-phase, cleavable microbeads to achieve simultaneous: i) toxicity prioritization, ii) detection, iii) extraction, and iv) identification of organic electrophiles in environmental water matrices. For toxicity prioritization, nucleophile probes that mimic the biological targets of organic electrophiles were immobilized onto microbead surfaces to capture only the reactive, electrophilic toxicants of interest. These nucleophile probes further served as derivatizing agents to enable ionization of the overall nucleophile-electrophile adducts for detection via LC-HRMS. As an additional advantage, the solid-phase microbeads offer a built-in extraction feature, where the target electrophiles form stable adducts with the microbeads, thus allowing for the rest of the sample matrix to be discarded. In addition to the detection and quantification of known organic electrophiles, this approach can also be used to identify previously unknown compounds in mixtures. To demonstrate the applicability of SPREx, carbonyl compounds were utilized as model organic electrophiles and were evaluated in terms of their stability with different nucleophile probes, recoveries, and detection from chlorinated model compounds, drinking water, and wastewater.

4.14.T-06 Direct Photolysis Studies of Photo Acid Generators: Experimental Challenges and Implications for Environmental Fate and Toxicity

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Photo acid generators (PAGs) are typically ionic substances used during the photolithography process of semiconductor manufacturing. With the passing of the Creating Helpful Incentives to Produce Semiconductors (CHIPS) Act, semiconductor production in the United States is expected increase, and recent consent agreements issued under the U.S. Toxic Substances Control Act (TSCA) require additional testing to understand the environmental fate and potential effects of PAGs released into the environment. Direct photolysis studies were conducted on five representative PAGs using OECD method 316 and radiolabeled PAG cations to partially satisfy these TSCA testing requirements. The PAGs generally had measured absorbance maxima at wavelengths well below 290 nm, and some PAGs had absorbances below the detection limit at wavelengths above 290 nm. Despite low absorbances at sunlight-relevant wavelengths, all five PAG cations photolyzed to some extent upon sunlight exposure in a solar simulator, and estimated half lives under natural environmental conditions ranged from 0.3 hours to 95 days. In contrast with the PAG cations, absorbance measurements of the PAG anions indicated that direct photolysis of the tested PAG anions is unlikely to occur in natural sunlight. While the PAGs are ionic compounds, all of the photodegradation products identified were non-ionic, and some of the major degradation products could not be identified. The differences in physicochemical properties between the PAGs and PAG photolysis products, including water solubility and octanol-water partition coefficient, will likely lead to major differences in their environmental partitioning (e.g., between water and soil), bioaccumulation, and toxicity.

4.15.P-We Navigating Environmental Assessments for Evaluating Consumer Products and Chemicals of Concern

4.15.P-We-155 Profiling the Potential Environmental Hazards of Coolant Chemicals using Computational Approaches

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Coolants, particularly the chemicals added to tobacco products for their cooling properties, have recently received increased attention due to their documented use as menthol alternates. However, detailed information on the types of menthol alternative chemicals used as coolants in tobacco products, their chemical structures, physical-chemical properties, and ecotoxicity potential are currently not available. In this study we (1) compiled a library of 187 coolants with unique chemical structures from multiple public data sources (literature, invention patents, and public databases); (2) investigated the coolants' structural similarities to each other and with menthol using unsupervised machine learning algorithm (Knowledge Discovery by Accuracy Maximization– KODAMA) and hierarchical clustering techniques; (3) compiled hazard categories combining experimental and predicted data for acute aquatic toxicity and bioaccumulation (EPA web-based Hazard Comparison Module- HCM); and (4) characterized the physico-chemical properties (Schrödinger Canvas) including log octanal-water partition coefficient (LogKow) and electrotopological state index (E-state Index) as indicators of the environmental fate and bioavailability. Of the 187 unique chemicals, 25 and 79 coolant chemicals were identified as very high and high toxicity, respectively, to aquatic organisms (fish, invertebrate, algae) per HCM. The ecotoxicity predictions were significantly positively associated with LogKow (generalized linear model GLM - $\chi^2 = 61.9$, df = 3, $p < 0.001$) and E-state Index (GLM - $\chi^2 = 58.1$, df = 3, $p < 0.001$). Mean LogKow and E-state Index values were significantly higher for chemicals with very high and high aquatic toxicity than those with moderate and low aquatic toxicity. The coolants were grouped into 6 main clusters based on their chemical structures and the predicted very high acute aquatic toxicity coolant chemicals were primarily in clusters having aromatic amides or alicyclic esters as the indicative functional groups. Additionally, three chemicals among those with high and very high acute aquatic toxicity were classified as bioaccumulative. Overall, our results help identify specific classes of coolant chemicals associated with high ecotoxicity potential for aquatic organisms. This research may inform U.S. Food and Drug Administration's environmental impact evaluations mandated by the National Environmental Policy Act as part of review of future tobacco product applications.

4.15.P-We-156 Analysis of Environmental Hazards of Personal Care Product Ingredients Utilizing U.S. Market Data from EWG's Skin Deep

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The potential environmental impact of personal care products is a significant concern due to the widespread use and release of these products into the environment. While there are many studies on the safety of personal care product (PCP) ingredients from a human health perspective, studies examining the environmental implications of PCP ingredients are limited.

The Environmental Working Group (EWG)'s Skin Deep® database, which includes over 100,000 products and over 80,000 ingredients and is used to represent the cosmetic products market in the United States. With this database, we identified the most common PCP ingredients with environmental hazard data. We then evaluated these ingredients based on their persistence, bioaccumulation potential, and ecotoxicology profile. Out of over 80,000 ingredients in Skin Deep®, 2,144 had environmental hazard data with the main sources being the European Chemical Agency, Environment Canada, and open scientific literature. The most commonly used ingredients with environmental hazards were ingredients used as emollients/emulsifiers, fragrance, and colorants. Our study highlights the environmental hazards in common personal care product ingredients. Though there are potential information gaps within Skin Deep, given its focus on the U.S. market, our results demonstrate the importance of advocating for sustainable practices in the personal care industry. Specifically, applying Skin Deep data as a screener for environmental hazards could be used to prioritize where efforts should go in seeking alternative chemicals for more sustainable formulation. More broadly, ecotoxicity screening tools can promote green chemistry by identifying less hazardous alternative ingredients within functional classes.

4.15.P-We-157 Evaluation of Fire Fighter Station Wear for Flame Retardants

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Flame retardants (FRs) are diverse groups of inorganic and organic chemicals used in clothing, fabrics, furniture, electronics, and other consumer products to purportedly mitigate fire safety risks. Their firefighting abilities, however, are ineffective and FRs cause unintended human health and environmental consequences. Exposure to polybrominated diphenyl ethers (PBDEs) and organophosphate flame retardants (OPFRs) has been associated with cancer, endocrine disruption, and reduced fertility. Fire fighters may wear station wear shirts (fire fighter uniform) which can be made from flame-resistant materials. This study analyzed parts (chest, armpit, and/or cuff) from eight short- or long-sleeve fire fighter station wear shirts for FRs. Each sample

was screened for the presence of the elemental indicators of common FRs and synergists, including antimony (Sb), bromine (Br), chlorine (Cl), and phosphorus (P) by X-Ray Fluorescence (XRF) spectrometer. The samples were also digested with nitric acid and analyzed for Sb and P by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). A trace amount of Br was detected in two shirts by XRF. Cl was detected in all shirts by XRF, three shirts having a trace amount and 5 shirts having high intensity. Sb was detected in five shirts ranging from 74.7 to 42,900 ppm by ICP-OES. P was detected in four shirts ranging from 42.7 to 1,730 ppm by ICP-OES. XRF and ICP-OES results indicated the possible presence of OPFRs and PBDEs in four shirts. The samples in these four shirts were extracted in toluene by sonication and analyzed by Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) for target analysis of 17 FRs (OPFRs and PBDEs). All 17 FRs were not detected, and the source of major elemental indicators of FRs was not identified by the target GC-MS/MS method. The sample extracts were further analyzed by GC-MS full scan (50-1000 amu) to search all chromatographic peaks against the NIST Mass Spectral Library. Aliquots of the samples were also extracted in methanol and analyzed by High Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry to perform a suspect screening against an in-house library of 92 FRs. Since the target and non-targeted analyses did not detect known FRs, further investigation is planned to determine if the source of the elemental indicators is from “unknown” FRs. The structures of “unknown” FRs should be explored by other analysis techniques (e.g., Raman spectroscopy).

4.15.P-We-159 Systemic Toxicity Screening of Real-life Mixtures in Consumer Products: An Effect Directed Analysis

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The exposure to chemical mixtures in consumer products is a common in daily life, presenting potential systemic hazards to human health. This study aims to bridge the gap in the regulatory framework concerning consumer products by assessing the hazards posed by real-life chemical mixtures. We evaluated the cytotoxicity of real-life mixtures found in over 20 commonly used consumer products, utilizing six human cell lines representative of systemic organs: liver (HepG2), lung (Beas-2B), brain (SH-SY5Y), immune system (Jurkat), and developmental stages (embryonic stem cells and neural stem cells). Initial findings highlighted product groups with concerning toxicity levels. A targeted chemical analysis of these groups confirmed the presence of significant concentrations of poly- and perfluoroalkyl substances (PFAS). Subsequently, we created artificial mixtures of PFAS at concentrations matching those found in the consumer products and exposed the same cell lines to these mixtures. The toxicity results of these artificial mixtures were then directly compared with those from real-life product exposures. This comparative analysis revealed that PFAS are major contributors to the toxicity of the mixtures. Our findings underscore the necessity of assessing real-life mixtures in consumer products, providing essential data that could inform regulatory changes and help mitigate environmental and human exposure to hazardous chemicals. Furthermore, this approach enhances scientific comprehension for employing effect-directed analysis on real-life samples, thereby contributing significantly to the implementation of the Green Deal project PANORAMIX. *This work was supported by Korea Environmental Industry & Technology Institute through “Core Technology Development Project for Environmental Diseases Prevention and Management”*

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4.15.P-We-160 Integration of ToxCast Bioassays to Hazard Assessment by Comparison of Human Equivalent Doses with In Vivo Data: Case Study with Phthalates in Consumer Products

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As regulations have banned major phthalates for their use, the industry has sought alternatives to those phthalates. Accordingly, they have been found in many consumer products and detected in increasing amounts in human biomonitoring data. However, their hazard to humans and the environment remains unknown, and there are many data gaps within the conventional risk assessment framework. In response, this study aimed to explore the potential of incorporating ToxCast data into hazard assessment of chemicals by comparing human equivalent doses (HEDs) from animal testing results for major phthalates and their alternatives. Firstly, we investigated the human equivalent doses (HEDs) of phthalates from international regulatory risk assessment reports. Secondly, we collected AC50 values from active ToxCast bioassays for each phthalate. DBP, DEP, DEHA, and TOTM, HEDs derived from in vitro AC50 values fell within the range of HEDs derived from animal studies. For DEHP and DnOP, the range of in vitro AC50-based HEDs was more protective than the animal HEDs-based HEDs. When compared by toxicity endpoint, HEDs from the developmental and reproductive toxicity-relevant assay groups could include or be close to the in vivo-based HEDs. In contrast, acute toxicity-relevant assay groups revealed a significant difference between in vitro and in vivo-based HEDs. Given the known toxic effects of phthalates, which are developmental and reproductive toxicity rather than acute toxicity, in vitro bioactivity may be correlated with potential modes of actions leading to the toxicity of phthalates, providing sensitive points-of-departure (PODs) values. We suggest that this mechanism-based assessment can support the identification of adverse effects in humans, disclose unknown side-effects, and provide regulatory direction for further assessment.

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4.15.P-We-161 Human Hazard Characterization of Active Substances in Biocidal Products Using the Weight of Evidence (WoE) Approach

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In the light of the need to reinforce the assessment of consumer products in the EU Chemicals Strategy for Sustainability, various methods are actively being developed for evaluating hazard by combining toxicity information and integrating the information with new approach methodologies (NAMs). The safety assessments of biocides in consumer products still relies on animal studies. However, animal welfare pressure will push to move the hazard assessment of biocides into the next phase using non-animal studies with the minimized animal studies. Thus, this study aims to examine the applicability of in vitro toxicity data in replacing the traditional animal-based human hazard data. We first reviewed in vivo hazard characterization data of 11 biocides (used as insecticides, algaecides, rodenticides and disinfectants) regulated by EU BPR and/or US FIFRA. In addition, we analyzed in vitro data corresponding to each traditional toxicological endpoint from the PubMed literatures and collected the cytotoxicity data from EPA ToxCast database. Finally, we compared the in vivo derived no-effect level (DNEL) to the human equivalent dose (HED) calculated from in vitro toxicity data. In the review of traditional toxicity, in vivo hazard data had a partial gap in information on test methods such as test period, test method, GLP status, and reliability. There were also the insufficient data on dermal POD, inhalation POD, neurotoxicity, immunotoxicity, and phototoxicity in most biocides. Although in vitro toxicity values obtained from literatures were mostly associated with genotoxicity, immunotoxicity, and endocrine disruption, there was a limitation to comparing in vitro hazard to in vivo hazard because the corresponding in vivo data are absent or qualitative. In conclusion, this study endeavored to integrate the information across different systemic toxicity with in vitro toxicity data and suggested that the unified cytotoxicity data from ToxCast could be more useful to produce new PODs for NAM. *This work was supported by a grant from the National Research Funding of Korea grant funded by Ministry of Science and ICT (NRF-2020R1A2C3006838).*

4.15.P-We-162 Inadvertent Polychlorinated Biphenyls (PCBs) in Consumer Products: Source Testing

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Production of inadvertently generated polychlorinated biphenyls (iPCBs) occurs during a variety of chemical manufacturing processes that involve carbon, chlorine, and high temperatures. PCB exposure is a known problem across the U.S. in schools and childcare settings. Product testing has identified iPCBs in children's products and evidence shows that inhalation of indoor air and ingestion of indoor dust via hand-to-mouth and hand-to-object-to-mouth pathways contribute to exposures. In this study, we obtained a total of 108 consumer products from the market between 2022 and 2023, some of which are often used by children, and vary by colors and scents. Among those products, 62 of them were purchased from bargain retail stores, such as Walmart, Dollar Tree, and Five Below. Twelve print ink products were donated by the Washington State Department of Ecology, and one plastic grocery bag product was donated by the Seattle Public Utilities. We treated the products with different colors, scents, and parts from the same product as separate product samples bringing the total number of product samples tested to 138. PCBs in product samples were extracted using methylene chloride solvent and sonication and analyzed in duplicate by a gas chromatography–mass spectrometry (GC-MS) calibrated for 209 PCB congeners to determine their PCB contents. Some of the products (e.g., detergents, stains, paints, and inks) needed further separation and clean-up following solvent extraction. Those products were processed through a Fluid Management Systems (FMS) packed sorbent column clean-up program developed to remove interferences. Three product samples were extracted both with the Soxhlet method and the sonication method to compare results. This presentation will provide an overview of PCB congeners detected in this large set of product samples. The dataset will be the foundation for further iPCBs fate and transport studies.

4.15.P-We-163 PCBs in School Air: Widespread Emissions of Airborne PCBs from Building Materials in 99 Schoolrooms in Vermont

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PCBs remain in school building materials since their manufacture starting in the 1930s despite evidence of their carcinogenicity, neurotoxicity, and endocrine disruption. Here we report the largest study to date of the sources and concentrations of airborne polychlorinated biphenyls (PCBs) in United States schools. Using simultaneous deployment of passive air samplers and passive emission samplers, we analyzed airborne PCBs with 159 air samples and 182 emission samples in 16 Vermont schools and 99 school rooms constructed prior to 1980. All 209 PCBs were quantified as 173 chromatographic peaks. Concentrations in school air ranged from 2 – 5700 ng m^{-3} and are comparable to Aroclor-based measurements reported from the State of Vermont. There is wide variability in PCB concentrations and emissions between different schools as well as between rooms in the same school. About 38% of Vermont schools built between 1950 and 1980 have elevated PCB levels above state regulatory levels. Among those school rooms with airborne PCB levels above regulatory levels, our emission samplers identified three distinct types of building materials with significant emissions including glass block windows (up to 30,000 ng m^{-2} d^{-1}), expansion joint sealant (up to 480,000 ng m^{-2} d^{-1}), and fireproof coating on steel columns (up to 830,000 ng m^{-2} d^{-1}). We examined congener distributions in emissions and air concentrations and found evidence of many different Aroclors not previously reported for school building materials including Aroclor 1221, Aroclor 1232, and Aroclor 1260. The most frequently detected were Aroclor 1254 and 1016/1242. Aroclor 1254 was often observed in the emissions from caulking and joint sealants, but other Aroclors are emitted from many different materials. We also confirmed that four dioxin-like PCBs (PCB 118, PCB 157, PCB 167, and PCB 189) are emitted from building materials. Our findings provide a path towards material-specific remediation that avoids school closures and reduces children's exposures to harmful PCBs in classroom air.

4.15.P-We-164 Novel Silicone-Based Passive Emissions Sampler (Si-PES) Measures Emissions of Polychlorinated Biphenyls (PCBs) from Concrete Immediately Adjacent to PCB Containing Materials in a School

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Polychlorinated biphenyls (PCBs) are a class of forever chemicals that exist in school air and threaten the health of children, who are especially vulnerable to the toxic and carcinogenic effects of PCBs. In the United States, mixtures of PCBs, called Aroclors, were added to building materials including flame retardant insulation and caulking from the 1950s to the 1980s, which was also a period of extensive school construction. PCBs are emitted to the air from Aroclor-containing materials and can also diffuse into surrounding building materials. Diffusion of PCBs into surrounding solids is well known, although it is not known if these materials may become secondary emitters of airborne PCBs. To evaluate this, we used passive emission samplers to measure PCB release in a school at points on gymnasium brick walls parallel to likely PCB sources. This study utilized a new, narrow silicone passive emissions sampler design called Si-PES. Si-PES consists of a thin aluminum covering and uses a slim strip of silicone separated by

an air gap to collect the PCBs emitted from a flat surface. The design of Si-PES allows for precise emissions measurements from small surface areas. We placed 16 Si-PES units in a school gym atop materials we suspected contained Aroclors, and atop concrete immediately adjacent to these materials. After 27 days, the Si-PES units were retrieved. PCBs were extracted from the silicone using pressurized solvent and analyzed for all 209 congeners using high resolution gas chromatography with tandem mass spectrometry.

4.15.T Navigating Environmental Assessments for Evaluating Consumer Products and Chemicals of Concern

4.15.T-01 A Framework and Case Study in Support of Risk-Based Prioritization of Additives and Polymer-Associated Chemistries (APAC)

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Widespread global use of polymers and plastic has led to increased stakeholder interest in the health & environmental assessments of these materials. Formal frameworks (i.e., RISK21, Canadian ERC) may require specific data types, developed with prescribed methods, and are largely focused on non-polymeric substances. These data are not always available and can result in inaction, or conservative decision making. This is due to the propensity for regulatory or policy decisions to rely primarily on hazard information, without comprehensively considering exposure information. Assessing and making risk-based decisions requires deliberate consideration of complex and dynamic suites of information. For assessments of large numbers of substances, approaches which screen and prioritize data inputs is a practical strategy for systematically addressing complexity in a stepwise approach. This framework for screening and prioritizing additives and polymer-associated chemistries (APAC) was developed to parameterize the large, diverse set of chemistries that are utilized in the manufacture and processing of polymers by considering relative chemical hazards and potential polymer-related exposure profiles together to form the initial basis for a practical and informed risk assessment. The framework leverages existing global databases and utilizes predictive modeling in a tiered approach to support fit-for-purpose polymers risk assessments. It is intended to be an accessible approach for all stakeholders with interest in polymers risk assessment. To evaluate the utility of the framework for a large, diverse dataset, a preliminary analysis utilizing the UNEP Chemicals In Plastics database (13,186) was performed. Substances were assigned to hazard and exposure tiers (based on the nature of available data for each substance) corresponding directly to the level of quantitative risk assessment which can be performed. For the UN Database: 78% of chemicals had sufficient data to support the development of quantitative screening level toxicological threshold values. Further, 65% of chemicals had sufficient structural and physical chemical data to support generalized exposure assessment. Additionally, the framework supports the user in developing “reasonable worst case” assumptions to further evaluate data poor chemicals (~ 22% of the database). The case study demonstrates the efficacy and utility of the framework to tackle complex challenges for a wide range of chemical substances.

4.15.T-03 The Importance of Data in Sustainable Product Formulation

Alexandra M. Gobeil *and Marie BenKinney, Exponent*

With the increasing regulatory demands and consumer expectations for sustainable and environmentally friendly products, the use of toxicological data and chemical data is crucial for informed decision-making in product reformulation. Data on human health and environmental characteristics of substances form the basis of hazard assessments for classifying raw materials. And while commercial databases exist to aid brands in identifying alternatives, they often rely on reported information rather than raw data and are not product-specific, underscoring the importance of scientific data for educated material selection. In this presentation, we will explore how scientific literature, regulatory and authoritative body chemical restrictions, and supplier provided information can be leveraged to identify safer chemical substitutes in consumer products. We will do this by sharing case studies and discussing the importance of data quality, strategies for handling data gaps or disingenuous information, and resources for obtaining robust toxicological and chemical information. Attendees will gain insights into using data to drive sustainable innovation, ensuring regulatory compliance, and meeting consumer expectations for safer, environmentally friendly products.

4.15.T-04 What's in Nail Products Sold in California? A Lab Study and Exposure Assessment Using USEtox

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Nail products contain a variety of chemicals with various functions, many of which are hazardous and may adversely impact product users, including women, children, and nail salon workers. However, due to limited regulatory oversight, there is a lack of publicly available data on the ingredients and their safety in nail products. To better understand and mitigate potential exposures to hazardous chemicals found in nail products, the California Department of Toxic Substances Control (DTSC) analyzed 156 nail products from 21 product categories in the California market for 52 organic chemicals and evaluated the accuracy of ingredient labels and marketing claims. We also used the latest USEtox 3.0 beta model to estimate the exposure and potential risk to consumers and nail salon workers. The study detected 29 target chemicals in the tested nail products, of which 24 are listed by various authoritative bodies as concerning. The highest concentration was detected for ethyl methacrylate at 840,000 µg/mL in liquid products and 2,220 µg/g in acrylic powders. Several highly concerning chemicals were detected, such as formaldehyde, toluene and methyl methacrylate (MMA), with maximum concentrations of 15,600, 187,000 and 8,760 µg/mL, respectively. Results showed that ingredient labels and marketing claims were sometimes inaccurate, with some chemicals at concentrations above 10,000 µg/mL not on labels. Additionally, products marketed to children did not necessarily contain fewer or lower levels of harmful chemicals than other products. Some products contained hazardous chemicals like N-methyl pyrrolidone (NMP), a developmental toxicant, at concentrations above 1,000 µg/mL, indicating their presence as intentionally added ingredients. USEtox modeling revealed that the noncancer risk from home use of a single nail product every 2-3 days was negligible, but the cancer risk from formaldehyde in two products would exceed the commonly acceptable risk level of 1e-5. Also, the exposure and health risk for nail salon workers and consumers using multiple products would be considerably higher and concerning.

This study will help inform regulatory efforts in mitigating potential exposures to hazardous chemicals found in nail products. It will also contribute to publicly available data on chemicals in nail products, and highlight the need for more accurate ingredient disclosure to increase public awareness.

4.15.T-05 Evaluating Risks from Cosmetics and Personal Care Products: Use of (in)Appropriate Testing Frameworks

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A proper evaluation of risk, including both hazard and exposure considerations, is critical to regulating chemicals and protecting the natural environment. However, when done poorly, risk assessments can come to conclusions that are either under-protective (e.g., allowing potentially harmful chemicals to be released) or over-protective (e.g., adversely restricting the use of safe chemicals). For example, the California Code of Regulations sets strict criteria for defining materials as toxic, which include acute toxicity testing using fathead minnows. This becomes potentially problematic for the evaluation of cosmetics and personal care products that would not have a direct release to the aquatic environment, and would thus have limited potential for exposure. Furthermore, this specific toxicity test relies on the organism being evaluated using laboratory exposures that may be unrealistic, consequently complicating estimates of hazard. Here, we outline the testing process, highlight deficiencies, and propose modifications that would better achieve the desired outcome of both environmental protection as well as preventing undue burden on the regulated community.

4.15.T-06 Development of a Rapid Screening Method for Detection of Hazardous Additives in Textiles

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Flame retardants (FRs), per- and polyfluoroalkyl substances (PFAS), antimicrobials, and dyes are intentionally added to textiles to provide protection from fire, stain/water resistance, odor control, and color. However, these additives pose a number of health concerns, including reproductive health risks, skin irritation, and increased risk of cancer. When these classes of chemicals are added to textiles used in uniforms that must be worn regularly, such as those of first responders, school children, flight attendants, postal workers, military personnel, food service and hospitality workers, they represent a public health or occupational health threat that is largely unstudied in the United States. Traditional analyses for the presence of PFAS, metals, and flame retardants in textiles typically require separate, specific extraction protocols and analysis using multiple instruments. In an effort to save analysis time and resources, the current work has developed a rapid screening methodology to identify hazardous additives in textiles, and applied the methodology to a variety of samples, including firefighter station wear. First, samples underwent the *ex-vacuo* techniques of particle-induced gamma-ray emission (PIGE) and particle-induced x-ray emission (PIXE) spectroscopy to perform nondestructive elemental analysis. In three minutes per sample, textiles were screened for F, Cl, Br, Cr, Zn, and Cu as a surrogate for the presence of PFAS, flame retardants, toxic dyes, or antimicrobials. Due to the presence of Br or Cl being from a FR or dye, samples shown to contain these elements then underwent a secondary thin layer chromatography screening method to differentiate dyes from

FRs. A subset of samples then underwent the following analyses to validate the screening methods: targeted LC-MS/MS analysis for PFAS, GC-MS analysis for FRs, HRMS for brominated/chlorinated dyes, and ICP-OES to confirm presence of metals. Preliminary results confirm a variety of concerning chemicals can be identified rapidly in firefighter station wear and other textiles.

4.15.V Navigating Environmental Assessments for Evaluating Consumer Products and Chemicals of Concern

4.15.T-02 Safety Assessment of Recycled Plastics: State of the Science and a Case Study

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Transitioning to a circular economy is vital to both preserving and protecting Earth's natural resources. The need for recycled plastics is growing and is expected to rise over the coming decades due to increases in society's demand for low carbon solutions, implementation of regulatory frameworks on mandatory use of recycled materials, and accelerated commitments on post-consumer recyclate (PCR) from brand owners. However, the bulk of publications to date have focused their investigations on the hazard potential of virgin or weathered virgin plastic materials. Even so, marginal consensus has been reached on the characteristics of plastic impacting toxicity potential. By extension, there are multiple challenges facing PCR hazard assessment: there is not a standard suite of analytical or toxicological assays employed for the determination of hazard (or consensus on what endpoints should be evaluated), nor have sufficiently diverse samples been robustly tested for meaningful hazard conclusions related to PCR to be drawn. In order to assess the hazard of PCR and to avoid regrettable substitutions, fit-for-purpose hazard and risk analyses of PCR materials are required. Thus, our goals were twofold, first, to survey existing literature on the hazard of PCR and related materials (virgin plastics), to summarize approaches employed for the determination of hazard, and to identify gaps in approaches and knowledge. Second, to share a case study on the Dow PCR recycling stewardship program: our approach to the hazard assessment of PCR, which takes into consideration the exposure scenarios for the intended use of the PCR and which ultimately feeds into the PCR safety assessment. This presentation will summarize the state of the science on PCR hazard assessment and present a case study of Dow's approach on PCR safety assessment.

4.16.P-Tu New Perspectives and Developments in Chemical (Bio)Degradation and Persistence Assessment

4.16.P-Tu-154 Prioritizing the Development of a Standardized International Approach to Assessing the Biodegradability of Cosmetic Formulations

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Consumer expectations and demands for biodegradable product formulations are increasing rapidly. Although biodegradability assessment of cosmetic formulations is included in various certification programs and environmental/sustainability scoring tools, no clear guidance is available to address how to assess the biodegradability of cosmetic formulations consistently. While acceptable approaches are available, a guideline will ensure these approaches are used in a consistent way. Therefore, the global not-for-profit science organization ICCS (International Collaboration on Cosmetics Safety) is seeking to support an internationally recognized process setting out a standardized approach such a standardized approach, a critical review of regulatory positions, existing criteria, and standards specifically for assessing the biodegradability of ingredients, formulations, and chemical mixtures is being performed and will be presented in this poster. The review includes cosmetics, personal care, and home care products. Based on the review results, suggestions regarding international standard development will be mapped, together with suggestions for how to calculate the biodegradability of a formulation based on single ingredients, A roadmap for gaining acceptance of a possible international standard will be highlighted.

4.16.P-Tu-156 Water Soluble Polymer Biodegradation Under Simulated Environmental Conditions

Kathleen McDonough*, Jennifer Menzies, Ashley Wilcox, Nigel Yates, Maura Hall, Kyle Roush and Kristin Connors, Procter & Gamble*

Due to proposed changes in polymer regulatory requirements, there is a need to understand the ability of OECD simulation assays to be used for polymer biodegradation assessments. The standard biodegradation simulation assays were developed for and have been largely applied to single constituent, small molecular weight molecules and their applicability to assess complex multi-constituent polymeric mixtures needs to be evaluated. When conducting simulation assays, radiolabeled test substances are commonly used to allow for quantification of primary and ultimate biodegradation rates at environmentally relevant concentrations in complex environmental media. Radiolabeled test substances also allow for mass balance and a thorough understanding of the fate of the test substance over the course of the study. The initial portion of this research evaluated different approaches to acquire and synthesize radiolabeled water soluble polymers. The second portion of the research focused on developing analytical approaches to characterize these polymers and metabolites in simulation assays. Once radiolabeled polymers were obtained and analytical approaches were verified, simulation studies were conducted to evaluate primary and ultimate mineralization under environmentally relevant conditions. This presentation will summarize this research as well as discuss data gaps and research needs in water soluble polymer biodegradation assessments.

4.16.P-Tu-157 Evaluating Biodegradation of Highly Microbial Inhibitory Materials: Octenidine Case Study

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Preservatives are important and common components of personal care products to provide resistance against microbial growth. However, these positive attributes may provide challenges when assessing biodegradation potential in OECD screening studies due to reduction and/or inhibition of the microbial population responsible for the degradation process. Octenidine was evaluated in OECD 301F, 302B, and OECD 306 at the lowest starting concentration allowed following the test guidelines (10 mg/L). At day 28, biodegradation results were 0% biodegradation in each of the three assays. Following this, a study was performed to assess what the inhibitory threshold was for octenidine within biodegradation testing medium. This was performed using a toxicity control in which octenidine was dosed at either 1 or 0.1 mg/L with 50mg/L carbon sodium benzoate in an OECD 302B study. While under these conditions the octenidine was below the threshold for detection of the analytical methods, it could be determined if octenidine was inhibiting the microbes at the concentrations tested based on the degradation of sodium benzoate. Sodium benzoate biodegradation at the 28-day timepoint was 86.7% and 92% for test concentration of 1 mg/L or 0.1 mg/L, respectively. This indicates inhibition did not occur at these concentrations and the threshold for microbial inhibition is between 10 mg/L and 1 mg/L. Since the analytical methods of OECD 302B cannot be used to determine biodegradation at these low concentrations, the test method was modified. Octenidine at concentrations of 5 mg/L and 1 mg/L were added to 1 L bottles containing waste-water sludge. At 0, 7, 14, 21, and 28-day timepoints, a sample was collected and analyzed under LCMS for octenidine concentration. Based on peak area results, a decreasing trend was observed. This study acts as a case study for a pathway to evaluate a threshold concentration for microbial inhibition for biodegradation testing, and analyze the degradation at the significantly lower concentrations that the traditional OECD screening assays can provide. This can provide testing conditions closer to actual environmental concentrations, as well as prioritize and streamline OECD screening assays for preservatives/microbial inhibitory materials.

4.16.P-Tu-159 Reflections on the Role of Environmental Persistence and Considerations Regarding Screening and Prioritization

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There is continuing activity among regulatory bodies to screen and prioritize chemicals used in commerce based on their potential to be persistent, bioaccumulative, mobile and toxic (PBMT). Reliable data are thus needed to inform a PBMT hazard assessment. Access to reliable and relevant data, however, represents a significant shortcoming and can hamper screening and prioritization activities. Specifically, concerns may arise regarding the potential for false positives and false negatives to be wrongly classified. In order to more effectively prioritize chemical substances with respect to their potential to be PBMT candidates, it would be helpful to better clarify an appropriate amount of time needed to acquire reliable and relevant information. It has been argued, however, that a chemical with ‘high persistence’ (i.e. has at least one degradation half-life in an environmental media exceeding a regulatory criteria) is sufficient to enable regulatory actions aimed at preventing additional environmental releases of the chemical. Adopting a P-sufficient approach would thus require immediate actions to be implemented, even where uncertainties may exist regarding the reliability and relevance of available data. Given the paucity of persistence data for the vast majority of chemicals used in commerce, it is highly likely that there will be instances where the question will arise as to whether or not restrictions on the use and manufacture of a substance can be delayed to allow time to obtain the necessary field and laboratory studies of a particular substance aimed at better understanding its environmental persistence? To address this question it is demonstrated that chemical partitioning property and environmental persistence information can be effectively combined to provide guidance for regulatory priority setting. Specifically, it is argued that substances that have media specific half-life values that exceed the regulatory threshold value for persistence under the Stockholm Convention on Persistent Organic Pollutants, for example, are more likely to have a 'legacy' associated with their use when the log KOA > 8, and when they are emitted to air or soil. Thus, precautionary actions aimed at preventing environmental releases of the substance may be warranted. Whereas substances emitted to air with log KOA < 6 and log KAW > -2 are less likely to have a "legacy" associated with their use. Thus precautionary actions in the absence of data may not be warranted.

4.16.P-Tu-161 Advancing PFAS Remediation: Insights from In Situ Chemical Oxidation and Biotransformation Studies

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Military bases and airports often face contamination from per- and polyfluoroalkyl substances (PFAS) due to the widespread use of aqueous film-forming foams (AFFFs) in various activities such as training exercises, equipment testing, and firefighting involving fuel- and solvent-based fires. Conventional ex situ remediation methods like pump-and-treat coupled with sorption processes are typically expensive and time-consuming, requiring decades of operation due to the protracted diffusion and dissolution of contaminants. In contrast, in situ chemical oxidation presents a more cost-effective approach, involving the direct injection of oxidants like persulfate and hydrogen peroxide into the aquifer to react with contaminants on-site. Heat-activated persulfate oxidation (HAPO) generates highly reactive sulfate radicals capable of mineralizing perfluoroalkyl carboxylic acids (PFCAs) and various polyfluoroalkyl substances found in AFFF. However, the efficacy of PFCA transformation may be compromised by the scavenging of sulfate radicals by solutes present in groundwater. To evaluate the feasibility of HAPO, experiments simulating source zone conditions were conducted, demonstrating that repeated treatment of AFFF-impacted aquifer solids with modest persulfate concentrations (50-300 mM) can convert over 95% of PFAS in AFFF to carbon dioxide, fluoride, and ultrashort PFCAs. Subsequent treatment with a strong base like NaOH is essential to neutralize acidity and convert dissolved metals into less mobile forms. Concurrently, experimental studies were undertaken to investigate the biotransformation of sorbed PFAS and precursors under varying environmental conditions. This comprehensive research enhances our understanding of the fate and persistence of PFAS in the environment, providing valuable insights for mitigating risks to human health and the environment through effective treatment strategies.

4.16.T New Perspectives and Developments in Chemical (Bio)Degradation and Persistence Assessment

4.16.T-01 The Challenge to Test UVCB Biodegradation and Persistence

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Persistence assessment of chemical substances is conducted through a tiered approach for biodegradation testing, from low tier screening tests through inherent biodegradation tests, to

higher tier simulation biodegradation tests. Standardized test methods, such as OECD test guidelines, have been developed for each tier. These standards are, however, not applicable for complex mixtures such as UVCBs (substance of Unknown or Variable compostition, Complex reaction products or Biological materials). UVCBs pose multiple testing challenges: (1) non-specific measuring parameters, such as carbon dioxide production, cannot discriminate the biodegradation of different constituents, and biodegradable major constituents could mask persistent minor constituents, (2) ^{14}C labeling of UVCBs is often not possible, and (3) many UVCBs contain volatile constituents that can be lost during testing. We have recently combined a whole substance biodegradation test with constituent specific analysis that target the challenges of UVCBs in simulation biodegradation testing. This work highlighted further challenges and paved the way for an intensified focus and work within persistence of UVCBs. The aim here, is to present approaches that handle the test challenges in biodegradation testing for persistence assessment of UVCBs. Screening and simulation biodegradation test challenges will be covered. The challenges and applicability of test methods will be illustrated by results from biodegradation tests of two essential oil UVCBs: Pepper Black and Lavender oil. Screening and simulation biodegradation tests were conducted in the period 2022-24. Biotic and abiotic test systems were used to determine biodegradation based on biotic/abiotic peak area ratios. Constituent specific analysis was conducted with SPME GC-MS. The experiments generally showed fast degradation of essential oil constituents. The study demonstrates how biodegradation can be determined for UVCBs with careful design of the experiments, and discuss the information obtained at different test tiers for UVCBs.

4.16.T-02 Unveiling the Microbial Defluorination: Enrichment and Metabolic Insights into n:3 Acid Biotransformation

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The widespread use of aqueous film-forming foams (AFFFs) containing highly persistent per- and poly-fluoroalkyl substances (PFAS) has led to increased PFAS levels in subsurface environments. Fluorotelomer-based AFFFs have been widely used in recent years after the PFOS phaseout, resulting in the presence of multiple fluorotelomers in contaminated sites. Partial biotransformation of fluorotelomers generates intermediate fluorotelomer acids with higher toxicity compared to perfluorinated carboxylic acids (PFCAs), emphasizing the need for effective PFAS remediation strategies leveraging environmental defluorination behaviors.

A biotransformation process known as the "one carbon removal pathway" has been repeatedly observed in studies, wherein microbial enzymatic actions sequentially shorten n:3 fluorotelomer carboxylic acids (n:3 FTCA or n:3 acid, n = 1-5) through the removal of one carbon, pioneering a hypothesis of complete fluorotelomer mineralization. Further understanding of the understudied microbes and enzymes involved in this pathway is essential for comprehending complete fluorotelomer mineralization. In this study, we have developed a novel enrichment culture from an activated sludge inoculum, optimizing for its efficiency in the defluorination of n:3 acids. We incubated the culture for 62 days for the degradation of 5:3 and 4:3 acids. The physiological conditions of microbial growth are monitored by optical density, oxygen consumption, and culture pH. The extent of defluorination is observed to be increasing over incubation. We observe a maximum drop in the initial concentrations of 4:3 and 5:3 acids by 46% and 84%, respectively. This reduction is attributed to the production of specific transformation products (TPs), the generation of fluoride, and another previously unknown process. Additionally, the intermediate TPs are identified as the unsaturated counterparts of 5:3 and 4:3 acids, α-OH 5:3 acid, α-OH 4:3 acid, and the terminal TPs were 4:3 acid, 3:3 acid and short-chain PFCAs with increasing concentrations. Furthermore, the total fluorine analysis validates the fluorine dynamics. The cultures are further screened for the microbial community associated with the defluorination and the presence of relevant genes through metagenomics approach. Our study explores the defluorination potential of aerobically enriched stable microbial consortia, further explaining the metabolic repertoire of these novel cultures proficient in this unique pathway.

4.16.T-03 River Channel Geometry Controls on Chemical Degradation and Persistence: Insights from a Comparative Field Study

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A number of mechanisms contribute to the removal of pollutants in rivers, including microbial transformation, photolysis, sorption to sediment and hydrolysis. These mechanisms, and their relative importance, are affected by intrinsic properties of individual chemicals (e.g. partition coefficients and chemical structure) and environmental conditions, such as temperature, pH, short-wave radiation flux and the location and competence of microbial communities. Some mechanisms will also be affected by hydraulic geometry (channel size and shape). For example, we have conducted a mesocosm study which showed that microbially-mediated transformation rates (nitrification and biodegradation) were inversely proportional to hydraulic radius (the ratio of channel cross-sectional area to wetted perimeter). This is explained by the fact that microbial transformations predominantly occur in fixed biofilms and that contact between the water column and biofilm on sediment will tend to be inversely proportional to hydraulic radius. Here, we test this hypothesis under field conditions. Rate constants of ammonium, linear alkylbenzene sulfonate and caffeine were measured downstream of sewage treatment plants in dye tracing campaigns conducted in two UK rivers with contrasting channel morphologies: (1) the River Maun (shallow, 1 m^3s^{-1} discharge), and (2) the River Calder (deep, 24 m^3s^{-1} discharge). Concentrations of sucralose, a conservative tracer which acts as a marker for domestic wastewater contribution and downstream dilution, were also measured. Higher rate constants were observed in the Maun compared with the Calder, supporting the hypothesized inverse relationship between microbial transformation rates and hydraulic radius. In-stream rate constants reported in the literature for the target chemicals along with estimated channel geometries are generally consistent with this relationship. This study highlights the need to consider geomorphology in modelling chemical behaviour and environmental risk.

4.16.T-04 Naphthalene and 2-Methylnaphthalene Biodegradation by Sediment Bacteria in a Tidally Influenced River in the Northeastern US

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Polycyclic aromatic hydrocarbons (PAHs) are a class of chemicals found in waterways with a history of industrial operations. At an urban, tidally influenced river in the northeastern United States, PAHs and other contaminants (e.g., PCBs, metals) in bottom sediments warrant remediation efforts. In this research, sediment cores sampled from two different locations were used to conduct a variety of tests to evaluate the potential for in situ contaminant biodegradation. Two low molecular weight PAHs (naphthalene and 2-methylnaphthalene) were selected as model contaminants. A bioprospecting approach consisting of both culture-based experiments and application of molecular biological tools (i.e., DNA sequencing and qPCR) were employed. Culture-based experiments utilized synthetic seawater medium augmented with the model contaminants in separate tests with either oxygen or sulfate as potential electron acceptors. After long-term incubation, PAH measurements via gas chromatography-mass spectrometry (GC-MS) revealed that microorganisms indigenous to the sediments are able to biodegrade >99% in roughly 200 days of both naphthalene and 2-methylnaphthalene. Sequencing of 16S rRNA gene amplicons in community DNA extracts from segmented cores and enrichment cultures after biodegradation occurred revealed the presence of a diverse assortment of bacteria potentially involved in contaminant biodegradation. Subsequent isolation efforts employing both marine agar and minimal medium supplied with naphthalene or 2-methylnaphthalene as sole carbon sources and grouping of isolates based on nearly complete 16S rRNA gene sequences recovered representatives from at least 20 species. It is anticipated that characterization of the PAH degradation capacity of the isolates in combination with qPCR assays targeting functional genes will facilitate selection of quantitative biomarkers that can be used to minimize uncertainty and aid in evaluating contaminant biodegradation at this site. Normalizing alternative complimentary approaches, such as bioprospecting and molecular biological tools, may be accretive to enhancing and modernizing how chemical biodegradation is assessed and may be useful in demonstrating natural attenuation.

4.16.T-05 Optimizing Bioaugmentation for Bioremediation of Dilute 1,4-Dioxane Plumes

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1,4-Dioxane (dioxane) is a prevalent and persistent groundwater contaminant often found in expansive, dilute plumes (< 100 µg/L) and that has an EPA health risk limit (HRL) of 0.35 µg/L. However, treating dilute plumes using pump-and-treat or advanced oxidation is often impractical due to the poor cost-to-mass removal ratio. Monitored natural attenuation (MNA) offers a more affordable alternative; however, it fails to meet the stringent cleanup targets within the necessary timeframe at many sites. As a result, there is a need to develop cost-effective in situ remediation techniques that reliably achieve regulatory compliance. Bioaugmentation is an attractive option for in situ treatment of dioxane-contaminated groundwater, as it is relatively inexpensive and well suited for dilute plumes. Yet, while various dioxane co-metabolizing bacteria have demonstrated potential in laboratory and field settings, they are often hindered by the need for primary growth substrates, which raises operational costs, increases non-target microbial growth, and potentially disrupts local ecosystems. Additionally, existing metabolic degraders are typically limited by their kinetic properties, such as high Monod constants (Ks) and the minimum substrate concentrations required for growth (S_{min}), which impede their ability to degrade dioxane at concentrations below 100 µg/L. Moreover, bioaugmentation strain survival and biodegradation activity can be substantially impacted by site-specific conditions, leading to unpredictable results. Our team has developed a promising bioaugmentation strain, *Rhodococcus ruber* RJ01, derived from *R. ruber* 219 through prolonged adaptation to low dioxane concentrations. This strain efficiently metabolizes dioxane to levels below the EPA HRL when supplemented with thiamine, even at concentrations < 100 µg/L. Notably, *R. ruber* RJ01 exhibits exceptionally low Monod constants (Ks = 0.015 ± 0.03 µg/L) and minimal required dioxane concentrations (S_{min} = 0.49 ± 1.16 µg/L), enabling it to grow and function effectively at low contaminant levels. To further broaden the utility of this strain for dioxane bioremediation, we are investigating encapsulation strategies to protect against competitors and provide a locally-available source of thiamine for sustained dioxane degradation. We expect this project to yield a cost-effective, scalable treatment strategy able to ensure performance under a wide range of environmental conditions.

4.16.T-06 Data Miner's Delight: How Bioprospecting Highly Enriched Microbial Cultures can be used to Address Environmental Pollution

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Microorganisms can catalyze innumerable biochemical reactions that can benefit society. The biodegradation of organic chemicals in the environment is one such application, with important implications for legacy contamination and public health. To make the most of the biodegradation potential of microorganisms, it is vital that we that (1) we make sense of the complex biology underpinning these transformation processes, and (2) incorporate key findings into existing environmental monitoring and modeling tools. Using current research on anaerobic benzene degradation as proof-of-concept, this talk will focus on the value of bioprospecting—the methodical exploration of biological systems—for improving our understanding and modelling of chemical biodegradation processes. Benzene (C_6H_6) is a carcinogenic petroleum chemical that is susceptible to biodegradation via aerobic and anaerobic microbial processes. Despite this, the hydrocarbon is notoriously persistent in many anoxic systems (e.g., deep sediments, groundwater). To get to the heart of this problem, we first needed a basic understanding of the biochemistry and ecology behind anaerobic benzene degradation. We began by establishing a series of anaerobic enrichment cultures from benzene-contaminated sites across North America, then used various analytical and molecular instruments to identify key microorganisms, functional genes, and biodegradation pathways. Working with enrichment cultures also enabled us to test individual factors that might slow or inhibit rates of anaerobic benzene degradation *in situ*, by designing experiments with high degrees of experimental control and replication. All data from these laboratory experiments is now being compiled into a metadata modeling tool to help inform and support applications of bioremediation at anoxic benzene-contaminated field sites. It is ultimately the simplicity of microbial model systems such as enrichment cultures that makes them such powerful tools for studying chemical biodegradation processes.

4.17.P-Th Point-of-Use Drinking Water Exposome and Potential Human-Health Effects

4.17.P-Th-053 Predicting Occurrence of Per- and Polyfluoroalkyl Substances (PFAS) in Groundwater at the Depths of Drinking Water Supplies

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Per- and polyfluoroalkyl substances (PFAS) exposure is associated with several adverse human health effects. Large-scale PFAS monitoring efforts at both the state and federal level are underway and are necessary to protect human health. However, PFAS analysis is expensive, time consuming, and rarely includes private well owners that are often in underrepresented rural areas. Predictive models overcome some of these challenges and can be used to identify areas of concern for PFAS in groundwater-based drinking water supplies and direct limited resources to areas of potential concern. Here we present a machine learning model to predict PFAS occurrence in groundwater at the depths of public and private drinking water supplies. Since 2019, the U.S. Geological Survey has sampled for PFAS through the National Water Quality Network groundwater assessment and California Groundwater Ambient Monitoring and Assessment Program—Priority Basin Project. The samples used for training the model (>1,200 groundwater samples) are grouped into over 30 networks (each with >20 wells) that cover a broad range of geographic regions, target either drinking water supplies within a principal aquifer or specific land uses, and largely consist of public supply, domestic, or shallow-monitoring wells. All samples were collected using consistent sampling methods and analytical protocols. The total sum of 24 PFAS compounds was converted to a binary dependent variable (detected or not detected) for modeling, as any PFAS detection indicates anthropogenic influence. We used only mappable factors to enable national predictions across the conterminous United States, allowing predictions in unmonitored locations where water quality data are unavailable. Potential predictor variables evaluated include land use, well depth, depth to water, precipitation, population density, soil characteristics, and many potential PFAS point sources on the landscape like fire training areas, metal plating facilities, plastic manufacturers, and airports. Results indicate that boosted regression tree modeling at the CONUS scale is feasible, and that urban land use and well depth are top predictors for PFAS occurrence. The model was validated using independent datasets for both public and private supply. This study provides information to water resource managers and private well owners to inform users not only where PFAS in groundwater is predicted to occur, but also where it is less likely to occur.

4.17.P-Th-054 Occurrence of Short- and Ultra-Short Chain PFAS in Drinking Water from Flanders (Belgium) and Implications for Human Exposure

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Short- and ultra-short chain PFAS (S- and US-PFAS) are increasingly used as alternatives for the long-chain PFAS which have become more regulated over time. These compounds have a high mobility in the environment and can easily reach drinking water sources. This implies that drinking water could be an important human exposure source to these compounds. Furthermore,

there have been growing concerns around the presence of PFAS in Flanders, as this region has been identified as a major PFAS hotspot in Europe. Because of this, human exposure of S-PFAS and US-PFAS through Flemish drinking water was investigated in this study and to this purpose, the presence of 2 S-PFAS (PFBS and PFBA) and 5 US-PFAS (PFPrS, PFEtS, TFMS, PFPrA, and TFA) was explored in 47 tap water samples, collected from different Flemish provinces, and 16 bottled waters purchased in Flanders. In addition, the levels of S- and US-PFAS in tap water were compared among different provinces, water supply companies, and following the use of a commercial filtration system. Out of the 7 target PFAS, 4 (PFBA, PFBS, PFPrS and PFEtS) were detected at concentrations above their limit of quantification (LOQ) in tap water. In bottled water, only TFMS was present above its LOQ. PFAS concentrations in all analyzed water samples ranged between <0.7–7.3 ng/L for PFBS, <0.03–15.0 ng/L for TFMS and <0.9–12.0 ng/L for PFBA. PFPrS was only detected once above its LOQ, at 0.6 ng/L. No significant differences in PFAS concentrations were seen in tap water among different drinking water companies, provinces, nor between the two types of analyzed bottled water (natural mineral water *versus* spring water). The use of a commercial carbon filter significantly reduced the median concentrations of the studied compounds in tap water, with an average decrease of 84%, 67% and 27% for PFBS, TFMS and PFBA, respectively. Finally, it was estimated that the presence of S- and US-PFAS in Flemish drinking water does not pose an immediate threat to human health, as concentrations were at least two orders of magnitude below the available guidance values.

4.17.P-Th-055 Analysis of Per- and Polyfluoroalkyl Substances in Nebraska Drinking Water

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Widespread environmental contamination of per- and polyfluoroalkyl substances (PFAS) poses significant human health risk and continues to threaten water quality. Despite ongoing nationwide monitoring of drinking water by various government agencies, there is limited information on the extent and quantification of PFAS in Nebraska drinking water, particularly from domestic wells. The primary objective of this research is to sample and measure PFAS in selected domestic wells to characterize the risk of PFAS exposure through drinking water. This study identified seven sites suspected to provide point source contamination of these compounds, four landfill and three military training sites. Using literature-supported protocol, samples for PFAS analysis were collected and analyzed using liquid chromatography tandem mass spectrometry following US Environmental Protection Agency (USEPA) Method 1633. Variables obtained from household- and individual-level surveys will be used to evaluate risk from PFAS exposure and a USEPA hazard index (HI) will help identify households with elevated risk. Preliminary results from surveys reported that 88% of the participants used private well as their primary household water source and 55% of participants reported that the tap water they consumed was treated/filtered. The most frequently reported chronic health conditions included asthma, depressive disorder, hypertension, and high cholesterol. Preliminary results provide evidence for measurable levels of PFAS in several well water samples. This project will provide data on concentrations and spatial patterns of PFAS in residential drinking water in Nebraska and examine associations between PFAS concentrations and social vulnerability variables and health outcome variables. The hazard index will inform public health decisions regarding the risk of PFAS exposure through drinking water.

4.17.P-Th-056 Spatial Distribution and Correlation of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water from Miami-Dade and Palm Beach in South Florida

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Per- and polyfluoroalkyl substances (PFAS) are widely used in industry and consumer products for their heat, water, and oil resistance. These properties make them essential in non-stick cookware, water-resistant textiles, and firefighting foams. Despite their utility, the environmental persistence and bioaccumulation of PFAS raise significant health concerns. Studies link PFAS exposure, particularly through contaminated drinking water, to various adverse outcomes, including developmental delays, immune dysfunction, and increased cancer risk. This study evaluates PFAS occurrence and concentrations in tap water from two counties in South Florida. 76 samples from Miami-Dade (19 zip codes) and 70 from Palm Beach (22 zip codes) are being collected between March- June of 2023 and May- June of 2024, respectively. These samples were evaluated using Solid Phase Extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) following the EPA method 1633. So far, 14%, 78%, and 100% of 50 drinking water samples from 14 zip codes in Miami-Dade County exceed the Maximum Contaminant Level established for PFHxSK, PFOA, and PFOS, respectively, in the recent Drinking Water Regulation established by the Environmental Protection Agency (EPA). Moreover, according to the same regulation, four of the 14 evaluated zip codes are over the ruled Hazard Index (maximum 1). Advanced statistical tools, including Cluster and Principal Components Analysis, will be applied to assess correlations between PFAS profiles and geographic distribution. Our findings will elucidate the extent of PFAS contamination in the region and contribute to the body of evidence on PFAS prevalence near known sources. Our future research will employ a case-control approach, examining banked human serum samples from liver cancer patients and controls in areas with the highest and lowest cancer mortality rates corresponding to the drinking water sample locations. This analysis aims to discern the health impacts of PFAS exposure, enhance our understanding of its implications, and guide public health interventions.

4.17.P-Th-057 Mixed Contaminant Exposure in Tapwater and Potential Human-Health Implications in Disadvantaged Communities: A Case Study in California

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Water is an increasingly precious resource in California as years of drought, climate change, pollution, as well as an expanding population have all stressed the state's drinking water supplies. Currently, there are increasing concerns about whether regulated and unregulated contaminants in drinking water are linked to breast cancer risk and other human-health effects particularly in disadvantaged communities. To begin to address this data gap, the study was designed to assess tap water samples, collected from households in Gold Country, the San Francisco Bay Area, two regions of the Central Valley (Merced Fresno and Kern counties), and southeast Los Angeles, for 251 organic chemicals and 32 inorganic constituents. Sampling was designed to include socioeconomically disadvantaged areas with suspected water quality challenges and elevated breast cancer rates. Mixtures of regulated and unregulated contaminants

were observed frequently in tap water throughout the communities sampled, and the types and concentrations of detected contaminants varied by region, drinking water source, and size of the public water system. Multiple exceedances of enforceable maximum contaminant level(s) (MCL), non-enforceable MCL goal(s) (MCLG), and other health advisories combined with frequent exceedances of benchmark-based hazard indices were also observed. Given the renewed focus on environmental justice and improving water quality in socially disadvantaged communities, our study highlights the importance of assessing mixed-contaminant exposures in drinking water to adequately address human-health concerns. Data from this pilot study provides a foundation for future studies across a greater number of communities in California to assess potential linkages between breast cancer rates and tap water contaminants.

4.17.P-Th-058 Shared Challenge: Private, Public, and Bottled Drinking Water Contaminant Mixtures

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In the United States, point-of-use drinking water is supplied via private tap water (predominantly private wells), public-supply tap water, and bottled water. Differences in management, monitoring, and messaging and lack of directly intercomparable exposure data influence the actual and perceived quality and safety of these different drinking water supplies and directly impact consumer decision making. A comparative analysis of point of use drinking water contaminant mixture exposures and corresponding potential human health effects of private supply, public-supply, and bottled drinking water was conducted by aggregating exposure results and harmonizing apical health benchmark weighted and bioactivity weighted effects predictions across studies conducted and published previously by this research group. Simultaneous exposures to multiple inorganic and organic contaminants of known or suspected human-health concern are common across all three drinking water supplies, with substantial variability observed in each and no systematic difference in predicted cumulative risk between supplies. The results emphasize the need for improved understanding of the adverse human-health implications of long-term exposures to low level inorganic/organic contaminant mixtures across all three distribution pipelines.

4.17.P-Th-059 Drinking-Water Exposome Research: Exposures and Risk in a PFAS Impacted Groundwater Community

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In the United States, private-supply tap water is rarely monitored. This data gap undermines individual and community risk management decision making, leading to increased probability of unrecognized contaminant exposures in locations that rely on private wells. The risk of unrecognized tap water contaminant mixture exposures are elevated for historically rural, actively urbanizing communities that depend on shallow, surficial aquifer private wells, due to limited private-well monitoring and proliferation of surface contaminant sources in urbanizing landscapes. A broad suite of 506 organics, 34 inorganics, and 3 microbial groups was assessed in 11 locations (residential, public) in a hydrologically vulnerable, Mississippi River, alluvial island community, with documented groundwater PFAS contamination. Potential human-health risks of individual and aggregate tap water exposures were estimated based on hazard indices of cumulative apical benchmark-based toxicity quotients and cumulative molecular-scale, *in vitro* exposure-activity ratio(s). Precautionary health based Hazard-Index screening levels were exceeded frequently. In locations adjacent to and downgradient of a municipal airport and corresponding PFAS source areas, tap water exposure risk was attributed primarily to PFAS. Other, more spatially-distributed tap water risk drivers included primarily groundwater (e.g., uranium, manganese, nitrate) and premise-plumbing (e.g., lead, copper) derived inorganics. This study supports the need for increased monitoring of private-well tap water employing a broad, environmentally informative analytical scope to reduce the risks of unrecognized contaminant exposures and to identify possible additional targets for simultaneous tap water risk mitigation.

4.18.P-Mo Quantitative Non-Targeted Analysis (qNTA): Bridging the Gap Between Characterization and Quantitation

4.18.P-Mo-105 Using U.S. EPA Tools for Emerging Contaminant Discovery and Screening-Level Assessment

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Non-targeted analysis (NTA) approaches often reveal a complex landscape of emerging contaminants including manufactured chemicals and associated transformation products in various environmental matrices. Ascertaining potential human health hazards associated with exposures to such emerging contaminants has historically been resource and time intensive. Researchers within the EPA Office of Research and Development (ORD) have therefore developed an efficient approach to screening-level assessments using a combination of quantitative non-targeted analysis (NTA) and chemical hazard profiling. First, for any NTA study, qNTA methods are used to estimate chemical concentrations with 95% confidence limits. As a conservative estimate, the upper confidence limit of each concentration prediction can be converted to a reasonable maximum exposure reference point, using relevant factors such as water intake rate, body weight, relative source contribution, and exposure event frequency. To characterize potential health risk, however, this converted exposure point (expressed in mg/kg-day) must be compared to a toxicological point-of-departure (POD, expressed in mg/kg-day). ORD has several tools that facilitate chemical hazard profiling, including the identification of PODs. These tools offer opportunities to surface existent human health relevant data from epidemiological or experimental animal studies where available, or, for more data-poor chemicals, semi-automated applications such as read-across. ‘Read-across’ considers structural, physicochemical, toxicokinetic, and biological similarities between data-poor target chemicals and more data-rich analogues from which PODs may be adopted as surrogate. When qNTA and media-specific POD values are each identified for a common chemical, a provisional margin-of-exposure (MOE) can be estimated by calculating the ratio of POD/qNTA exposure estimate. A MOE value greater than unity suggests a POD concentration greater than the qNTA upper-bound estimate (and thus, less concern for risk of health effects). A MOE value less than unity suggests possible exposure levels exceeding the POD concentration, thus indicating greater concern for health effects. Such a finding would warrant additional follow-up investigation to better inform human health risks to communities within the impacted areas. This presentation will demonstrate this combined ‘qNTA + hazard profiling’ workflow using example data from a recent environmental health study.

4.18.T Quantitative Non-Targeted Analysis (qNTA): Bridging the Gap Between Characterization and Quantitation

4.18.T-01 Longitudinal Assessment of Organic Chemicals and Prioritization of Chemical Tracers in Drinking water from Miami, South Florida by Non-Targeted Analysis

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Environmental contamination through ingestion, inhalation, and direct contact, are common routes of children's exposure to chemicals, in which through indoor and outdoor activities associated with common hand-to-mouth, touching objects, and behavioral tendencies, children can be susceptible and vulnerable to organic contaminants in the environment. We have previously developed and implemented an innovative non-targeted analytical (NTA) workflow using an online-SPE coupled to a Q-Exactive Orbitrap instrument for the screening of organic chemicals of concern in drinking water collected from Miami residents, Florida. Chemical features were annotated with Compound Discoverer (v3.3, SP2) using online database search such as mzCloud, Mass List search, and Chemspider incorporated into the software. Additional manual data processing and retention time model (using Log Kow) was applied to improve confidence of tentatively identified chemical features. Chemical features were prioritized based on their predominant abundance (peak area>500,000), detection frequency (in >50% of the samples), available information on their uses and potential toxicological effects. Specific tracers of drinking water exposure such as Isophorone and PFOS were selected and confirmed with reference standards. These tracers were estimated in drinking water samples and correlated with sociodemographic factors among South Floridan residents. We are proposing for the first time a long-term monitoring studies for these tracers in drinking water.

4.18.T-02 A Workflow for Defensible Quantitative Non-Targeted Analysis Using the United States Environmental Protection Agency's Non-Targeted Analysis WebApp

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Non-targeted analysis (NTA) is increasingly used for environmental characterization as it allows detection of novel chemicals and more holistic characterization of chemical exposures and cumulative risk. Software tools, such as the U.S. Environmental Protection Agency's Non-Targeted Analysis WebApp (hereafter the NTA WebApp), enable standardized processing of copious data collected via NTA experiments. While the NTA WebApp currently supports qualitative analysis of NTA data, it lacks functionality for quantitative estimation. We introduce here a workflow for processing NTA data in a manner that supports quantitative interpretations. The first part of the workflow facilitates the visualization and correction of sample and feature-level effects that can impact quantitation. Sample-level effects are examined via run sequence plots, multi-component sample quality scores, and quality score plots. Feature-level effects are examined via run sequence plots and log-log calibration curves (for pre-defined and prepared calibrants in matrix). After sample-level correction for batch and/or run sequence effects, calibrants passing feature-level quality thresholds are used for fitting predictive quantitative models. Specifically, calibrant features are the basis for quantitative prediction using a naïve *bounded response factor* method and/or an *ionization efficiency (IE) regression* method (if IE values are provided by the user). This processing workflow complements the existing quality assurance procedures and outputs of the NTA WebApp and is designed to provide defensible outputs from quantitative NTA (qNTA) experiments. *The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

4.18.T-03 Model-Based Selection of qNTA Surrogates to Optimize Predictive Accuracy, Uncertainty, and Reliability

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Quantitative non-targeted analysis (qNTA) combines analytical chemistry with statistical methods to estimate analyte concentrations based on their instrument response. Concentration estimation, when associated with successful chemical identification, enables better assessment of potential risk for prioritizing follow-up targeted studies. Despite the value of this emerging technology, qNTA faces the challenge that analytes of interest are not pre-selected, and thus, concentration estimation is based on the behavior of a selected group of known ‘surrogate’ standards. The relationships between instrument responses and known concentrations of the surrogate standards are used to estimate concentrations and associated confidence intervals for all analytes of interest. Our recent works have established accuracy, uncertainty, and reliability metrics that reflect qNTA performance, and demonstrated baseline utility of qNTA methods for environmental health applications. Herein we present research demonstrating statistical improvement of qNTA models when *a priori* calculations are used to optimize the coverage of suspected chemical space and direct the selection of desirable surrogate standards. We present on the influence of structural consideration on key metrics of qNTA model success, construct a quantity for capturing the coverage of a given set of surrogate standards for a suspected space, and validate our theory via simulated experiments using data from the EPA’s Non-Targeted Analysis Collaborative Trial (ENTACT). The results indicate that integrating structural consideration improves the baseline success of even simplest qNTA models. These qNTA surrogate selection methods are currently assisting NTA study designs for pesticide contaminated areas, with the goal of improving the quality of qNTA estimates for chemicals detected in soils, sediments, and environmental waters. *The views expressed in this abstract do not reflect the views of the United States Environmental Protection Agency.*

4.18.T-04 Evaluating the Performance of Two qNTA Approaches Using Independent Benchmark Values from Targeted Analysis

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Recent research indicates substantially more chemicals are present in water than historically measured using targeted analytical methods. It is impossible to fully understand source and treatment effects on human and ecological exposures and risks without comprehensive chemical characterization of water samples. Non-targeted analysis (NTA), using high-resolution mass spectrometry (HRMS), is well suited to facilitate a more comprehensive chemical characterization of water. To date, most NTA studies of water have generated qualitative results (identifying chemicals of interest), and few have pursued absolute quantitation with consideration for predictive accuracy, uncertainty, and reliability. Performance assessment of quantitative NTA (qNTA) methods can be accomplished through external validation, wherein qNTA estimates are directly compared to independent benchmark estimates. This presentation will focus on a direct comparison of two qNTA approaches, with targeted quantitative estimates serving as the benchmark for method validation. Approach 1 (A1) used a "naïve" *bounded response factor* method to estimate chemical concentrations and confidence intervals – this method does not rely on having confident structural identifications. Approach 2 (A2) used an ionization efficiency regression approach to estimate concentrations and confidence intervals – this method does rely on having confident structural identifications. Estimates from A1 and A2 were compared to independent benchmark values to yield estimates of absolute accuracy quotients (AAQs; measures of accuracy), confidence limit fold ranges (CLFRs; measures of uncertainty), and overall reliability percentages (ORPs; measures of reliability). Finally, this presentation will demonstrate the benefits of the implementation of qNTA in future water research and comprehensive chemical assessments. *The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency but do represent the views of the U.S. Geological Survey.*

4.18.T-05 Estimating Total PFAS Concentrations in Drinking Water Resources Through Application of qNTA

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While traditional targeted analysis methodologies allow researchers to generate both qualitative and quantitative measures of per- and polyfluoroalkyl substances (PFAS), they are naive to the thousands of PFAS that exist in the chemical exposome. Comparatively, non-targeted analysis (NTA) methodologies can identify thousands of potential PFAS in environmental samples but are generally reliant on targeted analysis to produce quantitative estimates. New quantitative non-targeted analysis (qNTA) methods are beginning to allow defensible quantitative estimation of individual PFAS in environmental samples. To date, most qNTA applications at the US EPA have focused on the use of surrogate chemicals to estimate individual compound concentrations, and the development of qNTA performance metrics to define predictive accuracy, uncertainty, and reliability. Despite rigorous development and testing of these qNTA methods, they still require preemptive development of surrogate calibration samples and produce concentration estimates for one chemical at a time. This presentation will describe a new approach for estimating total concentrations of measured PFAS in drinking water extracts using empirical PFAS sample concentrations from targeted analysis. An application of this approach to drinking water samples extracted and analyzed using common PFAS targeted methodology (e.g. EPA methods 533 and 1633) will be presented to investigate the performance of total PFAS concentration estimation.

4.18.T-06 Emerging PFAS in Household Exposure Media from the American Healthy Homes Survey II

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants associated with a wide range of potential health effects and known to be widespread in exposure-relevant media. Approximately twenty commonly identified "legacy" PFAS have been well characterized and examined in many environmental media, but there are known to be thousands of emerging PFAS in industrial and commercial products. These species are also able to transform into additional novel forms or even "legacy" PFAS themselves. As a result, relying solely on "legacy" PFAS using traditional analytical methods severely limits efforts to fingerprint PFAS sources and characterize the totality of PFAS exposures. Tap water is a major source of PFAS exposure to the general population with additional household sources, including textiles and consumer products, also contributing to PFAS exposure. House dust both aggregates persistent chemicals, such as PFAS, present in the home, and can serve as an additional exposure source. We applied non-targeted analysis (NTA) to identify PFAS present in 240 paired drinking water and house dust samples collected during the Department of Housing and Urban Development – Environmental Protection Agency American Healthy Homes Survey (AHHS) II, a nationally representative sample of permanently occupied, non-institutional housing in which children may live. We report on the presence of over forty emerging PFAS in drinking water and house dust, and apply network modeling to test for potential relationships between legacy and emerging species both within and across paired media. The network modeling reveals groups of emerging PFAS which are likely associated with specific source profiles. We further apply quantitative NTA methods to estimate PFAS concentrations for species lacking analytical standards using a collection of PFAS surrogates, and we assess relative body burden attributable to tap water versus house dust. *The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

4.19.A.T Spatial and Temporal Analysis of Organic Contaminants in Humans, Wildlife, and the Environment

4.19.A.T-01 Atmospheric Deposition and Bioaccumulation of Legacy Pollutants in the Great Lakes: A Ten Year Study of Air, Fish, and Herring Gulls, 2010-2020

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Legacy chemicals persist in the environment for a long time after they have been banned. Common legacy pollutants include polychlorinated biphenyls (PCBs),

dichlorodiphenyltrichloroethane (DDT), chlordanes, and polybrominated diphenyl ethers (PBDEs). In this study, we compared the temporal trends of PCBs, DDT, chlordanes and four PBDE congeners (BDE47, BDE99, BDE100, BDE154) in the Great Lakes basin during the period 2010-2020 using air s from the Integrated Atmospheric Deposition Network (IADN), fish from the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) and herring gulls' plasma from the US Fish and Wildlife Service monitoring program. The concentration of PCBs in each matrix showed a decreasing trend with halving times ranging between 5.3 - 9.0 years in air, to 3.4 - 23.1 years in fish, and 5.9 years in birds. BDE 47 was more persistent in fish than in herring gulls, with the longest half-life of 21.7 ± 11.1 years for fish samples from Lake Erie. These variations highlight the importance of considering different biological and environmental compartments when comparing the persistence and mobility of pollutants. Furthermore, air samples collected from two remote sites in the IADN project did not show significant trends for BDE 99, BDE 100, and BDE 154, unlike another rural site on Lake Erie, for which halving times were 8.3 ± 5.1, 7.4 ± 4.5, and 5.8 ± 2.6 years respectively. This disparity among locations emphasizes the impact of localized environmental conditions on the degradation rates of these compounds and underscores the variability in pollutant behavior across different regions.

4.19.A.T-02 PFAS Sources to Rivers: A Spatial and Temporal Analysis of Forever Chemicals in the First Industrial River Basin

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Locating sources of per- and polyfluoroalkyl substances (PFAS) to rivers, and quantifying the loading attributable to those sources, requires temporal and spatial analysis of PFAS loads across river basins. However, as most studies focus on the measurement of PFAS concentrations, there is a distinct lack of mass loading data for rivers worldwide. As a result, we do not have scientifically robust estimates of how much PFAS enter our rivers from different sources within a river basin or how much PFAS flows from rivers into the oceans. Here, we present a temporal and spatial analysis of PFAS loads in the River Mersey Basin, England; a heavily industrialized and urbanized river basin. Our primary aim was to locate and quantify sources of PFAS to the river and to elucidate the spatial and temporal dynamics of PFAS transport. Utilising a combined field sampling and numerical modelling approach, we provide the first temporally robust estimates of PFAS export for a European river system and highlight non-systematic temporal patterns in PFAS transport associated with point source effluents. Mass balance analysis of spatial patterns in PFAS loads within the river basin reveals approximately 50% of PFOS enters the river from wastewater treatment works effluents and there may be large-scale transformation and storage of PFOA in riverine habitats. Our work highlights the urgent need for temporally robust and catchment-scale PFAS mass loading data to help prioritize remediation and management interventions and to reliably estimate the flux of PFAS from rivers to the oceans.

4.19.A.T-03 Spatial and Temporal Distributions of PFAS in Galveston Bay, TX, USA

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Per- and polyfluoroalkyl substances (PFAS) continue to receive significant attention as they are often detected in natural waters and biota. Galveston Bay, TX, USA, is an urbanized estuary that receives river inputs from two highly populated and industrialized drainage basins (San Jacinto and Trinity Rivers). We present data on the concentration of PFAS throughout Galveston Bay waters from a quarterly sampling time series (2017 to 2023). This time series captures the dynamics and concentrations of PFAS across different seasons with different hydrographical conditions, as well as after extreme events such as Hurricane Harvey and the Deer Park Intercontinental Terminals Company (ITC) fire. Water samples were collected from surface and near-bottom waters from eight stations during quarterly cruises in Galveston Bay. The field sites are distributed along a salinity gradient and capture river inputs from the two major tributaries. We performed both targeted PFAS analysis and untargeted suspect screening in the water samples. In general, elevated total dissolved PFAS (C4 to C8) were observed in the upper Galveston Bay, near the San Jacinto and Trinity rivers. Significantly elevated perfluorooctanesulfonic acid (PFOS) and 6:2 fluorinated telomer sulfonates (6:2 FTS), compared to background concentrations, were also observed after the ITC fire.

4.19.A.T-05 Spatial and Ecological Factors Driving Differences in PCB Bioaccumulation Between Gulls and Cormorants in the Laurentian Great Lakes

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Most studies of polychlorinated biphenyls (PCBs) in wildlife have focused on singular bioaccumulation mechanisms of organism physiology, food web effects or environmental factors. We attempted to combine these multiple bioaccumulative pathways to predict the contaminant burdens of different species. We provide a synthesis of PCB bioaccumulation pathways in two Laurentian Great Lakes fish-eating birds, the herring gull (*Larus argentatus*) and the double-crested cormorant (*Nannopterum auritum*). We related PCB concentrations of bird eggs to their fatty acid and stable isotope signatures across environmental gradients and from 9 jointly occupied colonies (2014-2018) in the Great Lakes. Differences in egg PCB burdens between species paralleled levels of parental investment, with semi-precocial gulls accumulating 70% more PCBs than altricial cormorants due to their higher lipid content in eggs. Approximately 84% and 47% of the variation in PCB concentrations was site-linked in gulls and cormorants, respectively. Based on ecological tracers, gulls switched to foraging on lower PCB terrestrial prey in less productive lakes, while cormorants bioaccumulated the least in more productive lakes. Lastly, we speculate that up to 23% of cormorant egg contamination may be driven by subsidies from contaminated southern wintering grounds, which were proportional to winter severity and migratory flyway used. Our findings illustrate that contaminant patterns in avian predators have both compound and complex, and at times counter-intuitive origins, and must be understood in light of multiple life history, ecological and geographic mechanisms.

4.19.A.T-06 Volatile POPs in Air: Assessment of Spatial and Temporal Trends Under the GAPS Network

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Among the persistent organic pollutants (POPs) listed under the Stockholm Convention, Hexachlorobutadiene (HCBD), Pentachloroanisole (PCA, degradation product of pentachlorophenol), pentachlorobenzene (PeCB) and hexachlorobenzene (HCB) are the more volatile organochlorine pesticides listed thus far. HCB was among the original dirty dozen listed since 2004, whereas PeCB, HCBD and PCA were listed in 2009, 2015 and 2017, respectively. Despite the listing, their occurrence in the environment continues to be reported due to their unintentional release from industrial, combustion or degradation processes and given the volatility, they are subjected to long-range atmospheric transport with their presence reported in remote areas. However, monitoring data is sparse on their level in air over time to assess the effectiveness of imposed restrictions as well as spatial information to assess hot spots and regional and global transport. The Global Atmospheric Passive Sampling (GAPS) network has been monitoring POPs and POP-like chemicals since 2005. In 2018, GAPS Megacities (GAPS-MC) sub-network was established with an aim to investigate POPs in highly populous cities of the world. The Polyurethane Foam (PUF) and Sorbent Impregnated PUFs (SIPs) passive air samplers are applied under these networks to investigate the occurrence, transport and exposure of contaminants at different locations. SIPs were deployed at 20 sites under the GAPS Network in the years 2009, 2013 and 2015. In 2017, GAPS expanded the monitoring of volatile chemicals to more than 40 sites across the globe with further expansion in 2022 with deployment at all core GAPS and GAPS-Megacities sites (> 80 sites in total). The samples are currently being analyzed for volatile POPs including HCBD, PCA, HCB, and PeCB. Koblizkova et al. (2012) and Rauert et al. (2018) have previously demonstrated the applicability of SIPs for these chemicals. Air concentrations at 20 GAPS sites in North America and Europe ranged from 2.5-75 pg/m^3 for PeCB and 37-40 pg/m^3 for HCB (Koblizkova et al., 2012), while HCBD and PCA levels were up to 120 and 220 pg/m^3, respectively, in GRULAC region (Rauert et al., 2018). Ongoing analysis of the results will provide the first global scale data on the levels and trends of these volatile POPs from 2009-2022 stemming from a single sampling network.

4.19.B.T Spatial and Temporal Analysis of Organic Contaminants in Humans, Wildlife, and the Environment

4.19.B.T-01 Divergent Transport Dynamics of Alkylated Versus Unsubstituted PAHs at the Air-Water and Sediment-Water Interfaces at a Legacy Creosote Site

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Alkylated PAHs are abundant constituents of many PAH mixtures and are drivers of risk at many contaminated sites. Despite their abundance, the movement of alkylated PAHs remains understudied relative to their unsubstituted parent compounds. In the present study, passive sampling devices were deployed in the air, water and sediments at 11 locations across multiple seasons to capture spatial and temporal variability in the abundance and movement of alkylated PAHs at a legacy creosote site in Oregon, USA. Freely dissolved concentrations of 18 alkyl homologous series were quantified by gas chromatography-triple quadrupole mass-spectrometry.

Application of models of diffusive and advective flux revealed highly abundant 2 to 3-ring alkyl PAHs exhibiting seasonal differences in movement, particularly across the air-water interface. Alkylated PAHs were found to be more abundant than unsubstituted PAHs in all sampled media (sum PAH/APAH concentrations approximately 40% alkyl to >90% alkyl PAHs). Two locations alone contributed the majority of release from sediments with temporal patterns in release from these sites reflected in surface water concentrations throughout the study area. In many instances, alkylated PAHs, particularly C3 and C4 homologues, moved in the opposite direction as unsubstituted PAHs across both the air-water and sediment-water interfaces. While the heterogenous nature of sediments is well established, these data improve our understanding of the impact of small areas of sediment contamination on chemical movement and broader site conditions. The importance of season in the abundance and movement of alkylated PAHs reinforces the need for sampling designs which capture temporal variability and suggests patterns which can be used to inform future sampling at contaminated sites. The novel application of models of flux to freely dissolved alkylated PAH homologous series revealed key differences in sediment-water and air-water exchange relative to their unsubstituted parents. These findings reinforce the need to characterize alkylated PAHs at contaminated sites.

4.19.B.T-02 Global and Historical Per- and Polyfluoroalkyl Substances (PFAS) Exposure in North Atlantic Right Whales

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Per- and Polyfluoroalkyl Substances (PFAS) are of growing concern for their potential adverse environmental and human health effects. PFAS compounds demonstrate remarkable environmental stability and are globally dispersed. Baleen whales are a category of migratory whale species characterized by the formation of baleen used to filter small prey from seawater. Baleen whales are globally distributed and tend to migrate between high-latitude feeding grounds and low-latitude breeding grounds. North Atlantic Right Whales, NARW (*Eubalaena glacialis)* are found throughout the North Atlantic in European and North American populations, with the latter being the most populated. NARWs are critically endangered, with an estimated 360 individuals and only 70 breeding females. Therefore, it is vital to assess all threats to the species' survival. Whales have been documented to accumulate persistent organic pollutants (POPs) but are challenging to study. Current methods for assessing POPs in whales rely on soft tissue obtained from necropsies of beached whales. Baleen, composed mainly of keratin, accumulates PFAS, which has a documented affinity for proteins. By analyzing baleen sections, PFAS exposure can be evaluated throughout the life history of the whale. In addition, PFAS concentrations in the plate can be correlated with confirmed sightings to determine where the whale was when the contamination was absorbed. From this, we can determine not only the exposure profile of the whale but also regional and global PFAS distribution. Our study aims to determine spatial and temporal PFAS profiles in two female NARWs. Plates were sections and digested in 1.0 M KOH in MeOH for 24 hours. Samples were passed through an EnviCarb^{TM} SPE tube to remove solubilized protein and analyzed for 42 legacy and emerging PFAS compounds. A Shimadzu Triple-Quadrupole Liquid Chromatography-Mass Spectrometer in Multiple Reactions Monitoring (MRM) mode was used to quantify PFAS concentrations. PFAS content in baleen is correlated with recorded pregnancies, lactation periods, and geographic location as confirmed by coincident concentrations of the sex hormone progesterone in baleen and visual shipboard observations of whales to confirm pregnancies and the geographic locations of whales during their life history. A detailed life-cycle exposure of PFAS in each whale has been established.

4.19.B.T-03 Spatial and Temporal Variability of Tar Balls on the Texas Coast

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Oil slicks from natural seeps or spills become weathered through both biotic and abiotic processes (i.e., biodegradation and photooxidation) in the ocean. The fate of these hydrocarbons during weathering may be degradation (e.g., the loss of n-alkanes and aromatic compounds), transformation (e.g., the production of oxygenated compounds), or enrichment due to recalcitrance (e.g., 17α(H),21β(H)-hopane). In oil spill forensics, petroleum biomarkers are proven tools for evaluating weathering processes and oil source matching. Oil tar balls are highly weathered oils that typically have a sticky or hard surface and often wash up on the surrounding coastlines. These tar balls are not necessarily from an oil spill. For example, there are an estimated 914 natural oil seep zones throughout the Gulf of Mexico. Here, we utilize diagnostic ratios based on petroleum biomarkers and multivariate statistical methods to assess tar balls collected from the Texas coastline. Due to the range of potential oil sources from natural seeps, we may expect a diverse composition of tar balls under a no-spill scenario. Alternatively, if we observe an abundance of tar balls with similar biomarker compositions, it may suggest a possible unreported spill event or a consistent "natural" source to the region. In this study, the tar balls are extracted in dichloromethane and analyzed via gas chromatography-mass spectrometry to measure several biomarker ratios, also known as diagnostic ratios. Statistical similarity/dissimilarity metrics, model-fit parameters, and weighted-scoring methods are used to assess the temporal and spatial similarities between more than one hundred tar balls collected over the past several years. Finally, we are beginning to explore an untargeted approach to fingerprint these weathered oils.

4.19.B.T-04 Spatial and Temporal Trends in Emerging Organic Contaminants Using Dated Lake Sediment Cores

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This study applies multi-contaminant analyses in dated sediment cores from lakes across Canada. Contaminants of emerging priority inherently lack long term monitoring records. Dated sediment cores facilitate spatio-temporal analysis over multiple decades. Here we developed methods for the targeted analysis of organophosphate ester (OPE) flame retardants, per- and polyfluoroalkyl substances (PFAS), benzophenone UV filters (BP), benzotriazole UV stabilizers (BZT-UVs), and substituted diphenylamine antioxidants (SDPA) using ultrahigh performance liquid chromatography tandem mass spectrometry. Sediment cores were collected at lakes across Canada ranging from highly industrial regions to remote, sparsely populated sites. Sediment coring was conducted at the deepest point using gravity corers, and sectioning occurred on site in 0.5 to 1.0 cm intervals. Sediment sections were freeze-dried and homogenized prior to subsampling for dating and contaminant analysis. Depth-age scales were determined based on Pb-210 and Cs-137 profiles via gamma ray spectrometry and application of the constant rate of supply age model. Contaminants were extracted using a simple solvent extraction with methanol and methyl-tert-butyl ether. Concentrations were multiplied by annual sedimentation rate to evaluate temporal trends on contaminant depositional flux. The results indicated exponentially increasing contaminant deposition with annual fluxes as high as 4 ng cm^{-2} (trixylyl phosphate and UV-328). In each contaminant class, the highest deposition was observed for dioctyldiphenylamine (SDPA), UV-328 (BZT-UV), BP-3 (BP), and trixylyl phosphate (OPE).

4.19.B.T-05 Bioaccumulation of Heterocyclic Aromatic Compounds in a Lake Erie Food-Web

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Hetero-polycyclic aromatic compounds (HPACs) are a subclass of known environmental pollutants called polycyclic aromatic compounds (PACs). Even though the similarities of HPACs to the common PACs result in comparable environmental behaviors and toxic properties, these compounds draw less attention due to challenges like isomeric complexity and limited availability of standards. The increased polarity with the presence of hetero atoms like N, O, and S make these compounds more bioavailable to aquatic organisms compared to their homocyclic analogous. In this study, fifty-three HPACs were analyzed in a food web from Lake Erie using an in-house developed and validated gas chromatography coupled with a tandem mass spectrometric (GC/MS/MS) method. The bias of the method ranged from 54 % to 120 % and limits of detection and quantitation ranged from 0.5 to 2.3 ng/ g and from 1.7 to 7.6 ng/ g, respectively. The method exhibited acceptable inter- and intra-day repeatability for all the analytes. Bioaccumulation and biomagnification were assessed in biota representing four trophic levels from Lake Erie. According to our knowledge, this study represents the first comprehensive food web analysis of most of these compounds in the Great Lakes. The findings shed light on the environmental fate and behavior of HPACs and highlight the importance of studying these compounds in aquatic ecosystems.

4.19.B.T-06 Identification of Halogenated Organic Compounds (HOCs) and DDT+ in Deep Ocean Sediments and Biota from the Southern California Bight Using Nontargeted Chemical Analysis

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The marine ecosystem of the Southern California Bight (SCB) has among the highest concentrations of DDT in the world, with the primary source long believed to be wastewater discharge to the Palos Verdes Shelf, a nearshore region adjacent to Los Angeles and designated Superfund site. However, the recent rediscovery of offshore DDT waste dumping during the same period indicates that the offshore, deep ocean basins may also contain highly polluted sediments, thus increasing the uncertainty of prior estimates of total DDT in the region. Further, the occurrence of an extended suite of DDT related contaminants (DDT+), previously identified in marine mammals, in the SCB sediments is unknown. Here, we use nontargeted chemical analysis to identify halogenated organic compounds (HOCs), including DDT+, in sediments from a known deep ocean dumpsite designated Dumpsite 2. Additionally, we examine the chemical profiles of deep ocean invertebrates and fish from Dumpsite 2 to determine how DDT+

and HOCs move within the deep ocean food web. We detected 49 HOCs across all samples, including 15 DDT+ compounds in the sediment and 10 DDT+ compounds in the biota. We detected several unknown DDT-related compounds previously identified in SCB marine mammals, as well as DDT impurities such as *4,4',4"*-tris(4-chlorophenyl)methane (TCPM) and its isomers. High DDT+ body burdens were found in biota, irrespective of collection location, indicating widespread DDT+ contamination in the deep ocean of the SCB. TCPMs were detected in all biota samples except a single surface species, indicating that deep ocean sediment may be a source of DDT+ to the marine food web. We found no clear trends for DDT+ within deep ocean sediments, but further analysis of additional sediment samples from the Palos Verdes Shelf and extending outward to Santa Catalina Island are currently being analyzed to further understand DDT+ distribution. This study demonstrates that DDT+ is present in the deep ocean food web, and the analysis of the larger suite of DDT+ is critical to trace deep ocean pollution of DDT in the SCB.

4.19.P-We Spatial and Temporal Analysis of Organic Contaminants in Humans, Wildlife, and the Environment

4.19.P-We-165 Characterizing PFAS Environmental Behavior Using a Passive Sampler Toolkit

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Significant attention and concern have focused on the health and ecological implications of the widespread environmental distribution of compounds categorized as per- and poly-fluoroalkyl substances (PFAS). Filling knowledge gaps about the patterns of PFAS in various environmental compartments, their bioavailability, and bioaccumulation potential will improve predictability and contribute to minimizing the risks of legacy and novel PFAS. Laboratory and field studies to address these gaps can be advanced by developing rapid and effective methods to assess PFAS concentrations in PFAS hotspots and predict PFAS concentrations in biological tissues.

To this end, we developed and tested a toolkit of PFAS passive samplers for use in fresh and seawater, sediments, and air. Specifically, we validated microporous polyethylene tubes (PET), agarose diffusion in thin film (AGA-DGT), and polyacrylate solid-phase microextraction (PA-SPME) as aquatic passive samplers under laboratory and field conditions. We used this toolkit to characterize PFAS distribution in mesocosm and field studies to understand and predict the bioaccumulation of PFAS in various aquatic organisms (e.g., fish, mussels, plankton, invertebrates, and reptiles).

4.19.P-We-166 A Four-Hundred-Year-Old Record of Wildfire and Industrial Emissions in the James Bay Region of Northern Quebec

***Jason Ahad**, Josué J. Jautzy, Md Samrat Alam and Joëlle Marion, Natural Resources Canada*

Recent warming and drying have been linked to larger, more frequent and intense wildfires in the boreal forest—a trend that is projected to increase into the future. As these events are significant sources of atmospheric pollutants, boreal wildfire emissions of polycyclic aromatic hydrocarbons (PAHs) are also projected to increase. Understanding historical variability in PAH emissions from fire events is key to providing accurate estimates of future emissions in a warming climate, as is delineating wildfire emissions from other potential sources (e.g., fossil fuel combustion) that could contribute to PAH loadings to boreal regions. In this study, we examined a four-hundred-year-old record of PAHs in dated sediments from a lake in the James Bay region of Northern Québec, Canada (Lac Ménarik). Fluxes of total parent PAHs were low (~ 0.5 g cm^{-2} yr^{-1}) and relatively stable from the beginning of the 17th century to the middle of the 18th century, then increased fourfold to a relatively stable value around 2 g cm^{-2} yr^{-1} until the end of the 19th century. This increase coincided with several large peaks in retene—a marker commonly used for coniferous wood combustion—and with the charcoal record observed in a peat core collected near the eastern shore of Lac Ménarik, suggesting a period of enhanced wildfire activity. The flux of parent PAHs then increased sharply to a mid-20th century peak of ~ 5 g cm^{-2} yr^{-1}, followed by a subsequent decrease back to 17th century levels during the last several decades. These trends were similar to those found for Pb fluxes, which collectively suggest long-range transport of fossil fuel emissions, ostensibly with coal as the dominant source. This study demonstrates that while wildfires will continue to be a source of contaminants to the boreal region of Northern Québec, these emissions are unlikely to surpass historical inputs associated with the burning of fossil fuels.

4.19.P-We-167 Influence of Climate and Local Sources on Long Term Trends of Perfluorinated Alkyl Substances and Brominated Flame Retardants in Landlocked Arctic Char in the Canadian Arctic

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Landlocked Arctic char are the only top predators in most high Arctic lakes and they can serve as a sentinel species for changes in atmospheric inputs of persistent organic pollutants (POPs) to remote freshwater environments. Atmospheric measurements and studies of ice cap snow/firn/ice cores in the Canadian Arctic archipelago have shown continuing and, in some cases, increasing, inputs of polybromodiphenyl ethers (PBDEs) and poly/perfluoroalkyl substances (PFAS). We were interested to see if these trends were reflected in landlocked char as well. PBDEs (13 Br_3-Br_7 congeners), C_6-C_{14}-perfluoro carboxylates (ΣPFCAs) and perfluorooctane sulfonate (PFOS) were determined in muscle of Arctic char and compared results to published global emission estimates and to climate related parameters such as glacier runoff, air temperatures, summer ice free area of the lakes. We selected data from two remote lakes (Amituk on Cornwallis Is. and Hazen on Ellesmere Is.) spanning 14 to 21 sampling years from the early-1990s to 2023, with sample sizes ranging from 3 to 10 fish per year. Σ_{13}PBDEs significantly increased in char from Hazen from the 1990s to 2017 (6.6 %/yr) while showing a slight (non-significant (NS)) decline in Amituk from 2001 to 2017 (-2.6%/yr). However, Σ_{13}PBDEs have declined since 2017 in both lakes (~20%/yr; NS). Published modelled predictions of PBDE emissions from global waste stocks show a decline starting about 10 years earlier illustrating a delayed response at these background sites. ΣC_7-C_{14}-PFCAs in Hazen char increased from 2014 to 2022 (20%/yr) after declining from 2003 (-17%/yr), while in Amituk they increased (7.7%/yr) from 2009 to 2017 and then have declined to 2022 (-10%/yr). The increasing trends of ΣPFCAs and PFOS in char were surprising given the phase-out of perfluorooctanoic acid and related PFAS by 2014 as well as PFOS in the early 2000s. Nevertheless, the general trends of ΣPFCAs and PFOS in char are in agreement with published Arctic snow/ice and air measurements from the mid-2000s to 2015.

Climate related factors could be important. PFOS in char was positively correlated with increasing glacial runoff in Lake Hazen while Σ_{13}PBDEs were not. Annual summer ice free area or ice out date was not correlated with year to year variation of the PBDEs or PFAS. Significantly increasing length-at-age was found for char in Amituk Lake suggesting faster growth rates possibly influenced by greater primary productivity due to warming temperatures.

4.19.P-We-168 Source Apportionment of Heterocyclic Aromatic Compounds in Sediments in Lake Ontario

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Despite the potential risk they pose, hetero-polycyclic aromatic compounds (HPACs) have received less scientific attention compared to their homocyclic counterparts like polycyclic aromatic hydrocarbons and alkylated polycyclic aromatic hydrocarbons. In this study, we analyzed 65 sediment samples collected from Lake Ontario, Canada, from 2014 to 2023 to identify the source and spatiotemporal trends. HPACs profiles of the sediment samples and seven coal tar-based reference materials were obtained using a gas chromatography coupled with a tandem mass spectrometric (GC/MS/MS) targeted analysis method. The mass confirmation of the identified compounds was achieved using gas chromatography coupled with high-resolution time-of-flight mass spectrometry (GC-HRTOF-MS). The individual compound concentrations confirmed that the polycyclic sulfur heterocycles are in lower concentrations compared to nitrogen and oxygen analogous in most samples. 9-bromo acridine and 3,6 dichloro-9H carbazole were identified as the most abundant halogenated HPACs that were included in the targeted analysis method. Over the last decade, no significant difference in HPACs concentrations was identified in these samples. Diagnostic ratio calculations and principal component analysis (PCA) were used to identify the potential sources of HPACs. The results indicated that areas of coal tar-contaminated sediment are potential contributors to the overall contaminant burden of sediments in Lake Ontario.

4.19.P-We-169 Temporal Study of the Relationship Between Polycyclic Aromatic Compounds in Seabird Eggs and Shipping Traffic in the Canadian Arctic

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The Canadian Arctic currently has low levels of shipping traffic compared to other regions globally. However, as ship activity is likely to increase in this region due to ameliorated sea ice conditions and growing industrial activities, marine ecosystems in Canada along the major shipping routes are likely to receive increasing levels of oil-related contaminants in the coming decades. Importantly, areas that experience higher levels of shipping and oil and gas exploration often have higher levels of oil-related contaminants in the marine environment. This may occur through low level chronic exposure, and as a result of oil spills. Polycyclic aromatic compounds (PACs) are a specific class of toxic oil-related contaminants that are associated with shipping and can persist in the environment. This study focuses on examining how ship-based pollution may contribute to contaminant burdens in seabirds. Seabirds, including thick-billed murres (*Uria*

lomvia), are useful for monitoring contaminants like PACs because they are established bioindicators of marine ecosystems. In this temporal study, 180 thick-billed murre eggs have been collected from Coats Island and Prince Leopold Island, with collections ranging from 1993 to 2023. Sample processing will involve microbead extraction using an approach developed for the extraction and determination of PACs specifically in seabird eggs and PACs will be quantified by Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS). The changes in PAC concentrations in the thick-billed murre eggs during this time frame will be assessed and compared with regional vessel traffic. It is hypothesized that PAC concentrations will increase over time as shipping traffic increases in this region. This project will contribute to ongoing monitoring in the Canadian Arctic and will help track how levels of oil- and gas-related pollution change over time in relation to vessel traffic, as well as the evolving risks to wildlife and their habitats.

4.19.P-We-170 Per- and Polyfluoroalkyl substances in Three Beluga Whale Populations in the Canadian Arctic

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Inuit are Indigenous peoples, with the majority living in 53 communities across the Canadian Arctic. Inuit lead subsistence lifestyles that for many communities, include harvesting beluga whales. As part of a larger research and monitoring project evaluating contaminants in three core representative locations in the Canadian Arctic, we report spatial and temporal data on per- and polyfluorinated alkyl substances (PFAS) in beluga whales. Samples from beluga whales were collected by local communities in partnership with researchers in Hendrickson Island, (near Tuktoyaktuk Northwest Territories), and Sanikiluaq and Pangnirtung, Nunavut. Temporal trends were evaluated using liver tissue, however, PFAS were also analyzed in muscle, skin, and blubber in select years as requested by communities to better reflect exposure risks to Inuit. Demographic and morphometric data were also collected such as sex, size, age, and diet markers. Annual mean total PFAS concentrations from 2019 to 2023 were 30-200 ng/g for total PFAS in liver. In addition to C4-C14 perfluorocarboxylic acids (PFCAs) and C4-C12 perfluorosulfonic acids (PFSAs), we also consistently detected *x*:3 fluorotelomer carboxylic acids (FTCA), perfluoroalkylsulfonamides, especially perfluorobutanesulfonamide. The lowest concentrations were found in beluga whales in Pangnirtung. While beluga whales in Sanikiluaq and Hendrickson Island had similar total PFAS concentrations, the congener profiles varied based on the % PFCAs vs %PFOS. Concentrations of PFAS in liver were significantly higher than muscle, skin, and blubber. Lastly, we used a modified method to quantitatively target very long chain PFCAs from C15 to C20 in liver.

4.19.P-We-171 Spatial and Temporal Distribution of Per-and Polyfluoroalkyl Substances in House Sparrows in an Industrialised Urban Environment

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Per-and polyfluoroalkyl substances (PFAS) are prevalent in industrialized urban environments and are known to bioaccumulate in wildlife. However, understanding of the distribution of ecologically accessible PFAS and their transfer through terrestrial food webs remains limited. In this study, we investigate PFAS exposure in house sparrows inhabiting various sites along an industrialized urban land-use gradient in Melbourne, Australia. We targeted several likely PFAS point sources, including fire training areas at a military airbase, a wastewater treatment plant, a chemical manufacturing plant, and an oil refinery. From each site, we collected whole blood samples from adult and juvenile house sparrows to identify age-related differences in PFAS bioaccumulation. These samples were analyzed for 36 PFAS using UHPLC-MS/MS. Preliminary analysis revealed that blood PFAS levels were highest at sites where the home range of house sparrows overlapped with fire training areas associated with the historic use of aqueous film-forming foams (AFFF). At these locations, concentrations of compounds associated with AFFF use, such as PFOS, PFHxS, and PFOA, were the highest among detectable PFAS in house sparrows. Sparrows from sites outside these areas showed significantly lower overall blood PFAS levels, indicating a rapid decline in ecologically accessible PFAS outside AFFF source zones. In house sparrows sampled at sites downstream of AFFF source zones and around other urban PFAS sources, such as a wastewater treatment plant, a chemical manufacturing plant, and an oil refinery, blood PFAS concentrations consisted of a higher proportion of PFAS used in the manufacture of industrial and consumer products. These included PFNA, PFDA, PFuDA, PFDoA, PFTrDA, and PFTeDA. Higher blood PFAS levels were detected in juvenile house sparrows compared to adults, potentially due to the greater dietary intake of animal material during early development. Ongoing research aims to recapture house sparrows to identify temporal changes in blood PFAS concentrations outside the breeding season. Our findings provide insight into the distribution of ecologically accessible PFAS in an urban terrestrial environment and highlight susceptibilities to PFAS exposure based on maturity and possibly diet.

4.19.P-We-172 Distribution of Environmental Monitoring of PFAS in South Korea (2021-2022)

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PFAS (per- and polyfluoroalkyl substances) are substances that hardly decompose in nature due to strong carbon-fluorine bonds and do not accumulate in body fat due to their lack of fat solubility, but exhibit water solubility and tend to be concentrated by combining with plasma proteins and polymers. These substances were mainly used as insulators for wires, firefighting foam, Teflon coating (PTFE) of cookware, and Gore-Tex. Representative substances include PFOS (Perfluorooctanesulfonic acid) and PFOA (Perfluorooctanoic acid). In the 2009 Stockholm Convention, PFOS and PFOS salts were listed in Annex B, and in Korea, management began as a residual organic pollutant in 2013. In the 2019 Stockholm Convention, PFOA and its salts were listed in Annex A, and in Korea, management began as residual organic pollutants in 2015. In this study, sampling and analysis were conducted from 2021 to 2022 at 171 residual pollutant monitoring sites (38 in the atmosphere, 61 in the soil, 36 in the water, and 36 in the sediment) in Korea to analyze the distribution of PFAS concentrations. PFBA, PFHxA, PFOA, PFNA, and PFDA were analyzed as PFCAs (Perfluoroalkyl Carboxylic Acids), and PFBS, PFHxS, and PFOS were analyzed as PFSAs (Perfluoroalkyl Sulfonic Acids). From 2021 to 2022, environmental atmosphere analysis showed a high concentration trend at the Gumi Gongdan-dong branch and the Danyang Maepo-ri branch, and as a result of soil analysis, high concentration trend was shown at the Dalseong-dong branch in Daegu and the Ulsan Industrial

Complex branch. As a result of the analysis in water quality, high concentration trend was found at the Anseongcheon estuary point and the Kumho River branch. In sediments, it was generally not detected.

4.19.P-We-174 Understanding the Extent of PFAS Contamination in Red Drum (Sciaenops ocellatus) Across 9 Florida Estuaries

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The widespread distribution and persistence of per- and polyfluoroalkyl substances (PFAS) in coastal environments present significant risks to both wildlife and human health. This study focuses on PFAS contamination in a valuable recreational fishery from coastal South Florida. Red drum (*Sciaenops ocellatus*) are particularly susceptible to exposure to aquatic pollutants due to their relative proximity to human sources of pollution and high site fidelity within estuaries. The objectives of this study are to evaluate PFAS occurrence, composition and quantify the extent to which red drum are contaminated, to then assess the risk of exposure to humans via consumption based on PFAS concentrations. We propose to address this by using a total of 109 muscle samples collected from nine Florida estuaries and analyze the samples using liquid chromatography- triple quadrupole mass spectrometry (LC-MS/MS) to determine PFAS concentration levels. Univariate and multivariate statistical analyses will be used to evaluate the similarities and differences of contaminant compositions between estuaries. This approach addresses a knowledge gap in understanding the impact of PFAS on marine and estuarine ecosystems and calls for effective policy and management practices to reduce contamination sources.

4.19.P-We-175 Application of Passive Sampling for Characterizing Spatial and Temporal Variation of Persistent, Mobile, and Toxic Substances in Aquatic Environment

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The occurrence of organic contaminants in the water environment from anthropogenic activities poses deleterious effects on aquatic biota and human health, which necessitates meticulous surveillance and management plans. Spatial and temporal monitoring of organic contaminants offers insights into the spatial distribution patterns of contaminants within water bodies, thereby facilitating the identification of hotspots and vulnerable areas. It is challenging to capture fluctuating concentrations of organic contaminants because it requires a series of water sampling and subsequent extraction and instrumental analysis. In this study, spatial and temporal variations of organic contaminants are monitored using a passive sampling approach. Since contaminants are accumulated on passive samplers over a sampling period, such integrated sampling offers enhanced sensitivity, reduced sampling frequency, and broad spatial coverage. This study aims to elucidate the interplay between hydrological variables and contaminant dynamics in the water environment. Passive sampling campaigns were conducted along the river including the outflow of wastewater treatment facility during both dry and flood seasons. A broad spectrum of contaminants including pesticides and pharmaceuticals were analyzed and their spatial and temporal variations were investigated. Especially, persistent, mobile, and toxic (PMT) substances were targeted. The results of this research contribute to predicting and

mitigating the environmental risk of organic contaminants in the water environment. In addition, this research helps stakeholders implement targeted remediation measures to mitigate environmental risks effectively.

4.19.P-We-176 Ambient PFAS Concentrations in the United States: A Literature Review

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Due to per- and polyfluoroalkyl substances (PFAS)'s effects on human and environmental health, there has been increased interest in releasing guidance and regulatory values for these substances. Consequently, to provide a contextual basis for such values, an understanding of PFAS concentrations found in the environment is crucial, as these "forever chemicals" are known for their ubiquitous global distribution. Though many studies have focused on PFAS concentrations associated with contamination, less research has focused on "ambient" or "background" PFAS levels removed from sources of contamination for matrices other than drinking water. To demonstrate an approach to how this data can be collected and summarized, we conducted a literature review of PFAS ambient concentrations for 6 environmental matrices (rainwater, freshwater, marine water, freshwater sediment, marine sediment and soil). From these studies, reported concentrations of PFAS analytes listed in U.S. EPA method 1633 were entered into a database. Summary statistics and boxplots were generated to visualize data for six PFAS analytes—PFOS, PFOA, PFBA, PFHxA, PFHxS, and 6:2 FTS. The most studies were available for freshwater, with fewer studies reporting ambient PFAS concentrations in rainwater or in marine sediment and marine water. Overall, ambient PFAS concentrations were higher in freshwater than in marine and rainwater, with measured surface water concentrations occasionally exceeding the USEPA's PFOA and PFOS maximum concentration levels (MCLs). PFAS concentrations in soil and sediment were frequently below detection limits, with PFOS and other long chain analytes typically being predominant. Future research and review efforts are needed to help address data gaps and discuss important considerations to further the understanding of background PFAS levels.

4.19.P-We-177 Distribution Characteristics of Pentachlorobenzene in the Environmental Atmosphere and Soil

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Pentachlorobenzene(PeCBz) is a process by-product and classified as an organic chlorine pesticide, and has been banned from use since the 1970s. PeCBz was registered in Annex A and C of the Stockholm Convention in 2009, but studies on the concentration of PeCBz by land use in the measurement area are still insignificant. Therefore, this study attempted to analyze the concentration of PeCBz by land use in air and soil media. In addition, Persistent Organic Pollutants (POPs) detected in the atmosphere drop due to wet deposition, etc., and accumulate in the soil for a long period of time, affecting the concentration of pollutants in the soil. Therefore, in this study, 38 air measurement points in South Korea were classified into 5 land uses, and the trend of the concentration of PeCBz in the soil was analyzed for each use. First, the average concentration of PeCBz in both the environmental atmosphere and soil was the highest in the industrial area. In addition, as a result of correlation analysis using the SPSS statistical program, it was confirmed that in the case of soil, there was a significant difference in concentration by land use of soil classified into six categories. Next, it was intended to analyze the correlation between the distribution of PeCBz in the atmosphere and soil. To this end, 38 atmospheric monitoring network points were divided into five land uses: industry, commerce, housing, green land, and background. After that, 38 atmospheric measurement network points for each use were buffered in a radius of 10 km using QGIS, a spatial geographic information program, and the PeCBz concentration of soil contained therein was analyzed. As a result, the concentration of PeCBz in soil was measured the highest in the industrial and commercial areas, followed by the high concentration in the industrial, commercial, and residential areas. PeCBz is a material that is intentionally produced due to incineration and incomplete combustion. Therefore, it is believed that the drop in PeCBz in the atmosphere produced by process combustion, waste incineration, and fuel combustion in industrial, commercial, and residential areas affected the soil concentration.

4.20.P-Mo The Practicalities of Non-Targeted Analysis to Support Decision Making

4.20.P-Mo-107 Capacity Building on Non-Target Chemical Analysis for Identifying the Origins of Sudden Environmental Pollution—Japanese Collaborative Trial on Non-Target Screening of Organic Compounds in Water

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Sudden environmental pollution, such as strange odors, water coloring, and foaming, not only can harm living organisms, but may also be able to affect the health of residents. Once such pollution occurs, local administrative office in the polluted area is required to immediately discover the source of origin and take any technical measures. In that case, chemical analysis that allows to detect the causative chemicals varying from case to case is required. Here, to build chemical analysis capacity for identifying the origins of sudden environmental pollution, nearly 11 laboratories across national and local research institutes have participated in the collaborative trial on non-target screening of organic compounds in environmental water. Specifically, we used various mass spectrometers coupled with gas chromatograph (GC-MS) or liquid chromatograph (LC-MS). Also, 20 synthetic chemical mixtures consisting of analytical chemical standards that has previously detected as pollutants, such as pesticides and surfactants, as well as extracts of river water and sediment are prepared. Then, we examined performance of non-target screening methods operated by the participants. This presentation gives the purpose of our collaborative trial, materials and methods, and analytical results of the chemical mixtures.

4.20.P-Mo-108 Characterizing the Chemical Space of Groundwaters from the Biscayne Aquifer in Miami, Florida Using Multiple Analytical Techniques

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Groundwater is an important source for drinking water and has agricultural and industrial uses. South Florida ecosystems are heavily managed to prevent flooding and provide access to water for food production. This is an interconnected system with considerable interactions between surface water, groundwater, and coastal ecosystems. The introduction of organic contaminants and limiting nutrients into Biscayne Bay from highly urbanized watersheds can negatively affect the ecological and economic balance of a critical environment. Therefore, understanding the composition and overall quality of groundwater is critical to understanding the negative impacts of contaminants transported in it. High levels of total phosphorus, ranging from non-detectable limits to high levels of 2,076 mg/L, were detected in Miami-Dade groundwater in May and April of 2023. Phosphorus is the limiting nutrient in the watershed, and the high levels of nutrients entering the system are contributing to the deterioration of water quality. Currently, knowledge of the chemical space of the groundwater in this area is limited, impeding the identification of specific sources and means of transport of phosphorus into the bay. Performing nontarget analysis (NTA) with the use of online solid phase extraction high-pressure liquid chromatography, paired with high resolution mass spectrometry via a Q-Exactive Orbitrap (SPE-HPLC-HRMS) enabled the analysis of legacy and emerging contaminants. NTA was carried out with Compound Discoverer 3.3 and found variations in the presence of organic contaminants between sites. Using NTA, specific compounds were selected to be tracers for unique sources. The average concentrations of the anthropogenic tracers sucralose and caffeine were determined to be 535 ± 572 ng/L and 48.4 ± 31.8 ng/L, respectively, with considerable temporal and spatial variations. This work demonstrates the necessity of thorough analysis for monitoring known contaminants (organic and inorganic) and identifying unknown chemicals in environments heavily impacted by anthropogenic sources such as increased urban development and vulnerable wastewater systems to better understand the overall picture of groundwater quality in the Biscayne Bay Watershed.

4.20.P-Mo-109 Characterizing Chemical Space Coverage of Multiple Solid Phase Extraction Methods for Use with Non-Targeted Analysis in Environmental Waters

Laura D. Brunelle[1]*, Eva K. Stebel*[2] *and Angela Batt*[2]*, (1)ORISE Postdoctoral Fellow at the U.S. Environmental Protection Agency, (2)U.S. Environmental Protection Agency*

Non-targeted analysis is a powerful tool for studying environmental contaminants, with the ability to provide a more comprehensive analysis by allowing for the detection of hundreds to thousands of chemicals in a single method. While the presence of a detected chemical comes with a certain level of confidence, non-detection of a chemical of interest is more difficult to interpret with non-targeted analysis. To better inform decision making and data interpretation, characterization of the chemical space covered by extraction methods used for non-targeted analysis is needed. Knowledge on which classes of compounds are amendable to extraction and instrumental methods provides valuable insight on method biases and the chemical space covered within a given method. In this work, ~200 reference standards spanning multiple classes and uses, were first used as surrogate chemicals to explore extraction efficiencies in spiked

laboratory and environmental water samples. These standards included 100+ pesticides and pesticide metabolites (ex. organochlorines, organophosphates, carbamates, triazines, neonicotinoids), per-and polyfluoroalkyl substances, personal care products, drugs of abuse, and 100+ pharmaceuticals (ex. antibiotics, antidepressants, antivirals, veterinary drugs, commonly used medications). In preliminary studies, multiple solid phase extraction (SPE) cartridge chemistries and extraction methods were examined (HLB, WAX, MCX, GCB, WCX), including stacking SPE cartridges to combine multiple cartridge chemistries. Additional factors including use of chelating agents, pH of samples, acid type used for pH adjustment, and clean-up steps were also tested. The top three performing method conditions were further optimized and used for the extraction of environmental surface waters. The recovery efficiency of the surrogate chemicals was determined in spiked environmental water samples, and the total number and types of non-targeted features retained by each method were explored. The goal of this work was to characterize the captured chemical space to provide more confidence and improve interpretation of non-targeted results. An additional benefit of this work is providing a suite of non-targeted extraction methods with varying characterized chemical coverages for use in future non-targeted efforts.

4.20.P-Mo-110 Retrospective Non-Targeted Analysis and Suspect Screening of Pesticides in Stored Extracts from Surface Water Surrounding a Closed Ethanol Production Facility

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Non-targeted analysis (NTA) and suspect screening were used to identify pesticides and potential transformation products in the surface water surrounding a closed ethanol production facility near Mead, Nebraska. The facility previously used pesticide coated seed corn in the ethanol production process, resulting in high levels of several neonicotinoid pesticides being reported in various media on and around the site. The University of Nebraska at Lincoln has been monitoring the surrounding surface and ground water since 2021 with targeted methods for about 30 pesticides, including neonicotinoid insecticides and strobularin fungicides. Targeted pesticides were chosen based on market research to include pesticides that are frequently used to treat seed corn, however, the full range of pesticides and other chemical contaminants that may be present around the site is not known. Stored sample extracts from 2021 used for the targeted methods were retrospectively analyzed with a NTA workflow using ultraperformance liquid chromatography and high-resolution mass spectrometry (LC-Q-ToF) equipped with electrospray ionization. This workflow included a targeted screening of over 1000 pesticides and pesticide metabolites using an in-house experimentally collected database. Additional external database spectra searching for other suspected chemicals was also included, such as additional pesticides, in-silico predicted degradation products of neonicotinoids, perfluorinated chemicals, antibiotics, mycotoxins, and surfactants. Several challenges were encountered with the retrospective analysis, such as a limited amount of sample extract, different detection limits and dynamic range between the target and non-targeted methods, retention time alignment issues with heavily matrixed samples, and a need to characterize the chemical space coverage of the targeted extraction method. This work will discuss the results of the non-target and suspect screening of the stored extracts, and how retrospective analysis challenges were addressed.

4.20.P-Mo-111 Comparing Ecosystem Functionality of Agricultural and Natural Waterways: An NTA Approach

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Human activities have changed the functionality of landscapes from primarily supporting natural systems to primarily serving human needs (e.g., agriculture). Many studies have highlighted differences between agricultural and natural systems in terms of water quality, nutrient loading, and biodiversity, but these methods tend to be highly specific and do not capture the breadth of ecosystem functionality. Non-target chemical analysis offers a more holistic approach for assessing and comparing functionality between landscapes by capturing thousands of compounds in an environmental sample. This study aims to compare the ecosystem functions of natural versus agricultural landscapes using multivariate statistical tools. Non-target chemical composition of water from 10 locations in the Klamath Irrigation District, 14 locations along the Lost River, and 5 locations along the Klamath River below Upper Klamath Lake (OR) was analyzed using NMDS (non-metric multi-dimensional scaling) and NMF (non-negative matrix factorization). We expect that irrigation canal water is similar in chemical composition to river water and hypothesize that irrigation canals provide similar ecosystem functions as natural rivers.

4.20.P-Mo-112 Non-Targeted Analysis and Estimated Concentrations of Pesticides in Grab Samples Collected from Surface Water Surrounding a Closed Ethanol Production Facility

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Non-targeted analysis (NTA) was used to identify pesticides and possible degradation products in the surface water surrounding a closed ethanol production facility near Mead, Nebraska, which had been using pesticide coated seed corn in the ethanol production process. A wastewater spill and land-applied application of waste product resulted in extremely high levels of neonicotinoid insecticides and other pesticides used in treated seed being found in various media (wastewater, soil/sediments, surface water) at and around the site. The University of Nebraska at Lincoln has used targeted methods to monitor about 30 pesticides in the surrounding surface and ground water since 2021. For this work, grab samples were collected in September 2023 for NTA and targeted pesticide screening using an in-house database of over 1000 pesticide and pesticide metabolites, around 240 pharmaceuticals and personal care products (PPCPs), 70 per- and polyfluoroalkyl substances (PFAS), and other anthropogenic markers to provide insight on which contaminants and degradation products may still be present. The grab samples were extracted by solid phase extraction using multiple sorbent materials (HLB-WAX) to capture a wider range of compounds. The extraction method was characterized prior to sample collection using about 200 surrogate chemicals, including pesticides, PPCPs, and PFAS. Samples were spiked with isotopically labeled compounds before extraction, and a laboratory fortified matrix sample spiked with the 200 chemical surrogates was also included. The isotopically labeled compounds and surrogate chemicals were used to monitor extraction efficiency and allow for estimated concentrations for the included chemical surrogates and related compounds (neonicotinoids, triazines, azoxystrobin, metalaxyl etc). This work presents the non-targeted analysis, targeted screening, and estimated pesticide concentrations of the grab samples collected

in 2023 to provide a more comprehensive assessment of the chemical profile of run-off from the area impacted by wastewater and waste products.

4.20.T The Practicalities of Non-Targeted Analysis to Support Decision Making

4.20.T-01 EPA's Non-Targeted Analysis WebApp: A Web-Based Software Tool for Production-Level NTA

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Targeted methods have long been the standard for chemical monitoring, allowing for assessment and quantitation of environmental exposures that may impact public health. These methods undergo validation and standardization in order to generate reproducible and actionable results. The limitation of targeted methods is their pre-defined scope where the researcher is only informed on specific targeted chemicals. With an increasing need to discover and identify new and emerging chemicals of concern, chemical monitoring has shifted towards non-targeted analysis (NTA) approaches which inform on all chemicals detectable by the analytical instrument. Due to the relatively nascent nature of this field, there is a lack of standardization of methodologies used by researchers, which may involve multiple time-intensive and manually laborious steps. Because of these limitations, NTA methods have lagged behind targeted methods in their ability to generate actionable and defensible results. In order to address these limitations, the US EPA has developed a Python-based NTA web application (hereafter referred to as “the NTA WebApp”) that allows for efficient, transparent and reproducible processing of high-resolution mass spectrometry NTA data from multiple vendors. The NTA WebApp has the following primary functions: (1) evaluation and reporting of quality control metrics, (2) data calls to multiple EPA databases to support chemical identification and prioritization, (3) generation of standardized results and figures, and (4) documentation on all processing steps and associated user-defined parameters. The specific functions within the NTA WebApp were designed to be in accordance with suggested practices from multiple NTA communities, including the Best Practices for Non-Targeted Analysis working group (BP4NTA), the NORMAN Network, and the Metabolomics Quality Assurance and Quality Control Consortium (mQACC). To date, the NTA WebApp has been used in multiple NTA exposure monitoring studies and an example study will be presented to demonstrate the previously described functionalities. *Abstract does not necessarily reflect U.S. Environmental Protection Agency policy.*

4.20.T-02 Investigating the Impacts of Sample Replication: A Cost-Benefit Analysis of Non-Targeted Analysis Study Design

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Regardless of sample matrix or compound class(es) of interest, non-targeted analysis (NTA)

methods expand the measurable chemical space far beyond the capabilities of traditional targeted methods. Because of this capability, NTA methods are becoming an integral part of the analytical toolkit for environmental monitoring of contaminants. NTA methods yield data on thousands of chemical features, whereas targeted methods typically capture fewer than one hundred. The sheer volume of data generated by NTA methods limits researchers' abilities to perform manual review, and the time and financial cost of NTA methods can be prohibitive to large-scale studies. Data processing tools have been developed to assist researchers in determining reproducible and non-reproducible features, but filtering features by reproducibility or variability across samples, or by feature abundance above a minimum reporting limit in a blank sample, necessitates measurement of sample replicates. While sample replication can increase the confidence in features measured and reported in an NTA study, sample replication further exacerbates the time and financial costs of implementing NTA methods. To address this issue, the NTA team at the Environmental Protection Agency's Office of Research and Development (EPA ORD) has designed an experiment to evaluate whether sample replication is a necessity in NTA study design. Using the EPA NTA WebApp, a web application created to standardize QA/QC workflows and feature annotations for NTA studies, we simulate the results of many unique combinations of sample replicates and explore the data penalties, measured through noise gain and information loss, incurred as replication rate is varied. The results of these simulations, combined with the cost-benefits of replicate reduction, can be used to inform the necessity of replication in NTA studies and optimize replication rate to preserve data quality and potentially reduce the time and expense required to perform NTA.

4.20.T-03 Development and Application of Non-Targeted Analysis Workflows, Tools, and Resources for State Investigations of PFAS in Drinking Water Resources

***Heather Whitehead**, U.S. Environmental Protection Agency*

Public and environmental health concerns over per- and polyfluoroalkyl substances (PFAS) has prompted states to pursue environmental and biomonitoring studies to examine their occurrences and exposure routes. In addition to traditional targeted analysis methodologies employed in public health laboratories, states have begun implementing non-targeted analysis (NTA) methods to address the disparity in the relatively few PFAS measured through typical targeted analysis methods to the thousands of PFAS that exist in the chemical exposome. While the use of NTA can provide a more comprehensive assessment of PFAS environmental occurrence to inform public health protective strategies, there are significant barriers to state adoption of NTA. In addition to fundamental barriers like instrument cost and availability, barriers like access to and use of computational resources and analytical expertise further limit implementation of NTA. EPA's Office of Research and Development (ORD) has been working to develop methods, tools, and data processing workflows along with technical collaborations to enable NTA adoption and technology transfer within state agencies. Partner states have embraced these tools and resources to replicate ORD's NTA analysis and acquire independent capability, greatly expanding laboratory capacity to investigate environmental emerging contaminants of concern. This presentation will provide an overview of these tools and workflows and demonstrate their application in partner states to generate NTA results that support the identification of PFAS in drinking water resources.

4.20.T-04 Targeted and Non-Targeted Sampling and LC-MS Analysis of Surface Water for Environmental Contaminants

Mark W. Sumarah, *Justin Renaud, Lyne Sabourin and Natasha DesRochers, Agriculture and AgriFood Canada, Canada*

Agriculture and Agri-Food Canada has been collaborating with federal, provincial, academic, local conservation authorities, and Indigenous partners to bolster integrated analytical approaches to measure emerging environmental contaminants in mixed-use watersheds. Most monitoring and environmental surveillance programs rely on targeted LC-MS/MS analysis of surface water samples, resulting in high operating costs and an increased risk of missing emerging substances, including metabolites. This consortium proposes an approach which includes: passive sampling through the Canadian Passive Sampling Network (CaPSaN), coordination with Health Canada's National Water Monitoring Program for Pesticides (NWMPP), and the application of online SPE for sample prep, which has allowed for the collection of much small volume samples and reduced time and costs. The goal is to reduce the NWMPP's need for bi-weekly sampling, while still providing the critical data need for regulation. Using our approach, online SPE samples are analyzed for 600 environmental contaminants of concern by targeted LC-MS/MS, and then diverted for non-targeted analysis (NTA) on the same sample. We are committed to sharing these data with international partners such as NORMAN and the BP4NTA working group, to strengthen evidence-based policy making.

4.20.T-05 Application of Non-Targeted Analyses to Inform the Design of a Statewide Monitoring Program in California

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The Stream Pollution Trends Monitoring program (SPoT) is a statewide program initiated in 2008 under the Surface Water Ambient Monitoring Program of the California State Water Resources Control Board. SPoT was designed to evaluate trends in sediment toxicity and contaminants and identify their relationships with land use. The program provides critical information on the condition of California waterways to state, regional and local management programs. Sediment samples are collected annually from up to 100 sites, analyzed for several contaminants and tested for toxicity markers using the amphipod *Hyalella azteca* and the midge *Chironomus dilutus*. With an expanding list of emerging contaminants and increasing costs associated with targeted chemistry, new approaches are required to adequately address the monitoring needs in California. To this end, in a 2021 pilot project, 30 sediment samples were selected for non-targeted chemical analyses (NTA). Sediment extracts were analyzed using liquid and gas chromatography with high-resolution mass spectrometry (LC-HRMS and GC-HRMS). Features of interest were selected based on library matches and toxicity linkages which were identified using Spearman's rank correlations. Results from LC-HRMS provided a total of 245 features which correlated with toxicity. Out of 700 features detected in the GC-HRMS results, 424 library matches and 51 correlations with toxicity were observed. Interestingly, some of these correlations were in concordance with historic SPoT findings such as identification of pyrethroid-driven toxicity. In addition, contaminants including pharmaceuticals and personal care products were also found to be correlated with toxicity. Integration of NTA within existing monitoring programs, such as SPoT, enables identification of contaminants that are missed from routine targeted monitoring and enhances the program's screening power to prioritize contaminant monitoring on a statewide and regional level. Ongoing work includes application of this integrated approach to assess temporal trends, comparisons across environmental matrices, and linkages with sublethal toxicity markers. This work supports advancement of sediment and water quality monitoring approaches across California and assists in development of resource-efficient and comprehensive environmental assessments.

4.20.T-06 Non-Targeted Analysis and Risk-Based Prioritization of Emerging Contaminants in Two Common Effluent Treatment Plants (CETPs) in India

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In India, small and medium-scale industries send their effluent to common effluent treatment plants (CETPs). Prioritization of compounds in CETP effluent is necessary for focusing research and management efforts on the most critical compounds that are environmentally more hazardous to non-target species and human health. The priority compounds may differ based on the geographical location of the CETP, as their levels of occurrence can be shaped by the industries they serve, as well as demographic and regional factors. In this study, a simple qualitative analysis-based prioritization approach is proposed for selecting components for future site-specific monitoring of CETP effluents. This approach could account for the unique characteristics of each site, ensuring a more targeted and effective response to managing emerging contaminants. In the present study, micropollutants were extracted from the CETP effluent using solid-phase extraction followed by analysis in HRLC-MS Q-orbitrap. The overall scores signifying the risk levels were determined based on three criteria (1) occurrence (abundance), (2) exposure potential (half-life), and (3) ecological effects (bioaccumulation and ecotoxicity). The number and class of compounds identified varied significantly in the two CETPs and included antibiotics, steroids, antidepressants, anthelmintics, herbicides, opioids, sedatives, antivirals, NSAIDs, insecticides, fungicides, vasodilators, and stimulants. Numerous antimicrobials (antibiotics, antivirals, and anthelmintics), antidepressants, and NSAIDs were present in the CETP-1 effluent. The average scores were approximately 0.62, 0.54, and 0.54 for NSAIDs, antibiotics, and antidepressants, respectively. For antibiotics, the scores followed the trend azithromycin > erythromycin > benzothiazole > enrofloxacin > sulfamethazine, and linezolid. Meloxicam, cannabidiol, diclofenac, germacrone, and triptolide were the NSAIDs with the highest scores. Mirtazapine, desipramine, desmethylselegiline, sertraline, and clomipramine were the antidepressants with the highest scores. In CETP-2, the average score for antimicrobials and pesticides were 0.53 and 0.5, respectively. The pesticides with the highest scores included dinitramine, clomeprop, furmecyclox, flurochloridone, and chlorphoxim while the antimicrobials with the highest scores included azithromycin, nevirapine, raltegravir, ciprofloxacin, and benzothiazole.

4.21.P-We Understanding Environmental Reactivity: Kinetics, Mechanisms, and Transformation Products

4.21.P-We-178 Effects of Temperature, Water Depth, and Ferrous/Ferric Ions on the Indirect Photolysis of Dimethylsilanediol (DMSD) in Water

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Dimethylsilanediol (DMSD) is a major degradation product of commercially available methylsiloxanes. Because of its chemical properties, environmental multimedia models predict DMSD is preferentially distributed to the water column. The environmental fate of DMSD in water is not fully understood. Experimental studies have shown that DMSD will degrade in water through indirect photolysis under simulated solar light. This study investigated the effects of varying temperature, water depth, and dissolved iron concentration on the rate of indirect photolysis of DMSD. Aqueous samples containing 1 mg/L radiolabeled ^{14}C-DMSD and environmentally relevant concentrations of nitrate, ferric, and/or ferrous ions were prepared in quartz cuvettes. These samples were placed in a temperature-controlled recirculating water bath and exposed to full spectrum light using a solar simulator. Samples were taken at selected timepoints and analyzed for total radioactivity, DMSD concentration, the presence of degradation products, and silicic acid concentration to determine kinetics of DMSD degradation and total mineralization. Irradiance of simulated solar light and water temperature were monitored during the light-exposure period. Degradation was observed in samples exposed at all temperatures in the presence of nitrate and ferric/ferrous ions. The degradation rate constant exponentially decreased with increasing water depth and decreasing temperature in the presence of nitrate ions at environmentally relevant conditions. The observed half-life in shallow regions (0-15cm) at 5-20°C varied between 40-100 days. The presence of ferrous and ferric ions (0.5-2 mg Fe/L) resulted in faster degradation rates and shorter half-lives at 12°C. These findings show that DMSD can be fully degraded to the natural substances silicic acid and carbon dioxide under environmentally relevant conditions.

4.21.P-We-179 Catalyst or Oxidant? Exploring the Interplay of MnO2 and Oxygen in the Early-Stage Abiotic Humification Under Alkaline Air Conditions

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The abiotic humification pathway, involving metal oxides like MnO2, remains a key focus due to its widespread occurrence in soil and various environments. Despite the recognized importance of Mn oxides in humification, understanding of the involved mechanisms remains limited. This research explores the enhanced oxidation of catechol by MnO2 under alkaline air conditions, uncovering the pivotal role of surface Mn(III/IV)-catalyzed oxidation and oxygen-catalyzed oxidation in the abiotic humification of polyphenols. Combining instrumental analysis and kinetic modeling, this study identifies, for the first time, that both MnO2 (favorable at lower pH) and dissolved oxygen (DO) (favorable at higher pH) are the primary oxidants and catalysts in the early-stage humification. Surface-O2•- is identified as the major reactive oxygen species (ROS) in enhanced oxidation. The results also reveal that MnO2 facilitates polymerization, while DO promotes ring cleavage in polyphenol humification. Overall, this work significantly advances our understanding of the interplay between MnO2 and DO in the abiotic humification of polyphenols, holding critical implications for managing the carbon cycle of polyphenols and offering insights into treatment technologies for bio-wastes, biomass recycling, and carbon sequestration.

4.21.T Understanding Environmental Reactivity: Kinetics, Mechanisms, and Transformation Products

4.21.T-01 The Speciation and Transformation of Complex Phosphorus Species

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Phosphorus (P), an indispensable nutrient, is an increasingly depleted, nonrenewable resource on a global scale. Phosphorus-containing compounds, whether organic or inorganic, are detrimental water contaminants that contribute to eutrophication (even at phosphate concentrations > 0.02 mg/L). Because it is the most readily assimilated form of P among the various forms, inorganic orthophosphate (H_xPO4^{x-3}) is the principal subject of investigation in the field of P management. Nevertheless, a multitude of P species are present in the environment and possess the capability of transforming into orthophosphate, thereby posing potential ecological challenges. Therefore, it is crucial to comprehend the speciation and transformation of P species to facilitate their removal and recovery while pursuing P sustainability. To comprehend the biogeochemistry and human utilization of P resources, one must know about P chemical speciation. Different types of P molecules can be found in wastewater and environmental samples. The diameters, charges, hydrophobicity, and local bonds of these molecules vary. These distinctions significantly affect the utilization of P resources and the appropriate treatment of P refuse. Furthermore, this multitude of attributes intrinsically complicates the process of separating and detecting P molecules within intricate matrices. We characterize P speciation in effluent samples, environmental water samples, and compounds by employing ion chromatography (IC) with inductively coupled plasma mass spectrometry (ICP-MS) detection on the small-molecule fraction (1000 Da). In comparison to conductivity or UV-visible absorption detection, mass-based detection of $^{47}PO^+$ offers enhanced selectivity and sensitivity, with typical detection limits of less than 20 ppb P. We utilized cerium oxide, a relatively inexpensive material, as a catalyst for the transformation of model P compounds in this investigation. Multiple inorganic and organic P species can be converted to orthophosphate in less than three hours, according to our findings. This research demonstrates that the recovery of phosphorus from waste streams that contain complex P species can be made more straightforward with catalyzed hydrolysis and adsorption for simultaneous removal and recovery.

4.21.T-02 Roles of Reactive Oxygen and Nitrogen Species in Transforming Organic Compounds to Nitrogenous Products in Aqueous-Phase Photolysis of Inorganic Nitrogen Species

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Inorganic nitrogen species such as nitrate and nitrite are present in natural aquatic environments and engineered water and wastewater treatment processes. Reactive oxygen and nitrogen species generated by the photolysis of inorganic nitrogen-containing water play important roles in determining the fate of organic compounds. Nitrated and nitrosated transformation products that may be formed from the photolysis are of serious concerns due to their potential toxicity to human health and ecological systems. In this study, we used three representative ultraviolet (UV) wavelengths (far UVC at 222 nm, conventional UVC at 254 nm, and near visible range at 365 nm) to investigate the different roles of reactive oxygen and nitrogen species in the transformation of target organic compounds to nitrogenous products. Excited state computational calculations provided mechanistic insight into the experimental observations of photolysis of inorganic nitrogen species and their photochemical products. Experimental quantifications of various nitrogenous transformation products of target organic compounds indicate the key roles of inorganic nitrogen species during photolysis at different wavelengths. Coupling experimental measurements and a kinetic modeling elucidated the transformation mechanisms for formation of nitrogenous products in the aqueous-phase photolysis of inorganic nitrogen species. This study advances our understandings of photolysis of inorganic nitrogen species and implicates the toxicity of transformation products.

4.21.T-03 Combining Sulfidated Zero Valent Iron and Subsurface Bacterial Communities for Enhanced TCE Remediation

*Kayleigh Millerick and **Nofil Khan**, Civil, Environmental, and Construction Engineering, Texas Tech University*

Zero valent iron (ZVI) can abiotically reduce chlorinated ethenes such as trichloroethene (TCE, C_2HCl_3) to innocuous products such as acetylene, ethene, and ethane but negatively impact indigenous microbial consortia in groundwater environments. Sulfur-modified ("sulfidated") ZVI similarly degrades TCE but is less likely to inhibit microbial activity. We hypothesize that ZVI-mediated and biotic TCE degradation can be pursued concurrently for sustained aquifer attenuation when ZVI is sulfur-modified. In this work, we compare reaction kinetics and TCE transformation product distribution in abiotic ZVI incubations (sulfidated and as-synthesized) to ZVI incubations amended with bacterial cultures. Bench-scale experiments utilize meso- and nanoscale zero valent iron and pure culture *Desulfovibrio desulfuricans*, a sulfate-reducing bacterial culture enriched from aquifer sediments, or patented dechlorinating microbial culture SDC-9 as model cultures. Results show that as-synthesized nanoscale ZVI can inhibit biological sulfate reduction activity in short-term experiments, but the inhibitory effect is suppressed when ZVI is pre-sulfidated, which passivates the reactive surface of the ZVI particles. In longer experiments, ZVI of all sizes either improves or imposes no adverse effects on sulfate-reducing activity, and biological sulfate reduction kinetics are greatest in the presence of sulfidated ZVI. TCE is degraded to the greatest extent in incubations containing both SDC-9 and sulfidated ZVI, compared to incubations without microbial amendments. However, degradation products identified in combined systems are more consistent with biological dechlorination than with abiotic transformation, and a mass balance on degradation products is currently underway. Overall, our study suggest that combined sulfidated ZVI-bacterial strategies may be a viable *in situ* TCE treatment approach that leverages synergy between ZVI and subsurface microorganisms.

4.21.T-04 Disinfection Byproduct Formation, Transformation, and Relative Toxicity in Conventional and Direct Potable Reuse Drinking Waters

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Disinfection byproducts (DBPs) are a challenging contaminant in water treatment due to their human health risks and continued prevalence and formation in the distribution system (DS). Of the 700+ identified DBPs, only 11 are regulated by the USEPA. Unregulated DBPs (urDBP) pose additional toxicological risks, with haloacetaldehydes (HALs), nitrogenous DBPs (N-DBPs), brominated DBPs (Br-DBPs), and iodinated DBPs (I-DBPs) as key toxicity drivers. As climate change advances, increasingly impaired water quality can be expected from a growing fraction of secondary treated wastewater effluents in drinking water sources due to drought (de facto reuse). De-facto reuse increases the availability of N-, Br-, and I-DBP precursors. This study investigated DBP formation, transformation, and relative toxicity in two source waters at the extremes of the de facto reuse spectrum: unimpacted surface water and advanced treated municipal wastewater (direct potable reuse). To simulate conditions in the DS, the influence of pH (7-10) and reaction time (0-5 days) was evaluated. Analysis covered 52 DBPs, including a suite of 43 urDBPs. Relative risk was assessed using the calculative additive toxicity. Initial water quality is a critical consideration for DBP formation and relative toxicity. DBPs were more abundant and more toxic in the direct potable reuse water compared to conventional drinking water. Toxicity was influenced by available precursors. N-DBPs and HALs were primary toxicity drivers in the direct potable reuse and conventional drinking waters, respectively. In both waters, as pH increased, overall DBP concentration increased, but toxicity behaved differently. In conventional drinking water, toxicity remained stable over the range of tested pHs. In the direct potable reuse water, toxicity decreased with increasing pH; this behavior was driven by base-catalyzed hydrolysis reactions that transformed toxic DBPs into less toxic products. Over time, at pH 7, the toxicity of the conventional drinking water decreased. The lowest toxicity occurred at 5 days. This decrease in toxicity over time was driven by N-DBP and HAL transformations. At pH 10, toxicity remained constant over time. This research provides critical insight into DBP formation, transformation, and associated toxicity post-treatment and enables utilities and regulators to understand potentially unaccounted for risk in the DS (urDBPs) and to implement risk reduction strategies based on initial water impairment.

4.21.T-05 Enhanced Adsorption and Hydrolysis of Insensitive Munition Formulation IMX-101 By Pyrogenic Carbonaceous Matter in Range Soils

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Military training with insensitive munition (i.e., IMX-101) deposits a significant amount of post-detonation residues on range soils that may contaminate groundwater. This study investigated the effectiveness of pyrogenic carbonaceous matter (PCM) as a soil amendment to retain and possibly transform IMX-101 constituents, namely 3-nitro-1,2,4-triazol-5-one (NTO), nitroguanidine (NQ), and 2,4-dinitroanisole (DNAN). We conducted batch experiments to evaluate the adsorption and hydrolysis of IMX-101 in three soil types amended with various PCMs at pH 8 and 25°C. Our results indicated that all PCMs increased the removal of NTO, NQ, and DNAN from the aqueous phase compared to the soil control, with an enhancement factor of 50 to 300. Despite being an anionic compound under environmentally relevant conditions, NTO (pK_a of 3.76) exhibited the highest partition coefficients (K_d) in PCM-amended soils compared to its counterparts (e.g., 735.5 L kg^{-1} for NTO vs. 124.9 for DNAN vs. 130.5 L $kg^{-1}$for NQ). Among five PCMs, activated carbon grafted with quaternary ammonium (F400-QA) performed the best, followed by the unmodified activated carbon and biochars. We evaluated the synergistic effects by comparing K_d values of individually spiked NTO, NQ, and DNAN with the same compounds in the IMX-101. For IMX-101, K_d values of NTO, NQ, and DNAN were slightly lower by 4 to 10%, possibly due to adsorption competition from other constituents. The treatment pH was evaluated at 8, 10, and 12 at 25°C. No NTO decay was observed at all pH conditions with or without F400-QA. The change in pH also did not impact K_d values of NTO. By contrast, up to 13% of NQ disappeared, but only at pH 12. Up to 20% DNAN underwent hydrolysis in the presence of PCM at pH 8, whereas 45-90% DNAN decay occurred at pH 10 and 12 over 7 days in all soil types with F400-QA. To investigate the long-term effectiveness of F400-QA, pH 10 was selected. We found the 2% F400-QA dose to be the most effective, maintaining its adsorption capacity and reactivity over three consecutive IMX-101 additions. The presence of soil organic carbon enhanced the degradation of DNAN. Our column tests confirmed that 2% of F400-QA amendment in sandy soil significantly delayed the NTO, NQ, and DNAN breakthrough by 7.2, 5.0, and 8.5 times, respectively. These findings demonstrated the effectiveness of modified PCM in retaining and destroying IMX residues, providing a strategy for in-situ remediation to sustain the military operation.

4.21.T-06 Transformation of PFAS in Landfills: Filling Target Gaps and Using the TOP Assay to Understand Fate

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Per- and polyfluoroalkyl substances (PFAS) are commonly found in municipal landfill leachates, where degradable PFAS likely transform into shorter chain, more persistent, and mobile compounds. A recent study of PFAS in landfill leachates (Capozzi et al. 2023) used a combination of target PFAS measurement and the TOP assay to understand occurrence and fate of PFAS in landfills and found that the "the TOP assay approximates some redox processes which occur in landfills, including chain-shortening reactions." In this study, we expand on this work to include several PFAS precursors and terminal acids such as trifluoroacetic acid (TFA) that were not a part of the previous study scope. To further understand potential PFAS transformation in landfills and to examine similarities between natural transformation mechanisms and the TOP assay, leachate samples were collected from both mature and new landfill sites. The samples were analyzed for a total of 74 PFAS and PFAS precursors using newly developed analytical protocols. These included 13 perfluoroalkyl carboxylates, 8 perfluoroalkyl sulfonates, 4 fluorotelomer sulfonates, 3 fluorotelomer carboxylates, 8 sulfonamides, 5 sulfonamidoacetic acids, 2 perfluorooctane sulfonamide ethanols, 11 per- and polyfluoroether carboxylates, 3 ether sulfonates, 5 fluorotelomer carboxylic acids, 1 cyclic PFAS, 2 nafion byproducts, 3 perfluoroalkylphosphonic acids, 2 polyfluoroalkyl phosphate mono esters, 3 perfluoroalkylphosphinates, 2 polyfluoroalkyl phosphate di-esters, and 5 ultrashort chain PFAS. To further probe the PFAS transformation potentials, selected samples were also analyzed using total oxidizable precursor (TOP) assay. The TOP assay protocol was modified to incorporate ultrashort PFAS that are formed as part of the chain-shortening oxidation. The study will identify predominant PFAS in both types of leachates and delve into the relative distribution of PFAS precursors to terminal acids and longer chain PFAS to short chain and ultrashort PFAS in the two types of leachate samples with a view to understanding potential transformations. The relevance of the modified TOP assay to approximate natural degradation processes will also be discussed.

4.22.A.T Understanding the Ecological Effects and Rolling Out Solutions for Tire Road Wear Particles and Related Chemicals

4.22.A.T-01 Analysis of Tyre Wear and Other Road-Derived Microplastics Collected from the Urban Roadside via Atmospheric Deposition Sampling

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Tyre and road wear particle (TRWP) emissions contribute significantly to microplastic concentrations in the urban environment. Tyre materials are extremely complex, containing multiple polymer types (natural rubber-NR, styrene butadiene rubber-SBR, and nylon), inorganic fillers (silica, carbon black), crosslinking agents, vulcanization agents, and additives (up to 10%) at various concentrations. Other emissions can include brake wear particles, road surface wear particles, road paint materials, and polymer modified bitumen. Although TRWP account for 30% of the terrestrial microplastic burden, analytical challenges exist for their detection and quantification. To address this gap, environmental samples were collected adjacent to a busy urban road (the Bristol Road (A38) close to the University of Birmingham, UK) via atmospheric deposition across the summer of 2023/2024. Samples were collected for different intensities of traffic flow and across a range of different precipitation events to understand how individual environmental factors affect the transport of TRWP to the immediate roadside environment. TRWP contained in these 70 samples were extracted, and organic debris digested using an established and robust protocol developed in-house. Analytical methods based on optical microscopy and pyrolysis gas chromatography mass spectrometry (py-GCMS) were developed to characterize and quantify the TRWP. This dual mode of detection determines the size of microplastics present (optical microscopy) and the concentrations of polymer types present (e.g., NR and SBR by py-GCMS) as an estimate of the mass of particles recovered. To target tyre wear particles (TWP) specifically, a py-GCMS method was developed. Exploration of the appropriate pyrolysis products to measure TWP was conducted, and 4-vinylcyclohexene (4-VCH) and dipentene (DP) were found to be the best suited markers for SBR and NR polymers, respectively. Utilizing this information, stock polymer reference materials were obtained, dissolved in the appropriate solvents, and serial diluted to obtain calibration standards in the nanogram/microgram range. A range of sample preparation techniques were explored for TWP analysis by py-GCMS, including microwave assisted extraction. The workflow developed for roadside sampling, extraction of TRWP from filters, and analysis by optical microscopy and Py-GCMS presented here represents a step-change in analysis capability for these hard-to-measure and quantify particles.

4.22.A.T-02 Quantifying 6PPD-Quinone in Water Samples from the Sacramento-San Joaquin Delta, 2018-2024

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After the discovery of 6PPD-quinone (a transformation product of 6PPD, an antiozonant tire additive) and its lethal effect to Coho salmon (*Oncorhynchus kisutch*) at low levels (LC50 = 41 - 95ng/L), there has been an increase in investigations looking to identify and quantify it in waters

around the world. Additionally, researchers have evaluated the effect of 6PPD-quinone (mortality and a variety of sub-lethal effects) on other species such as Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*Oncorhynchus mykiss*) which are native to the Sacramento-San Joaquin Delta in California. However, to date, no data have been reported on the concentrations of 6PPD-quinone in the Delta. We quantified 6PPD-quinone in archived Delta water sample extracts collected from 2018 to 2024. 6PPD-quinone was detected between 0.2 – 14.0 ng/L (detection limit = 0.2 ng/L) and it was detected only in samples collected between December and March, which coincides with California's rainy season. These results are the first to report historic environmental concentrations of 6PPD-quinone in the Delta and serve as a starting point for a more in-depth evaluation of different source inputs of 6PPD-quinone into the ecosystem.

4.22.A.T-03 Analytical Quantification of 6PPD-Quinone in Fish Tissue by LC-MS/MS

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6PPD-quinone (6PPDq) is an ozonated transformation product of the widely used vehicle tire rubber preservative N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). Due to normal tire wear, both 6PPD and 6PPDq are frequently detected contaminants in stormwater runoff with the latter compound identified as the causative agent of a phenomenon known as Urban Runoff Mortality Syndrome, which describes rapid death of Coho Salmon (Oncorhynchus kisutch) in the Pacific Northwest. Since the identification of 6PPDq, it has been the focus of much research to identify how toxic it is to O. kisutch and other sensitive salmonids, which presents an opportunity to study its bioaccumulation potential in fish. The work presented here describes a novel sample preparation and analytical detection method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for 6PPDq quantitation in fish tissue. The developed method includes fish tissue homogenization, addition of both extracted and non-extracted non-radioactive isotopic internal standards, addition of an extraction solution, sonication, centrifugation, supernatant decantation, cleanup with solid phase extraction, sample evaporation with nitrogen gas, then solvent reconstitution. Analytical validation with LC-MS/MS utilizes an isotope dilution approach, inclusion of multiple reaction monitoring channels and the employment of eight-point matrix-matched calibration standards ranging in concentration from 0 to 2,000 pg mL^{-1}. Calibration curves show coefficients of determination (R^2) >0.998 for 6PPDq. Results of method validation indicate detection limits <8 pg mL^{-1} in fish tissue sample extracts. This developed method is being applied to ongoing studies investigating the bioaccumulation potential of 6PPDq in salmonids including O. kisutch, Rainbow Trout (O. mykiss), and Brook Trout (Salvelinus fontinalis), and in conducting non-targeted analysis to identify transformation products relating to 6PPD and 6PPDq in plasma of Smallmouth Bass (Micropterus dolomieu) collected from sites within the Potomac River, Virginia, the Cheat River, West Virginia, the Mahantango Creek, Pennsylvania, and Lake Memphremagog, Vermont.

4.22.A.T-04 Laboratory Assessment of 6PPD-Q Exposure in Tolerant and Sensitive Salmonid Species

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Several chemicals are used during tire manufacturing process to protect tire rubber from oxidizing and fast wearing, such as 6PPD. These chemicals eventually leach out and can be widely present in urban stormwater runoffs. A 6PPD quinone transformation product (6PPD-Q), found in environmental samples, is one of the key compounds responsible for the pre-spawn mortality of coho salmon (*Oncorhynchus kisutch*). An understanding of 6PPD-Q exposure among salmonids with diverse sensitivity to 6PPD-Q is important to help unravel the physiological basis for toxicity. Our study focuses on assessing 6PPD-Q uptake and distribution in a highly sensitivity species, coho salmon, and a relatively less sensitive species, steelhead (*Oncorhynchus mykiss irideus*). Groups of juvenile coho (n = 5) and steelhead (n = 4) were exposed simultaneously to 6PPD-Q via a static water system at a temperature of 12.5 – 14.7 ºC. Water samples were collected throughout the experiment and fish were euthanized after 53 and 76 hours of exposure, followed by sampling of blood (for plasma analysis), bile, liver, and carcass. We have developed methods of analysis for measuring 6PPD-Q in different fish tissues and water, all showing adequate analytical efficiency, sensitivity and reproducibility. All exposure tanks had initial mean concentration of 6PPD-Q in water ranging between 25.3 – 28.8 ng/L. The water concentration of 6PPD-Q had a mean percentage loss of 63% in tanks with coho salmon and 57% in tanks with steelhead at the end of the experiment. Preliminary studies of 6PPD-Q stability in the exposure tanks without fish indicated no more than 3% was lost over 76 hours. Concentrations of 6PPD-Q in bile (mean 0.3 and 0.4 ng/mL) and plasma (0.16 and 0.19 ng/mL) were similar between the 2 species (coho and steelhead, respectively). The average liver concentration of 6PPD-Q was 50% higher in steelhead (0.014 ng/g) than in coho (0.0097 ng/g). Mean concentration in coho carcass samples was near two-fold higher than steelhead (0.15 and 0.08 ng/g, wet wt.). Mass balance calculations of recovery of 6PPD-Q indicated approximately 60% was disappeared during coho exposures compared to 49% for steelhead, most likely associated with biotransformation. Ongoing in vitro and in vivo experiments are seeking to determine interspecies differences in biotransformation and what extent the metabolism contributes to the elimination of 6PPD-Q in salmon.

4.22.A.T-05 The Impact of 6PPD-Quinone on Aerobic Metabolism and Swimming Performance in Juvenile Lake Trout (*Salvelinus namaycush*)

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6PPD-quinone, an environmental oxidation product of the rubber tire antioxidant 6PPD, has recently gained recognition as a chemical of concern. Frequently detected in road runoff and surface waters, a variety of studies have reported this compound to cause acute lethality in several salmonid species at extremely low concentrations, including lake trout (*Salvelinus* *namaycush;* 24 h LC_{50} = 0.51 µg/L). Following exposure, sensitive species show characteristic symptoms and atypical swimming behavior, such as gasping, spiraling, increased ventilation, loss of equilibrium, erratic movements, and tumbling. However, there is currently a paucity of research targeted at understanding sublethal toxicities of 6PPD-quinone exposure, especially concerning swimming capability and metabolic function. To evaluate potential effects of 6PPD-quinone on swimming performance and aerobic metabolism, juvenile lake trout were exposed for 20 hours to aqueous concentrations of 0 or 0.46 µg/L in a swim tunnel respirometer to assess temporal changes in standard metabolic rate (SMR) compared to unexposed controls. Following exposure, fish underwent a swim trial to determine critical swim speed (U_{crit}), oxygen consumption (MO_2), active metabolic rate (AMR), aerobic scope (AS) and cost of transport (COT), followed by analysis of white muscle triglyceride and glycogen concentrations. Data showed that 6PPD-quinone exposure resulted in decreased swimming performance, evident by a decrease in U_{crit}. Furthermore, exposure resulted in significant decreases in AMR and AS, although alterations in SMR were not observed. In addition, decreases in concentrations of white muscle triglycerides of swam fish were also observed. These results suggest that aqueous 6PPD-quinone exposure at environmentally relevant concentrations causes dysregulation of aerobic metabolic capacity, producing adverse effects that can diminish endurance and maximum swim speeds, which may affect survival of fish populations.

4.22.A.T-06 Lethal and Sublethal Effects of 6PPD-Quinone on Coastal Cutthroat Trout

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Urban stormwater runoff carries hundreds of pollutants including pharmaceuticals and pesticides into the surrounding freshwater systems. One such pollutant prominent in urban watersheds is 6PPD-quinone (6PPD-Q), a transformation product of 6PPD which is an antioxidant globally used in vehicles to significantly extend tire life and increase safety. Recently, 6PPD-Q was implicated in inducing mass pre-spawn mortality events in adult coho salmon in the Pacific Northwest (PNW) United States. Since then, studies have reported highly variable toxicity among salmonids, while generally hypothesizing that early life stages may be more susceptible. Coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) is a widespread salmonid species in PNW watersheds that are impacted by stormwater pollution. However, the impacts of 6PPD-Q on this species has yet to be studied. Here, we investigate the acute toxicity of coastal cutthroat trout from 24-hour 6PPD-Q exposure in early-stage alevin and young of the year juveniles, and both 24-hour and 96-hour toxicity in 1+ year animals. Toxicity is measured as concentrations necessary to induce 50% mortality in 24 hours or 96 hours, and we analytically verify exposure concentrations via ultra performance liquid chromatography-mass spectrometry both at the start and end of each exposure. So far, we have found that 6PPD-Q exposure to early-stage alevins had a 24-hour LC50 concentration of 267.4 ng/L and young of the year juveniles had a 24-hour LC50 of 93.5 ng/L. Analytical confirmation of exposure concentrations for determination of the 1+ year juveniles' 24-hour and 96-hour LC50s are in progress. Alterations in overall morphology or heart rate were not observed in surviving alevins, and all juveniles showed urban mortality runoff symptoms (URMS) prior to mortality. These results demonstrate the life-stage dependence of 6PPD-Q toxicity and suggest that coastal cutthroat trout is a sensitive species to 6PPD-Q exposure at environmentally relevant concentrations. In addition to acute mortality, we are currently investigating sublethal effects on immune system function including potential effects on disease susceptibility to opportunistic pathogens, as well as swimming fitness after 24-hour chemical exposure in coastal cutthroat trout. Our results will provide data to inform the environmental risk assessment of 6PPD-Q in the context of an economically and ecologically important fish species.

4.22.B.T Understanding the Ecological Effects and Rolling Out Solutions for Tire Road Wear Particles and Related Chemicals

4.22.B.T-01 Sub-Chronic and Acute Toxicity of 6PPD-Quinone in Early Life Stages of Two Salmonid Species

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The rubber-tire derivative N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-q) has been identified as a possible driver of Urban Runoff Mortality Syndrome, a phenomenon in which mass die-offs of pre-spawn salmon occur in association with urban settings. Tire-wear particles left on the road's surface are swept into roadway runoff during precipitation, resulting in pulses of 6PPD-q in aquatic systems. Sensitivity to this toxicant is highly variable across fish species, and with environmental concentrations meeting or exceeding sensitivity thresholds, the potential for toxicity in sensitive aquatic species is significant. In addition, there is little known regarding chronic effects, nor effects on early life stages of fishes, which are typically more sensitive to toxicants than adults. Rainbow trout (*Oncorhynchus mykiss*; RBT) represent a model species which is known to be sensitive to 6PPD-q, and lake trout (*Salvelinus namaycush*; LT) are a native species of concern for which the sensitivity is unknown. Sub-chronic exposures to 6PPD-q were conducted in both species starting at hatch, with RBT and LT exhibiting a 28-day LC_{50} of 0.56 µg/L and 45-day LC_{50} of 0.39 µg/L, respectively. These experiments also indicate sub-lethal effects which occur at this sensitive life stage, including yolk sac edema, spinal curvature, and pooling of blood in the eye and caudal fin. Follow-up juvenile studies were conducted with older fry, finding 96-hr LC_{50}s of 0.47 µg/L (RBT) and 0.50 µg/L (LT). These studies demonstrate the potential risk of 6PPD-q to early-life stage inland salmonid species, which may have significant effects on already vulnerable populations.

4.22.B.T-02 Evaluating the Relative Acute Toxicity of PPD Parent Chemicals and Their Transformation Products on Coho Salmon (*Oncorhynchus kisutch*)

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6PPD, a critical component of automobile tires, reacts with ozone in the environment to prevent rubber from cracking. The identification of 6PPD-quinone, a transformation product of 6PPD, as the causal toxicant of pre-spawn mortality syndrome in coho salmon (*Oncorhynchus kisutch)* has led to increased efforts to find an alternative for 6PPD use in tires. Other chemicals in the PPD family are at the top of the priority list in the search for alternatives because they are likely to provide a similar antiozonant property. However, little is known about the toxicity of the PPD compounds and their transformation products. In this project we investigate the toxicity of six chemicals in the PPD family; 6PPD, 7PPD, 77PD, 44PD, IPPD, and CCPD. Coho salmon were exposed to these chemicals for 24 hours to estimate median lethal concentrations (LC50s). The nominal estimated 24-hour LC50 is 509.5 ± 98.3 μg/L for 6PPD, 706.5 ± 123 μg/L for 7PPD, and 159.6 ± 27.7 μg/L for 77PD. Definitive toxicity tests for 44PD, IPPD, and CCPD are currently being performed. Following the initial toxicity tests, the PPD chemicals with equal or lower toxicity than 6PPD will be ozonated. Ozonation will be performed using a novel gas phase method that allows for large masses (>100mg) of PPD to be ozonated at once. After ozonation, the concentrations of the parent compounds and transformation products will be measured using LC-MS/MS. Fish exposures will be performed using the eluent from the plates that contains a mixture of unreacted parent compound, quinones, and other transformation products. The acute toxicity tests will allow us to assess the toxicity of the mixture of transformation products and help to guide further investigations in the search for alternatives to 6PPD.

4.22.B.T-03 Mechanistic Evaluation of 6PPD-Quinone Toxicity in Fish

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High rates of mortality have been reported in coho salmon (*Oncorhynchus kisutch*) entering urban rivers in Seattle, Washington. The behavioral symptoms preceding mortality include erratic swimming, gasping, and loss of equilibrium. Through laboratory testing, it has been confirmed that 6PPD-quinone (6PPD-Q), the transformation product of the tire anti-degradant, 6PPD, is capable of causing the same effect at low doses. The precise adverse outcome pathway (AOP) leading to 6PPD-Q-induced mortality is still not fully understood, however, cardiorespiratory function appears to be affected. Increased vascular permeability, hematological effects, and mitochondrial impairment coincide with mortality. The potency of 6PPD-Q differs substantially between fish species (even those that are phylogenetically similar), with some salmonids demonstrating relatively high tolerance to the chemical in comparison to coho salmon. Dissimilar responses between subpopulations of the same species (e.g., westslope cutthroat trout and coastal cutthroat trout) have also been reported. Thus, specific inter- and intraspecies genetic variations may offer clues about the molecular underpinnings of 6PPD-Q-induced mortality. Tolerant fish species tend to exhibit higher levels of hydroxylated metabolites when exposed to 6PPD-Q, but no specific biotransformation enzyme has been implicated. Determination of the molecular initiating event(s) and/or early key event(s) in the AOP leading to 6PPD-Q-induced mortality could significantly accelerate the search for a suitable alternative chemical and could help researchers screen for additional potentially sensitive species to inform better targeted mitigation strategies. Here, we assemble the current body-of-evidence on 6PPD-Q toxicity in fish, including both sub-lethal and apical endpoints, using a matrix-style approach with relevant parameters such as effect concentration, species, life stage, test duration, and study quality. We present the priority evidence holistically and identify potential AOPs that warrant further investigation.

4.22.B.T-04 Comparing the Effects of 6PPD and a Mixture of Atmospheric Transformation Products on Immortalized Chicken and Double-Crested Cormorant Hepatic Cell Lines

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N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) is an antioxidant added to tires to prevent degradation. 6PPD reacts with atmospheric oxidants and creates transformation products (TPs) either on tire surfaces or on particles emitted into the atmosphere. One of its transformation products, 6PPD-quinone (6PPD-Q), is toxic to certain aquatic organisms, especially coho salmon in the Pacific northwest. However, little is known regarding the toxicological properties of 6PPD and/or its complex TPs in birds. In this study, cell viability, reactive oxygen species (ROS), mitochondrial membrane potential, and changes in mRNA expression were determined in immortalized chicken and double-crested cormorant hepatic cell lines, LMH and DCH22, cultured as 3D spheroids following exposure to 6PPD, 6PPD-Q, and four mixtures of TPs formed under various typical atmospheric conditions. The TPs were generated in the presence of ultraviolet radiation (UV), ozone (O3), hydroxy (OH) radical, and nitrogen oxides (NOx; in the presence of OH) using a custom oxidation flow reactor. Neat TP extracts, as well as 6PPD and 6PPD-Q, were dissolved in DMSO, and serial dilutions were prepared in DMSO. Cell viability and ROS were evaluated using intracellular ATP concentration and oxidation of 2,7-dichlorofluoroscin diacetate (DCFDA) fluorogenic probe by ROS, respectively. Mitochondrial membrane potential was determined using a commercially available kit. Customized species-specific PCR arrays, comprising 48 genes, were used to determine changes in mRNA expression. The four TPs and 6PPD-Q were more cytotoxic than 6PPD in both LMH and DCH22 spheroids. Exposure to 6PPD, 6PPD-Q and the four TPs increased ROS and resulted in the depolarization of mitochondrial membrane potential in both cell lines in a dose-dependent manner. For ROS and mitochondrial membrane potential, 6PPD-UV and 6PPD-O3 elicited the greatest effect. Key genes dysregulated by 6PPD and the five TPs included ALAS1 and BATF3 in LMH spheroids, which are involved in mitochondrial signaling and inflammation, lending further support to the cell-based assays. Gene expression data for DCH22 spheroids are pending. Based on the prevalence of these compounds, this study generated much needed toxicity data for avian species.

4.22.B.T-05 Differences for Effects Between Marine and Freshwater Amphipods Exposed to Tire Wear Particles

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Amphipods are a typical benthic invertebrate in aquatic environment. The species inhabit fresh and marine water areas close to the thousands and are an important prey for a lot of organisms as fish. Generally, because they are very sensitive to chemicals, the serious polluted sites possibly threaten their biodiversity. Therefore, we have to carefully monitor the pollutions in sediments. Recently, some research groups showed that much amount of tire ware particles (TWPs) has been carried into the aquatic environment. Tires are composed with many kinds of chemicals and possibly affect to the aquatic organisms especially inhabit the benthic environment. The present study investigated the effects of Cryo-Milled Tire Tread (CMTT), obtained from U.S. Tire Manufacturers Association, on freshwater amphipod, *Hyalella azteca*, and marine ones, *Ptilohyale barbicornis*. Furthermore, we examined the differences in impact between two species amphipods, and to elucidate the effects of TWPs on the organisms at the base of the food chain in both freshwater and marine environments. The ultimate goal is to provide the essential effects data to enable continuous evaluations of TWP risks from freshwater and marine environments. The effects to *P. barbicornis* are caused by the particles and elution into water column; more than 80% of amphipods died within 7 days when exposed to CMTT with a diameter of approximately 100 micrometer, added to rearing water at 0.5 mg/mL or higher; and when exposed to the saturated elution, more than 80% mortality was observed within 48 hours at concentrations equivalent to 2.5 mg/mL or higher of the particles. More than 80% mortality of *H. azteca* exposed to CMTT particles was observed within 7 days at 0.1 mg/mL or higher. The particles with 0.1 mg/mL killed 80% *P. barbicornis* by the exposure for 12 days. As a result, the effects for the particles were roughly similar between freshwater and marine amphipods. On the other hand, more than 80% mortality was achieved in *H. azteca* at the 8 days exposure to the eluates the equivalent of 2.5mg/mL of the particles. Because of persistence of TWPs for the long period in the sediments, and considering their chronic effects to the benthic organisms, the effects between freshwater and marine amphipods are roughly similar. Additionally, it was deemed that acquiring the toxicity of TWPs for either species would be adequate for extrapolating the risks from freshwater to marine environments for the entire amphipods.

4.22.B.T-06 6PPD-Quinone and 6PPD: Development of Aquatic Life Acute Screening Values by U.S. Environmental Protection Agency's Office of Water

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The U.S. Environmental Protection Agency Office of Water has published aquatic life screening values for acute exposures to two widely distributed rubber-tire compounds, 6PPD-quinone (6PPD-q) and 6PPD. Detections of 6PPD-q and 6PPD in waterways across the U.S. and elsewhere indicate short-term exposure to these compounds may present a risk to aquatic organisms. 6PPD-q, the transformation product of 6PPD, has been found to be highly toxic to adult salmonid species, especially coho salmon. As emerging contaminants for which understanding of toxicity is rapidly evolving, the currently available data did not fulfill EPA's requirements for deriving national recommended 304(a) aquatic life ambient water quality criteria for 6PPD-q and 6PPD in freshwaters or estuarine and marine waters. The EPA will continue to monitor literature and toxicity data to evaluate the protectiveness of this screening value. These screening values serve as estimates of the concentrations of 6PPD-q and 6PPD based on the latest scientific knowledge that support protection of freshwater aquatic communities from acute effects including the endangered species, coho salmon. The EPA developed these screening value in accordance with Section 304(a)(2) of the Clean Water Act (CWA) to provide states, authorized Tribes, and other stakeholders with the best available information on the toxicity of 6PPD-q and 6PPD to aquatic organisms.

4.22.P-Mo Understanding the Ecological Effects and Rolling Out Solutions for Tire Road Wear Particles and Related Chemicals

4.22.P-Mo-113 Chronic Exposure to 6PPD-Quinone (6PPD-Q) Has Concentration-Dependent Effects on Developing Coho Salmon (*Oncorhynchus kisutch*) Embryos

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Stormwater runoff is known to carry hundreds of pollutants into surrounding waters such as streams and lakes. One such pollutant prominent in urban watersheds is N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD)-quinone (6PPD-Q), a transformation product of 6PPD, a globally ubiquitous car rubber tire antioxidant additive. 6PPD-Q was recently implicated in inducing mass pre-spawn mortality events in adult coho salmon (*Oncorhynchus kisutch*) in the Pacific Northwest, USA. Since then, studies have reported life stage susceptibility differences with acute exposures. However, the toxicological effects of chronic exposure to 6PPD-Q during early development in salmonids are still unknown. Here, we conducted a 13-day exposure of three concentrations of 6PPD-Q, 100, 500, and 1000 ng/L (n = 4 replicated treatments, with 40 embryos each), using a semi-static system. Daily observations of mortality, hatch, and blood pooling were made, and any hatched living fish were monitored in fresh water. While no mortality was observed with 100 ng/L 6PPD-Q, 500 and 1000 ng/L induced significant mortality, with majority of mortalities occurring by day eight. Timing of hatching was significantly perturbed by all three 6PPD-Q concentrations, and exposed individuals tended to hatch before controls. Interestingly, with 1000 ng/L, ~50% of the mortalities occurred during hatching ("partial hatch mortalities"). Significant blood pooling was visible in embryos exposed to 500 and 1000 ng/L 6PPD-Q starting at day three, but this phenotype was minimally observed with 100 ng/L. In addition, at both the high concentrations, hatched fish that survived showed significant deficiencies in their "touch response" several days post chemical exposure, and mortalities continued to occur for up to one week after removing fish from chemical exposure. Our data so far suggests that the chorion offers some level of protection from 6PPD-Q to coho salmon embryos, and significant effects on hatching occur even at 100 ng/L, an environmentally relevant concentration. Current and future investigations will examine differences in gene expression during chemical exposure, delayed behavior abnormalities in buttoned up exposed fish, and morphometrics markers related to 6PPD-Q exposure. Our study is relevant to environmental scenarios where coho salmon embryos develop and hatch in stream habitats impacted by urban stormwater runoff.

4.22.P-Mo-114 Ecotoxicity Evaluation of Tire Particles Using Common Carp

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This study investigated the chronic toxicity of tire dust on fish to contribute to the ecological risk assessment of tire dust. Cryo-Milled Tire Tread (CMTT) samples were obtained from the United States Tire Manufacturers Association (USTMA). Deteriorated CMTT was prepared by irradiating with ultraviolet light for 8 weeks using a xenon lamp accelerated weathering tester. In this study, three independent exposure tests were conducted. In Test 1, three groups of carp were fed diets containing 0, 2.5, and 10% CMTT for 4 weeks, respectively. In Tests 2 and 3, four groups of carp were fed diets containing 0, 0.625, 1.25, and 2.5% CMTT for 2 weeks, and in Test 3, 0, 1.25, 2.5, and 5% deteriorated CMTT, respectively. In all tests, the standard body length and weight of carp were measured and blood samples were collected at the end of the exposure period. The blood was used to count the total number of blood cells and measure hemoglobin (Hb) concentration and hematocrit (Ht) values. In addition, in Test 1, spleen tissue sections were stained with Berlin blue and observed. There were no deaths in any of the tests during the test period. In Test 1, there were no significant differences in standard body length, weight, body mass index, or growth rate between treatment groups. This result suggests that chronic exposure to CMTT does not affect the growth of carp. On the other hand, Hb concentration and Ht values were significantly lower in both concentration groups compared to the control group. In the spleen, many hemosiderin deposits were observed, confirming that red blood cells were damaged. In Test 2, Hb concentration was significantly lower only in the 2.5%

group compared to the control group. The results of Tests 1 and 2 revealed that CMTT administration causes anemia in carp, and its lowest observed effect level (LOEL) and no observed effect level (NOEL) were 0.75 and 0.375 mg/g-b.w./day, respectively. In the exposure test with CMTT deteriorated by UV irradiation in Test 3, no significant changes in Hb concentration were observed in any treatment group. From the above, it was inferred that chemicals derived from tire additives are the main cause of anemia.

4.22.P-Mo-115 Rolling Along: State of the Science for Tire Related Chemicals 6PPD and 6PPD-Quinone

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In 2020, scientists at the University of Washington-Tacoma and Washington State University identified a chemical that causes pre-spawn mortality in coho salmon: 6PPD-quinone. 6PPD-quinone is a transformation product of 6PPD, an antiozonant used in tires. Currently, 6PPD is used in all tires, is found in recycled tire products, and can contaminate stormwater anywhere tires are used. A team of the nation's leading experts, formed under the Interstate Technology & Regulatory Council (ITRC) as the Tire Anti-Degradants (6PPD) Team, was assembled in early 2023 to advance the science and provide education and guidance on the emerging contaminant of concern, 6PPD-quinone, and its parent compound, 6PPD. Based on the acute toxicity to coho salmon and the rapidly emerging science, the team aims to provide timely guidance to federal, state, and tribal governments that manage or regulate water quality, toxics, solid waste, fish and wildlife, and transportation. The team's second product, a guidance document with anticipated issuance 9/2024, presents the latest science on toxicity, fate and transport, and assessment and mitigation strategies for 6PPD and 6PPD-quinone. The ITRC 6PPD Team will present a poster that will utilize the ITRC's 6PPD and 6PPD-quinone guidance document to 1) promote awareness of the rapidly emerging contaminants 6PPD and 6PPD-quinone, and 2) provide the latest innovations regarding stormwater best management practices, alternative chemical assessments to eliminate or reduce 6PPD and 6PPD-quinone, research to determine toxicological mechanisms within organisms, cutting edge guidance on monitoring and analytical methods for measuring these chemicals in water and aquatic organisms, and current research efforts and projected next steps to finding a solution to 6PPD and 6PPD-quinone.

4.22.P-Mo-116 Microbial Degradation of Tire Waste

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Tires pose a significant threat to environmental health, contributing to pollution in air, water, and land. In the United States alone, approximately 317 million tires are discarded annually, with 75% ending up in landfills and the remainder improperly disposed of in bodies of water or on land. The release of toxic chemicals from tires further exacerbates pollution in the environment, posing a substantial risk to water and soil quality and consequently human health. This project focuses on the development of various enrichment cultures to assess the degradation of tire particles using microbial activity. Microbial tire degradation offers a promising alternative that is both cost-effective and environmentally sustainable. Microorganisms sourced from discarded

tires in El Paso, Texas, and Fairbanks, Alaska, were cultivated and incubated with tire wear particles (natural rubber) and isoprene, the monomer of natural rubber present in tires, for the development of mesophilic and psychrophilic enrichment cultures, respectively. The degradation process was monitored by assessing microbial growth every couple of days using optical density at 600 nm (OD600), while isoprene content in the enrichment cultures will soon be analyzed via gas chromatography. The mesophilic and psychrophilic cultures are stored on a shaker at 160 rpm, maintained at both room temperature and 4°C. Analysis of OD600 data reveals successful microbial growth utilizing tire particles as the sole carbon source. Future work includes the characterization of microorganisms using 16S rRNA sequencing, assessment of the degradation kinetics, and characterization of tire wear particle degradation products via gas chromatography-tandem mass spectrometry. This research aims to provide insights into microbial efficacy in degrading tires and the release of potentially toxic chemicals into the environment, contributing to the design of bioreactors and microbial consortia able to convert tire additives and rubber into safe products.

4.22.P-Mo-117 Optimization of Techniques and Evaluation of Kinetic Parameters for 6PPD-Quinone and Other Tire Wear Chemicals in POCIS

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Passive sampling has become a widely accepted technique for obtaining information on the average concentrations of chemicals in the water column over prolonged durations. This data can be used as a proxy for evaluating the potential exposure of dissolved chemicals to aquatic organisms. The Polar Organic Chemical Integrative Sampler (POCIS) was designed to sample moderately polar to polar organic chemicals and has been used for a variety of chemical types including pesticides, waste indicator chemicals, pharmaceuticals, and PFAS. Recent concerns about the presence of chemicals leaching from tire and road wear particles and the potential risk to aquatic life has raised the interest in developing sampling techniques. One of the chemicals of concern, 6PPD-quinone (6PPDq), a transformation product of a widely used tire rubber preservative (6PPD), has been shown to be highly toxic to Coho Salmon and may pose a risk to other fish and aquatic species. POCIS has been used on a limited basis to sample 6PPDq in the Pacific Northwest and in the Great Lakes; however, conditions for the sampling and processing of 6PPDq and other tire wear chemicals have not been fully evaluated. We will present optimized methods for the extraction and analysis of 6PPDq and other tire wear chemicals from POCIS along with results from laboratory calibration studies to evaluate various kinetic parameters (such as sampling rates) necessary for estimating time-weighted average concentrations of chemicals in the environment.

4.22.P-Mo-118 How Abiotic Factors Influence 6PPD-Quinone Toxicity in Juvenile Brown Trout (*Salmo salar*)

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Within the field of environmental toxicology, we often reduce complexity by focusing on one factor at the time, at optimal condition and in relation to toxicity. However, it is well known that abiotic and environmental factors can influence the toxicological outcomes of exposure to xenobiotics in aquatic organisms. One emerging xenobiotic of concern is 6PPD-quinone, a chemical associated with tire-wear and road runoff, which is known to be non-lethal to certain fish species (e.g., brown trout; *Salmo salar*) and lethal to others (e.g., Coho salmon; *Oncorhynchus kisutch*) at environmentally relevant concentrations. Another group of chemicals strongly associated with road-runoff and known to induce toxicity in salmonids are polycyclic aromatic hydrocarbons (PAHs). Yet, it is unknown how abiotic factors, such as temperature and dissolved oxygen, or how PAHs influence the toxicity of 6PPD-q in non-sensitive aquatic species. The goal of this research is to investigate (1) how increased water temperature, alongside decreased levels of dissolved oxygen, influences the toxicity of 6PPD-q, and (2) how co-exposure of 6PPD-quinone and the PAH, phenanthrene, influence and contributes to the formation of toxicity in juvenile brown trout (*Salmo salar*). Hypoxia tolerance, upper thermal limit, and responses of the transcriptome of gill and liver tissues were investigated in relation to exposure and abiotic factors. By combining multiple toxicological endpoints in a 6PPD-q tolerant species, we aim to contribute to a greater understanding on how road run-off can affect wild salmonids in an ever-changing world.

4.22.P-Mo-119 Quality and Reliability Evaluation of 6PPD-Quinone Surface Water Occurrence Data and Considerations for Use in Risk Assessment

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6PPD-quinone, a transformation product of 6PPD, is an emerging chemical of interest because of its adverse health effects on aquatic species such as coho salmon. Given its recent discovery, there is a need to characterize the distribution and occurrence of 6PPD-quinone in the environment. One primary area of study is 6PPD-quinone occurrence in the aquatic environment, including surface water. Research is actively ongoing and therefore, there is a need to understand the quality and reliability of surface water occurrence datasets, especially when used in risk-based decision making. We reviewed available surface water occurrence data from the peer-reviewed literature and monitoring databases, assessing them for quality and reliability. In this exercise, we evaluated surface water datasets using two frameworks: (1) the USEPA Draft Systematic Review Protocol Supporting TSCA chemical risk evaluations and (2) the SETAC Criteria for Reporting and Evaluating Exposure Datasets (CREED). In general, both frameworks rated the individual studies as reliable and of sufficient quality for use in risk assessment. The two frameworks generally aligned, although the CREED framework was more comprehensive and applicable to environmental monitoring datasets. Criteria from both frameworks that had the greatest impact on overall reliability and quality of the datasets were related to sampling and analytical methods, reporting of results, and the handling of censored data. While these frameworks provide a systematic approach with specific criteria to evaluate occurrence data, reviewers' conclusions may vary; therefore, multiple reviewers are needed to increase confidence in reliability conclusions. Further, there are limitations when evaluating the combined surface water dataset holistically, such that existing data gaps prevent characterization of 6PPD-quinone occurrence across broad geographic regions and under varying environmental conditions (e.g., dry vs. wet conditions). Therefore, region-specific risk assessments that consider regional differences in aquatic species present, as well as effects of urbanization, climate, and stormwater infrastructure and best management practices, are warranted.

4.22.P-Mo-120 Investigating the Prevalence of 6PPD-Quinone and Tire Wear Particles in Southcentral Alaska

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Tire wear particles (TWPs) and their leachates are emerging as a significant source of environmental pollutants. In particular, 6PPD-quinone (6PPDQ), a derivative of a tire rubber additive, has been shown to be acutely toxic to certain salmonid species. This study aimed to gain further knowledge on the environmental fate and distribution of these pollutants in Southcentral Alaska, where salmon populations are heavily relied on for both commercial and subsistence purposes. Snow dump sites were examined as a potential point source for both TWPs and 6PPDQ by collecting environmental samples of three different sample matrices. Snow samples were taken during the snowmelt season, water samples were acquired from runs produced from the snow thaw, and soil samples were collected from the soil surface. Additionally, to further establish the spatial extent and temporality of TWPs and 6PPDQ in the area, stormwater drains and salmon-bearing streams were examined over the spring and summer of 2024. Samples were analyzed following established analytical techniques. TWPs were identified and quantified using pyrolysis gas chromatography-mass spectroscopy (Py-GC/MS), and 6PPDQ was extracted following a solid phase extraction procedure and quantified using liquid chromatography-tandem triple quadrupole mass spectroscopy (LC-MS/MS). The information gained from this study may ultimately be used for the assessment of targeted mitigation strategies while also providing further knowledge for decisions made in regard to the conservation of Alaskan salmon populations.

4.22.P-Mo-121 Transcriptomic Disruption of Northern Leopard Frog Tadpoles (*Lithobates pipiens*) by 6PPD-Quinone

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The rubber-tire derivative N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-q) has recently emerged as the causative agent of Urban Runoff Mortality Syndrome. This syndrome refers to the widespread deaths of pre-spawn salmon occurring near roadways. Tire-wear particles deposited on road surfaces are washed into runoff during rainfall, introducing pulses of 6PPD-q into water bodies. Thus far, widely varying degrees of sensitivity have been observed across fish species. With environmental concentrations surpassing some sensitivity thresholds, there is a notable risk of toxicity for susceptible aquatic species. Thus far, no research has been conducted to evaluate the potential toxicity of this compound to amphibians. Larval-stage amphibians often inhabit small, temporary bodies of water near roadways, and other roadway contaminants such as copper and road salts, are a known detriment to amphibian populations. An acute (96 hour) exposure was conducted using newly hatched *Lithobates pipiens* (Northern leopard frog; NLF) tadpoles. Treatment included nominal concentrations of 6PPD-q of 0.2, 2 and 20 µg/L, as well as a 0.01% dimethyl sulfoxide control in triplicate. Upon termination of the exposure, whole-body tadpoles were flash-frozen, and total RNA extracted from three pooled individuals per replicate. qPCR was performed using the EcoToxChip NLF v0.1 and analyzed using EcoToxXplorer. No mortality was observed during the exposure, but transcriptomic disruption was evident. At a log2fold-change of 0.5, and a p-value of <0.05, nine genes were significantly dysregulated (8 up-regulated and 1 down-regulated). Down-regulated genes included thyroid-related transcripts (type III iodothyronine deiodinase and thyroid hormone receptor beta) as well as apoptotic genes (caspase-9 and regulator bcl-2) and the lipogenic enzyme cyl-CoA desaturase. These gene expression changes suggest potential for 6PPD-q to activate biological pathways in NLF larvae after an acute exposure, and further study into the exposure routes and potential sub-lethal effects of 6PPD-q in amphibians is needed.

Track 5: Environmental Risk Assessment

5.01.P-Mo-124 Toxicity of Individual and Combined Effect of Crop Protection Safener, Mefenpyr di-Ethyl and Its Co-Herbicide, Fenoxaprop-P-Ethyl, to *D. rerio*

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The quest to increase food production to sustain the ever-increasing human population has led to the use of herbicides to combat unwanted pests. However, these herbicides have been found to adversely affect the yield of crops, Safeners were then formulated with specific herbicides to protect these crops from the potential adverse effects of herbicides without interfering with its ability to combat pests. Mefenpyr diethyl (MEF), a safener that protects cereal crop plants and other grains and a supposed inert chemical used in herbicide formulations with active ingredients such as Fenoxaprop-P-ethyl (FEN), has been found in surface water of river harboring several animals. Since the potential risks of this safener, individually and in combination with its herbicide, toward non-target aquatic organisms, were not known, therefore, acute, and chronic studies were conducted on various endpoints in embryos of *D. rerio*. Endpoints during acute exposures included mortality and hatchability. During chronic exposures, the growth and survival of larvae were determined. Exposure to concentrations of MEF > 3 mg/L alone significantly decreased the rate of hatching, while exposure to FEN alone to > 3 mg/L had no significant effect on the rate of hatching. However, exposure to each of the chemicals individually caused some delay in hatching. When exposed in combination with FEN, adverse effects of MEF on the rate of hatching were mitigated in a dose-dependent manner. During the acute exposure to 3 mg/L, mortality was caused by MEF than FEN. During chronic exposures to 0.1 or 1.0 mg/L, MEF was more toxic than FEN. Both compounds caused some abnormalities, including pericardial edema, spinal curvature, tail malformation, and edema of the yoke sac. Based on the activities of SOD and GST, both MEF and FEN caused oxidative stress. FEN reduced the toxic potency of MEF when exposed together, but there were more deformities of greater severity in embryos exposed to MEF than those exposed to FEN. Molecular docking showed that both chemicals could potentially inhibit the activity of hatching enzymes. These results demonstrate that while classified as inert, the safener, and FEN can cause various effects on aquatic organisms, including molecular responses and lethality and should be monitored and regulated to ensure a safe environment for all.

5.01.P-Mo-126 Assessment of Heavy Metals in Vegetables and Fruits and Their Effect on Health

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The food safety issues due to heavy metals contamination has attracted attention worldwide. The present study was carried out to evaluate the essential metals and toxic metals As, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mn, K, Mg, Na, Ni, Se, Pb and Zn levels in different vegetables and fruits and to assess the possible human risks to consumers. The locally grown vegetables and fruits samples were collected from the local farmer market. The overall all studied metal accumulated levels in different vegetables and fruits showed followed decreasing trend: spinach > tomato > sugar beet > white eggplant > kale > cucumber > green chili > green bean > dill soya > potato > capsicum > onion > corn. Overall, K and Mg were noted in higher while Pb and Hg were found in lower in studied vegetables. On average basis in all vegetables, metals showed the following decreasing trend: K > Mg > Ca > Na > Fe > Zn > Mn > Cu > Ni > Cd > Se > As > Co > Cr > Pb > Hg. In micro elements (Ni, Mn, Cu, Se and Co), Mn and Cu were the dominants while in toxic elements (As, Cd, Cr, Hg and Pd), Cd and As were the higher. Highest Cd concentrations (0.99 µg/g) was noted in spinach while highest As concentration (0.44 µg/g) was found in cucumber followed by white eggplant. The highest level of K, Mg, Fe, Mn, Zn, Cr, Pb, and Hg was noted in spinach. The highest level of Cu, Na, Se, Co and Ni were found in tomato, sugar beet, dill soya, green chili, and green bean, respectively. Most of the metals were found to be lower than maximum allowable concentrations set by international agencies among the analyzed fruits and vegetables. Health risks associated with the intake of these metals were evaluated in terms of estimated daily intake (EDI), and non-carcinogenic risks by target hazard quotient (HQ) and hazard index (HI). EDI values of all the metals were found to be below the maximum tolerable daily intake. The HQs of Cd were noted to be higher > 1 in most of the analyzed vegetables, followed by As, suggesting health hazards for population. HQs value for Zn and Mn were also higher than 1 in spinach. HI of Cd, As and Mn was comparatively very high as compared to other metals in studied vegetables. The findings of this study reveal the health risks associated with Cd and As through the intake of selected fruits and vegetables in consumer. Lastly, the levels of these metals should be regularly monitored in vegetables especially leafy vegetables like spinach for pollution control and human health.

5.01.P-Mo-127 Toxicity Assessment of the Beta-Adrenergic Receptor Agonist/Antagonist, Lubabegron, to Fathead Minnow using Traditional and Molecular Endpoints

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Lubabegron is a commercially available veterinary drug approved for use to reduce ammonia gas emissions from cattle and their subsequent waste in agricultural settings. However, data regarding the chemical's potential impacts on aquatic environments and surrounding biota is sparse. This study evaluated the effects of lubabegron on the ecologically relevant freshwater fathead minnow (*Pimephales promelas*). Three exposures were conducted: a 72-hour flow-through (~45 mL/min) with adult males, a 72-hour static renewal with adult males in 10 L aquaria, and a 24-hour 96-well plate based high throughput exposure with one day post-hatch larval fish. Throughout the adult exposures, survival and water quality were monitored. Given the nature of the chemical, water samples were collected for ammonia analysis daily. There was no significant dose dependent impact on ammonia concentrations within the aquaria for either the static renewal or flow-through exposures, nor was survival impacted. At the end of each adult test, tissues were isolated from each fish and RNA was extracted to be used in EcoToxChips, a 384-well qPCR array that focuses on a core set of genes, and additional targeted qPCR. EcoToxChip analysis identified 16 differentially expressed genes (DEGs) across all tested concentrations in liver samples from the adult flow-through exposure. Five of these (*nr2e1, il1b, zmat3, pkma, mgmt*) were consistently impacted by the top two test concentrations of 0.1 and 0.032 mg/L. The EcoToxChip data were also used to derive a point of departure of 0.01 mg/L, which is higher than predicted environmental concentrations. From the high throughput assay, a 24 h LC50 of 0.65 mg/L was estimated for larval fathead minnows, and RNA was extracted for transcriptomic sequencing. This study demonstrates the use of traditional and high throughput assays for generating toxicity data for emerging environmentally and commercially relevant chemicals. *The contents of this abstract neither constitute, nor necessarily reflect, official US EPA policy.*

5.01.P-Mo-128 Assessing Potential for Exposure of Native Bees to Neonicotinoid Soil Residues in Restored Lands

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A large proportion of land use in the Midwest/Great Lakes region is agricultural and it is estimated that 150 million pounds of pesticides are applied annually in the Midwest alone. While pollinator habitat restoration is needed, it is also important to understand if restored habitats could be facilitating pollinator exposure to pesticides and creating an ecological trap. Much of our understanding of bee exposure to pesticides is based on research with European honeybees (*Apis mellifera*) which are very different ecologically and have different life history strategies compared to native bee species, the majority of which are ground-nesting. If there are pesticide residues in the soil, particularly in fallow cropland that is being converted to pollinator habitat, soil could be a direct route of exposure to pesticides for ground-nesting bees. Neonicotinoid pesticides are persistent in soils and can elicit sublethal and lethal effects in native bees. The primary goal of this project was to identify the presence of neonicotinoids in soils at Ottawa National Wildlife Refuge (NWR) in Ohio at 3 different sites: fallow cropland that is planned to be restored, cropland that has already been restored to grassland/prairie, and a “control” area that has not previously been cropland and has a low probability of neonicotinoid exposure. Grab soil samples were collected from three sites and bee surveys were completed at each of the sites to confirm the presence of ground-nesting bees. Only clothiandin was detected at the fallow cropland site and the presence of ground-nesting bee species (*Agaopostemon* spp., *Lasioglossum* spp., *Andrena* spp.) was confirmed at all soil sampling locations. For honeybees, clothiandin has the potential to be highly toxic through both contact and oral exposure, and concentrations observed at the fallow cropland site (average 9.33 ppb) were below the LD_{50} for contact exposure. Lack of neonicotinoid detections at sites where soils were collected likely indicates enough time has passed since restoration for natural attenuation. Additionally, many of the soils that were sampled are frequently inundated with water, which may facilitate movement of neonicotinoids out of the soil and into surrounding water bodies/sediments.

5.01.P-Mo-130 LC-MS/MS Study of Hydrolysis Kinetics of Mancozeb

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Mancozeb is a dithiocarbamate, a non-systemic agricultural fungicide with multi-site, protective action, used on a large scale. It inhibits enzyme activity in fungi by forming a complex with metal-containing enzymes including those that are involved in the production of ATP. The active component and its main metabolite, ethylene thiourea, has been related to health issues. Robust, fast, and reliable methodologies to quantify its presence in water are of great importance for environmental and health reasons. The determination of mancozeb, is a challenge, due to the nature of the compound, a manganese and zinc complex of ethylene bis dithiocarbamate and to the general instability of the dithiocarbamate. In this Study we have investigated the degradation profile of mancozeb in aqueous solutions to at different pH, and temperature. The aim of this study was to propose kinetic study suitable for reproducing and predicting mancozeb conversion to by-products as a function of the operational conditions. Existing methods of mancozeb analysis involve multiple steps and complex reaction and taking longer time. Due to sparingly nature of mancozeb and low recovery rate a derivatised approach is proposed in combination with LC-MS/MS analysis. The proposed approach is to use chelating agent, 10 mM EDTA disodium salt in the presence iodomethane solution. C18, 100 mm × 4.6 mm, inner diameter 5 µm column and methanol: 10 mM Ammonium Formate mobile phase used to separate the derivatised product. According to the results of LC-MS/MS analysis for dithiocarbamate, method was validated using linearity range of 0.025 to 0.311 mg/L with LOQ 0.10 mg/L. The current study concludes that during stability study mancozeb found is unstable in aqueous solutions, particularly at an acid pH, in addition to presenting both severe light and lower temperature sensitivity. LC-MS/MS was the most reliable and precise technique and was able to determine the main degradation product of Mancozeb, at the same time. The proposed method resulted to be simple, fast, green, economic, and suitable for residue analysis in water samples.

5.01.P-Mo-131 UPLC-ESI-MS Based Approach for the Quantification of Fungicides, Insecticides, and Plant Growth Regulator in *Mangifera indica* Using QuEChERS Extraction with d-SPE Clean-Up

Meet Rajeshbhai Patel[1]*, Nadeem Ahmad Khan*[2]*, Alpesh Hirjibhai Patel*[3] *and Mayank Pandya*[4]*, (1)JRF International, Barbican, London, United Kingdom, (2)Jai Research Foundation, India, (3)Chemistry, Jai Research Foundation, India, (4)Chemistry, RK University, Rajkot, India*

In conventional agricultural systems, chemical fertilizers are commonly employed to maximize output. The constant and excessive use of chemical fertilizers contributes significantly, directly and/or indirectly, to changing environmental circumstances and health hazards at higher than Maximum Residue Levels (MRLs). This work presents a sensitive, cost-effective, accurate, and precise method for the monitoring of multi-pesticide residue in *Mangifera indica* (also known as Alphonso). The method involves applying the QuEChERS technique along with d-SPE clean-up for the sample preparation and liquid chromatography associated with tandem mass spectrometry (UPLC-ESI-MS/MS) for the analysis. The analytical method validation was executed by using the area ratio of the internal standard (IS) approach to determine the Multi pesticide residues of fungicides, insecticides, and plan growth regulator in *Mangifera indica* following the SANTE/2020/12830 guideline. The matrix-matched calibration curve designed for the quantitation of the significant matrix effect (% ME) was evaluated. The linearity of the technique was adequate (coefficient of correlation (r) ≥ 0.99) with a concentration range of 0.115 to 265.362 µg kg $^{-1}$. The ranges for the limit of detection (LOD) and limit of quantitation (LOQ) were 0.115 to 10.614 µg kg $^{-1}$ and 5.759 to 530.724 µg kg $^{-1}$, respectively. The range of mean recoveries for this method was within the range of 87.10 to 113.24%, with relative standard deviations (RSD) of 0.58% to 13.34%. The different pesticide residues were examined using the

UPLC-ESI-MS/MS technology on farm samples of *Mangifera indica*, using the validated method. As compared to the standard multi-component analysis approach, the proposed method can be more dependable and cost-effective.

5.01.P-Mo-132 Contamination Characteristics and Human Health Risk Assessment of Potentially Toxic Elements in Dust from Different Land Use Areas

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Investigations were conducted into the features, hazards, and origins of potentially toxic elements found in dust from various land use areas. A field portable X-Ray fluorescence analyser was used to measure the amounts of potentially hazardous components in 107 dust samples that were collected from seven distinct land use regions. Pb (3.5 – 42.1), As (3.0 – 4.6), Zn (43.2 – 272.9), Cu (12.1 – 54.6), Fe (12792.1 – 28202.3), Mn (101.9 – 321.4), Cr (44.4 – 127.4), V (53.8 – 123.7), Ti (1918.8 – 3746.9), Ni (16.0 – 18.9), and Cd (5.2 – 5.9) were the mean concentration ranges (in mg kg^{-1}) of toxic elements in the dust at different land uses. All of the potentially hazardous element levels in the dust, with the exception of cadmium, were found to be below background, WHO, and Dutch target guideline limits. The geo-accumulation data showed that the dust in Sekyere South was moderately polluted with V, severely polluted with Cd, and unpolluted to moderately polluted with Pb, As, Zn, Cu, Mn, Cr, and Ti. Zn was found to be moderately enriched, V to be highly enriched, and Cd to be greatly enriched, according to the enrichment factor calculation; the remaining metals showed deficient to little enrichment. The site has a moderate degree of contamination, with Cd making up 57% of the contamination, according to the modified degree of contamination data. The places that have been contaminated are as follows: car repair shop > residential > school > lorry park > market > road > playground. In the dust, only Cd presented a very high potential ecological concern. Three separate components of the elements were found using the principal component analysis. The first component (Ti, V, Cr, and Fe) may result from wear on automotive components or from natural sources. Component two, which includes Mn, Cu, Zn, As, and Pb, may have originated from natural or human sources (such as traffic emissions); component three, which includes Cd and Ni, may have come from paints, pesticide application, and trash incineration. Compared to adults, children were exposed to higher risk levels. According to the study, none of the potentially toxic elements that were examined were at dangerously high concentrations or expected to endanger human health. Since the study discovered a sizable load of cadmium pollution in the locations it examined, appropriate pollution control measures need to be put in place

5.01.P-Mo-133 Environmental and Health Risk Assessments on Mercury in Cocoa Beans (*Theobroma cacao*) and Agricultural Soil in Artisanal Small-scale Gold Mining (ASGM) areas, Ashanti Region, Ghana

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This study evaluate mercury pollution from ASGM(Artisanal small-scale gold mining) in Ghana to agricultural soil and cocoa beans. Mercury in agricultural soil and cocoa beans in four ASGM communities in the Ashanti Reion of Ghana were analyzed with mercury analyzer (MA-3000). The concentration ranged in agricultural soil was 7.0-125µg/kg whiles that of cocoa beans was 8.05 to 60.87µg/kg. The results were used to assess pollution in the mining communities by using contamination factor and potential ecological risk index indicating mercury pollution in the mining communities ranging from low to considerable contamination and low to considerable ecological risk respectively. Correlation analysis indicates that the concentration of Hg in cocoa beans and agricultuaral soil is somehow correlated. The bioacculation factor ranged from 0.08 to 0.5. According to hazard quotient (HQ), children were at a greater risk than adults; however the hazard quotient was less than the safe limit (HQ = 1) indicating that the cocoa beans from the study area poses no potential risk through oral route.

5.01.P-Mo-134 Rubric to Assess Ecosystem and Health Risks of Agricultural Enhanced Weathering Projects for Carbon Dioxide Removal

***Phyllis Fuchsman**, Carla Beals, Bharti Ujjani and Alma Feldpausch, Ramboll*

Mitigating climate change will require a combination of emission controls and carbon dioxide removal from the atmosphere. However, safeguards are needed to avoid unintended negative consequences of potential carbon dioxide removal technologies. One such technology enhances the weathering of silicate rocks and minerals such as basalt and olivine by applying them in fine-grain form to agricultural land, where they react with rainwater and convert carbon dioxide to stable bicarbonate. Like lime, these silicate materials can serve as agricultural amendments to increase soil pH, with the added benefit of carbon dioxide removal. We assessed ecosystem, human health, and safety risks that might be associated with agricultural soil amendments for enhanced weathering and developed a rubric for proponents and funders to safeguard against the identified risks. With respect to human health and safety, rock and mineral sources must be screened to avoid geologic sources contaminated with asbestos, and airborne dust suppression measures are necessary to avoid silicosis risk for workers. A primary ecological consideration is the potential for nickel (a common impurity) to build-up in soil to levels that could adversely affect sensitive crops. To protect against this risk, we identify simple calculations to estimate the added soil nickel concentration from the mineral nickel content and application rate. We recommend comparing the result to a protective benchmark from a threshold calculator for metals in soils (https://arche-consulting.be/tools), which can be customized based on soil characteristics. Chromium is another impurity of note but occurs in silicate rocks and minerals in minimally toxic forms and thus is not of concern. Other considerations for enhanced weathering projects include protection of groundwater and surface water from nickel leaching and runoff, soil nutrient balance, potential environmental impacts of silicate material sourcing, project governance, and community engagement. With appropriate safeguards to manage the identified risks, enhanced weathering through agricultural applications is a promising technology for carbon dioxide removal.

5.01.T Agriculture and One Health: Toxicology and Ecological Health Risk Assessment of Metals, Pesticides, and Other Agricultural Inputs

5.01.T-01 Synergistic Toxicity of Pesticides and Heavy Metals in *Apis mellifera L.* and Implications for Human Health

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The global decline of *Apis mellifera L.* populations over the past two decades has raised concerns about the impact on pollination services and ecosystem health. This study examines the role of chemical stressors, particularly pesticides and heavy metals, on bee populations and their potential effects on human health. Direct sampling from urban and agricultural areas revealed no spatial variation in metal concentrations in pollen and honey, nor in pesticide concentrations in pollen. The study further investigates the synergistic effects of the most abundant contaminants—Imidacloprid (IMI), Piperonyl butoxide (PBO), Lead (Pb), and Selenium (Se)—on cytotoxicity and genotoxicity at the cellular level in mammalian cells. Elevated cytotoxic effects of PBO were observed in poly (ADP-ribose) polymerase (PARP) deficient Chinese hamster ovary (CHO) cells, with enhanced effects when combined with IMI, Pb, or Se. These findings underscore the complex interactions between environmental pollutants and biological systems, highlighting the need for a One Health approach to address the interconnectedness of environmental, animal, and human health. Further research is required to elucidate the toxicological mechanisms of these combinations, including at concentrations below regulatory limits, to inform risk assessments and policy decisions.

5.01.T-02 Uptake and In-Vitro Bioaccessibility of Toxic Metals in Cocoa Beans: Human Health Risks

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Cocoa-growing areas in Ghana have experienced a rise in mining activities affecting cocoa cultivation and increased concentrations of potentially toxic metals in the soil, which can accumulate in cocoa beans. This study evaluated potential toxic metal contamination in cocoa beans and soils from cocoa farms in both mining and non-mining areas in Ghana. Metal concentrations were determined by X-ray fluorescence following an inductively coupled plasma–mass spectrometry and Zeeman mercury analyzer for mercury analysis. The farm soils had soil pH (4.08 – 6.86), electrical conductivity (29.16 – 870.50 µS/cm), and soil organic matter (4.78 – 7.38%). Generally, metal concentrations in the soil were within the Canadian Soil Quality Guidelines for Agricultural soils, Dutch Target and Intervention Values and World average world soil for unpolluted soils but varied between study areas. Cocoa beans had toxic metal concentration ranges of arsenic (1.20–1.33 mg/kg), cadmium (2.68–3.16 mg/kg), chromium (9.31–11.73 mg/kg), copper (59.69–70.88 mg/kg), mercury (0.008–0.017 mg/kg), manganese (18.90-23.68 mg/kg), nickel (10.19-11.76 mg/kg), lead (1.71-1.86 mg/kg) and zinc (80.20-87.34 mg/kg). Cadmium had high contamination in soil; geoaccumulation (Igeo<3), contamination factor (CF<18), and enrichment factor (EF<70.21) while Cu and Zn had high bioaccumulation

(BF<4). Arsenic, Cd, Cr, and Cu showed a potential health risk with target hazard quotient (THQ<1). Farm soils were found to be highly enriched with toxic metals which further accumulated in cocoa beans at levels which can pose a potential non-carcinogenic risk to consumers. Elevated levels of toxic metals in cocoa beans suggest regular monitoring to mitigate the accumulation of toxic metals.

5.01.T-03 Pharmaceuticals and Personal Care Products (PPCPs) in the Terrestrial Environment: What's the Fuss?

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Pharmaceuticals and personal care products (PPCPs) are perceived as contaminants of emerging concerns due to their inherent ability to induce physiological changes in humans and animals at low doses. Despite the increased use of PPCPs, their eco-toxicological profile in the environment is yet to be established. This study focused on appraising the potential ecotoxicological effects of PPCPs on the terrestrial ecosystem and the corresponding impacts on ecosystem service delivery. A total of 2678 publications were identified in a systematic review exploring the antimicrobial fate, occurrence and effects in soil environment. A total of 857 soil entries were found in the Umweltbundesamt database (UBA 2021) and 70 antimicrobials had monitoring data in soils. The most highly monitored antimicrobials in soils were tetracycline, oxytetracycline and chlortetracycline while the antimicrobials detected at the highest concentrations were enrofloxacin, ciprofloxacin and chlortetracycline. Highest measured environmental concentration of antimicrobials was found in South America, Asia and Europe. Effects data were found for 11 of the top 15 antimicrobials monitored. Of these, only 2 had data on all key ecological components of the soil environment (microbes, invertebrates and plants). Based on the ubiquity of these PPCPs, a risk assessment was carried out using the risk quotient approach. Risk characterization of these chemicals indicates that sulphonamides, tetracyclines and fluoroquinolones are of environmental concern as they have risk quotients (RQs) greater than 1 i.e (RQ > 1). Furthermore, several knowledge gaps have been identified. This include lack of toxicity data for chemicals and the degree to which ecotoxicological effects of PPCPs could disrupt ecosystem service delivery is yet to be well elucidated. Laboratory studies were carried out on five active pharmaceutical ingredients (APIs) with RQs above safe limit based on the risk assessment that was conducted. Organization for Economic Co-operation and Development (OECD) protocol was employed in a bid to determine the impact of the APIs on microbial nitrogen transformation in soils. The results generated have shown the probable toxicological effects of these antibiotics on terrestrial microbes. Chemical analysis is currently ongoing to determine the degradation of the antibiotics in soil. The results from this study would inform policies on the management of potential threats from PPCPs.

5.01.T-04 Determining Fungicides' Impact on the Spread of Antibiotic Resistance Genes in Plant-Surface Bacterial Communities

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It is estimated that antibiotic resistant (AR) bacterial infections cause 1.27 million deaths annually. Research shows that soil and earth worms exposed to fungicides contain higher relative abundances of AR genes suggesting that they may be driving the spread of AR. Plants are exposed to many of the same agricultural inputs as soil but have not received the same attention as potential reservoirs for AR genes despite the apparent human health risk. Our research aims to determine if fungicides act as a driver for the spread of AR in plant-surface bacterial communities through both field and laboratory studies. The field portion of this work was conducted on creeping bent grass in Madison, WI during the summer of 2022 and 2023. Leaf material was collected prior to fungicide application, as well as 4 hours, 24 hours, 96 hours, and one week post application. qPCR and 16s sequencing results were used to determine abundance of genes known to confer antimicrobial resistance and bacterial community composition respectively. Additionally, we are conducting lab studies in tomato plants to determine if fungicides impact the frequency with which plant-surface bacteria transfer these AR genes. This work will increase understanding of the impact fungicides have on plant surface bacteria communities and may lead to novel AMR management strategies.

5.01.T-05 Salinity and Organochlorine Pesticide Contamination in Drinking Water in the Aral Sea Region of Karakalpakstan, Uzbekistan

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The rapid shrinking of the Aral Sea, largely due to unsustainable agricultural practices, is widely considered one of the greatest ecological disasters in modern history. The crisis has inflicted damage on human and environmental health in the region, as water, soil, and dust are heavily contaminated with pesticides such as dichlorodiphenyltrichloroethane (DDT) and lindane, salts, and other contaminants. The Republic of Karakalpakstan was once home to the thriving Aral Sea economy, but is now laden with high rates of cancer, neurological disorders, and diarrheal and pneumonic diseases. In 2022, TerraGraphics International Foundation partnered with Médecins Sans Frontières, the Ministry of Health of Karakalpakstan (MOHK), and local researchers to begin a preliminary environmental health risk assessment; findings indicated that the extent of drinking water contamination is not well understood, as historical monitoring results are maintained on paper in regional district-level offices. These files contain at least 7 years of salinity and organochlorine pesticide results for routine drinking water monitoring by sample location and district. In June 2024, project partners will aggregate and digitize the existing monitoring data into a database to be managed by MOHK. In addition to manual data entry, optical-character recognition artificial intelligence (AI) software is being explored to improve efficiency of tabulating and digitizing thousands of individual sample records. The resulting database will enable partners to obtain temporal trends in water quality over time, potentially identifying whether exposure levels correlate with the agricultural seasons, climate patterns, increasing water scarcity, and district location. When utilized with ongoing environmental assessment and analysis, the outcomes of this project will identify trends and rates of change of water quality and highly exposed subpopulations. This will establish a more robust picture of environmental health risk and ultimately be used to develop a targeted health intervention strategy in the Republic of Karakalpakstan. Results from the June 2024 digitization and AI efforts will be presented, along with preliminary statistical analyses of the database, including temporal trends identified. This analysis and intervention strategy may be applied to similar communities in the Aral Sea basin and other inland seas that are affected by agricultural chemicals, climate change, and water diversion issues.

5.02.A.T All Things Related to Endangered Species Assessment

5.02.A.T-01 The Feasibility of Testing Non-Crop Plants to Develop a Refined Risk Assessment for the EPA Herbicide Strategy for Threatened and Endangered Species

Henry O. Krueger *and Joshua R. Arnie, Eurofins Agroscience Services*

EPA's issuance of the draft Herbicide Strategy has resulted in an even more comprehensive evaluation of plants in the risk assessment and registration of herbicides. In the development of seedling emergence and vegetative vigor guidelines there was a consensus among regulators and scientists that there was sufficient evidence to support that crop species were good surrogates and were as sensitive as non-crop species, and more practical to work with than non-crop species. However, inclusion of non-crop species has continued to be a topic of concern. Part of the additional ESA analysis of the existing plant data includes determining the IC25 of the most sensitive endpoint of the test and establishing a species sensitivity distribution (SSD) of those values. In some cases, it may be necessary to test non-crop plant species more closely related to an endangered species of concern in order to conduct a more refined risk assessment and determine where they fall within the SSD. One of the limitations of conducting seedling emergence tests with non-crop species is finding commercial supplier of untreated seed sources with a high and consistent emergence rate that is needed to obtain reliable and accurate inhibition concentrations from the control for emergence. An argument can be made that emergence testing with 10 crop species adequately serve as surrogates. Vegetative vigor tests offer a much better opportunity for testing non-crop species. Since it is likely that most endangered plant species are off-field, spray drift would be the most likely route of exposure and the direct spraying of young seedlings in a vegetative vigor test would represent a worst-case scenario. The restriction of testing in sandy loam soils limits species that can be tested. Those soils were selected to keep organic matter low to reduce the potential binding of the chemical with the organic matter in the soil which would reduce exposure. However, having more choices of soil types would greatly increase the number of species that could be tested. In conclusion, we would only recommend testing additional non-crop species as part of a refined risk assessment to address special circumstances that would address a specific question that is driving the risk assessment. Careful consideration should be used in the selection of non-crop species and preliminary work should be done on small scale experiments to be used in the justification to regulators for their approval of the additional work.

5.02.A.T-02 Using Species Sensitivity Distribution Models and Surrogate Species to Estimate Pesticide Toxicity and Risk to Listed Lepidopteran and Coleopteran Insects

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There are nearly 100 insect species listed under the Endangered Species Act. Most of these species belong to the orders Lepidoptera (butterflies and moths) and Coleoptera (beetles). Currently, for insects, only bee (particularly honeybee) toxicity data are required for pesticide registrations. Thus, there is a dearth of non-bee toxicity data, and the issue is further compounded by the lack of standardized toxicity tests for non-bee insect species. As listed species cannot be directly tested, we have attempted to evaluate toxicity and risk for terrestrial Lepidoptera and Coleoptera by using and integrating available toxicity data and by estimating exposure. Specifically, we have developed species sensitivity distribution (SSD) models and employed the AgDRIFT model to estimate risk. Additionally, we have undertaken chemical and biological pesticide toxicity studies with monarch butterflies (*Danaus plexippus*), a species of conservation concern, and are determining if it can serve as an effective surrogate for listed lepidopteran species.

5.02.A.T-03 Automated Probabilistic Spatial Co-Occurrence Assessments for Aquatic Endangered Species

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The Endangered Species Act requires the U.S. Environmental Protection Agency to consult with the U.S. Fish and Wildlife Service and the National Marine Fisheries Service over potential impacts of pesticides on federally listed species. The deterministic methods currently used to analyze the spatial co-occurrence of endangered species and pesticide applications do not account for the spatial and temporal variability of either phenomenon, or the uncertainty inherent in modeling them. Probabilistic methods that account for variability and uncertainty to improve the accuracy of co-occurrence assessments have been recommended by the National Research Council but have not yet been implemented due to the higher complexity of the calculations and the volume of additional data required. The Automated Probabilistic Co-Occurrence Assessment Tool (APCOAT) provides access to both the data and methods required to rapidly conduct probabilistic spatial co-occurrence analyses and can be used to facilitate the transition from deterministic to probabilistic co-occurrence analyses. Here, we use APCOAT to study the potential co-occurrence between aquatic endangered species and atrazine applied to corn in the continental U.S., when considering both in-watershed and upstream pesticide usage. We modeled flowing aquatic habitats (rivers and streams) for 189 species drawing from a pool of 197 environmental variables, and static aquatic habitats (ponds and lakes) for 29 species drawing from a pool of 148 environmental variables. Species habitat models (SHMs) were generated iteratively, with variables being removed on the basis of correlation with other variables and contribution to the model, while minimizing reductions to model fitness as measured by the area under the receiver-operator curve (AUC, the chance that a randomly selected species presence location is ranked higher than a randomly selected background location). The resulting SHMs show high model fitness and parsimony, with flowing habitat SHMs having AUC of 0.86 - 0.99 (median 0.99) based on a median of 8.5 environmental variables, and static habitat SHMs having AUC of 0.92 – 0.99 (median 0.99) based on a median of 5 environmental variables. Across all species, habitat types, and pesticide transport methods, 65% of co-occurrence analyses showed < 5% probability of co-occurrence, 9% of analyses showed 5% - 10% probability of co-occurrence, and 26% of analyses showed >10% probability of co-occurrence.

5.02.A.T-04 Recent Experiences and Lessons Learned During the Development of Endangered Species Biological Opinions for Insecticides

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Biological Opinions developed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service are designed to protect threatened and endangered species. However, there is a potential for a significant impact on U.S. agriculture following implementation of these BiOps. Methods, policies, and risk mitigation strategies continue to evolve rapidly with every new federal action around ESA. Additionally, the role of EPA in Jeopardy and Adverse Modification (J/AM) analysis, Pesticide Use Limitation Area (PULA) development and mitigation implementation continues to expand. New methodologies from multiple stakeholders are being put forward to minimize assessment uncertainties and to develop targeted protective measures for listed species and their habitats. Policies must consider safeguarding endangered species, potential assessment uncertainties due to resource constraints and implementation with grower communities. In this presentation, we aim to offer insights into the development of tools that can be used to refine and improve risk assessment for carbamates and other crop protection products. We will delve into integration of new methods and ongoing feedback from stakeholders regarding these assessment refinements. Our discussion will also encompass efforts to expand mitigation options and improve connectivity between risk assessment and necessary mitigations. Lastly, we will outline the challenges and opportunities emerging from these evolving practices and experiences.

5.02.A.T-05 Refinements to Use Data Layers (UDLs) Used in Endangered Species Assessments of Pesticides

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In their Endangered Species (ES) assessments for pesticides, the US Environmental Protection Agency (EPA), US Fish and Wildlife Service (FWS) and National Marine Fisheries Service (NMFS) use datasets called Use Data Layers (UDLs) to spatially delineate potential pesticide use sites. For agricultural uses, the UDLs are currently generated from the US Department of Agriculture's (USDA) Cropland Data Layer (CDL) raster dataset, which is created using a machine learning classification algorithm applied to satellite imagery, agricultural ground truth data, and other ancillary datasets. The current approach for generating agricultural UDLs sources 5 years of CDL datasets which are processed by grouping the more than 100 individual CDL cultivated classes into 13 general UDL classes. While this approach is robust, there are some challenges. One challenge with generating agricultural UDLs is the presence of "spurious pixels" or highly fragmented areas of the UDL that are known to be false-positive classifications (i.e., non-agricultural raster cells classified as agriculture) carried through from the source CDL dataset. The presence of spurious pixels can result in inaccurate estimations of co-occurrence between UDLs and listed species locations, and it is desirable to remove spurious pixels to the greatest extent possible. However, it is difficult to identify and filter spurious pixels in a programmatic way given the nature of the remotely sensed source dataset. Furthermore, it is undesirable to remove actual agriculture from the UDLs, which can occur if agricultural pixels are misidentified as spurious. To address this issue, we propose to mitigate the presence of spurious pixels by incorporating additional processing steps to generate more accurate UDLs. This method builds on the USDA's refinement of the base raster CDL data into a vector Crop Sequence Boundary (CSB) dataset that uses publicly available data and employs a transparent yet efficient processing workflow. This new methodology virtually eliminates spurious pixels from the UDLs and provides the added benefit of significantly improved processing efficiency leading to a reduction in processing time from weeks for the original CDL based approach to overnight using the new CSB based approach.

5.02.A.T-06 Challenges with Crop Mapping to Support ESA

__Gerco Hoogeweg__[1], Raghu Vamshi[2], Nick Friendenberg[1] and Taisha Venort[1], (1)Corteva Agriscience, (2)Environmental Safety, Corteva Agriscience

Assessing the potential exposure, effects, and risk to species listed under the Endangered Species Act (ESA) is a required component of pesticide registration and registration review in the United States. The overlap between species and their designated critical habitats (CH), if available, and agriculture is a critical step in determining if U.S. Environmental Protection Agency makes May Affect, Likely to Adversely Affect, and subsequently Jeopardy/Adverse Modification determinations. An ESA overlap assessment contains two critical datasets: (1) species range or CH, and (2) crop location. If selected thresholds are exceeded, additional steps can be undertaken to refine the potential risk for each species and/or CH and use. Currently agencies and registrants rely on use data layers (UDL) that are based on aggregates of historical cropland data layers (CDL) from 2013-2017 in conjunction with the 2017 Census of Agriculture. Although easy to use, these UDLs have several drawbacks including not representing current cropping patterns, not accounting for multiple crops within a field, are overly conservative due to a 5-yr aggregation of crops, a lack of crop specificity, and the presence of significant noise in the form of spurious pixels. Recently USDA released the Crop Sequence Boundaries (CSB) dataset. This dataset contains various improvements over the CDL including increased spatial resolution and roads to limit crop distribution, as well as a minimum field size threshold of 1 ha. Currently this dataset is being considered for use in refined overlap assessments. Building upon the existing EPA approach, this presentation will include possible paths for improving the UDLs by incorporating more current datasets, state-of-the-art data processing algorithms and mapping pipelines, crop specific information, the elimination of non-ag areas and the incorporation of local cropping data layers.

5.02.B.T All Things Related to Endangered Species Assessment

5.02.B.T-01 Identification of Agricultural Best Management Practices Using Remote Sensing

Andy Jacobson[1], __Zechariah Stone__[1], Nicholas Guth[1], Maura Roberts[1], Sarah Terrell[1] and Richard Brain[2], (1)Waterborne Environmental, (2)Syngenta Crop Protection

The United States (U.S.) Environmental Protection Agency (EPA) has proposed a menu of runoff mitigation measures, as part of both their Endangered Species Act (ESA) workplan and their draft herbicide strategy, to protect listed species identified as potentially at risk from predicted pesticide exposures, based on the Agency's screening-level, risk assessment approach. To evaluate the potential ramifications of the Agency's proposed mitigation measures, an inventory of existing, agricultural best management practices (BMPs) for pesticide runoff in the contiguous US is needed. However, due to the number of funding sources available for conservation practice implementation, including federal, state, local, non-governmental organization (NGO), and private sector sources, as well as the fact that agricultural BMPs are often voluntarily adopted by growers without the support of conservation programs or funding, it is impossible to get an accurate accounting of implemented BMPs. Furthermore, federal and state reporting of BMP adoption/implementation is often limited to state-level aggregation, or county-level at best. Therefore, due to recent improvements in satellite imagery, in both resolution and re-visit period, remote sensing and artificial intelligence (AI) were utilized to develop a comprehensive system for the unsupervised identification of existing, implemented agricultural BMPs. While a remote sensing approach may not be applicable to all agricultural BMPs, it can be used globally to improve future inventories by identifying both permanent (e.g., grassed waterways, terraces) and non-permanent (e.g., cover crops, conservation tillage) practices. Using existing datasets of identified BMPs and their corresponding locations, the developed system was trained, tested, and evaluated based on its accuracy for BMP identification. Ultimately, the BMP identifications resulting from this developed system may be utilized to evaluate the potential ramifications of the EPA's proposed menu of mitigation measures, as well as to refine future pesticide risk assessments.

5.02.B.T-02 Evaluating Field-Specific Pesticide Runoff and Erosion Mitigation Need and Mitigation Practice Effectiveness Using the Pesticide Mitigation Assessment Tool (PMAT)

__Michael F. Winchell__[1], Jody Stryker[1], Tony Burd[2], Lula Ghebremichael[3], Jane Tang[4], Richard Brain[2], Robin Sur[5] and Tilghman Hall[6], (1)Stone Environmental, (2)Syngenta Crop Protection, (3)Bayer Crop Science, (4)Bayer AG - Crop Science Division, (5)Environmental Safety, Bayer AG - Crop Science Division, Germany, (6)Bayer CropScience

Mitigations are now being required by regulatory authorities in the US as an a priori mechanism to offset potential risks posed by the use of agricultural pesticides to "listed" (threatened and endangered) species. Mitigation requirements have been proposed to reduce off-site pesticide transport via both spray drift and runoff/erosion processes. The runoff and erosion mitigation options available to growers provide some flexibility in meeting the requirements for pesticide use; however, the current process includes limited consideration for field-specific conditions that would guide mitigation selection. Determining both the level of mitigation required and the effectiveness of mitigation options is challenging but necessary given the proposed regulatory framework. Many site-specific factors contribute to the variability in both requirements and effectiveness, including weather conditions, soil characteristics, topography, and cropping systems. While generalized mitigation requirements and effectiveness assumptions can be made and implemented into a regulatory framework with beneficial results, the advantages afforded by a site-specific mitigation effectiveness assessment make a compelling case for adopting such an approach. The Pesticide Mitigation Assessment Tool (PMAT) is a site-specific agronomic modeling tool that predicts pesticide runoff and erosion from agricultural fields based on site-specific conditions. Based on the USDA-supported APEX model, PMAT evaluates both the mitigation requirements for a field and the mitigation effectiveness of a range of common agricultural conservation practices, including combinations of mitigation practices. PMAT automatically evaluates the effectiveness of all feasible mitigation practice combinations, providing the user with a menu of options that meet the requirements for pesticide use and protection of endangered species. The site-specific approach offered by PMAT enables more efficient use of conservation resources, guiding the appropriate level of mitigation and avoiding unnecessary changes in agricultural practices when ecological protection goals are already met. An example application of PMAT will be provided with a case study context.

5.02.B.T-03 Creating More Refined Pesticide Mitigation Areas Informed by Our Familiarity with Species Spatial Data in Support of Carbamate Biological Opinion Development

Dana Christian[1]*, Matthew E. Kern*[2]*, Christopher M. Holmes*[1]*, Steve Kay*[3]*, Kevin Henry*[4] *and Jim Cowles*[4]*, (1)Applied Analysis Solutions, (2)Balance EcoSolutions, (3)Pyxis Regulatory Consulting / Generic Endangered Species Task Force, (4)Tessenderlo Kerley*

Pesticide mitigation areas are designed to ensure a pesticide's use will be protective of listed endangered or threatened species and their critical habitat. A species range can be geographically broad and can include areas unsuitable for habitat due to various environmental factors. There is a need to create more spatially refined pesticide mitigation areas (e.g., Pesticide Use Limitation Areas, PULAs) to better identify habitats where mitigation may be required. We have implemented a process during recent work with carbamates which has allowed us to explore more detailed habitat definitions to refine areas within a species range that, according to the most recent documentation from FWS, are required for a species' continued recovery. We have worked to develop a "core map" representing locations of the species where mitigations are needed based on species range, critical habitat, known locations, and suitable habitat. We have focused on using the best publicly available spatial datasets in conjunction with the most recent FWS species documentation to build detailed core maps for a wide variety of endangered species. Experience has shown us that, while challenges with resources and uncertainty exist, this range refinement process is possible and valuable for assessment of risk to many endangered species. This presentation lays out the lessons learned and choices made while developing more refined pesticide mitigation areas, based on our extensive experience with species range, critical habitat, potentially suitable habitat and known locations in support of the carbamate BOs.

5.02.B.T-04 Refining Ranges for Endangered Species: Implications for Pesticide Use Limitation Area (PULA) Development and Pesticide Registration

John Marton *and Gerco Hoogeweg, Corteva Agriscience*

Pesticide registration and re-registration in the US requires that EPA complete risk assessments for threatened and endangered (i.e., listed) species to comply with the Endangered Species Act (ESA). One critical component of the assessment is determining how much of a given species' range and designated critical habitat (CH), if available, overlaps with agricultural fields. If the overlap is above 4.44% and subsequent refinement steps do not result in a no jeopardy and/or no adverse modification determination, pesticide use limitation areas (PULAs) are developed to limit potential exposure, thereby reducing potential risk to listed species and their CHs. Large, generalized species ranges can lead to unnecessarily large PULAs, putting undue burdens on growers while having little to no meaningful benefit to the species. In this presentation, we demonstrate how to refine the ranges of two terrestrial plants, the pondberry (*Lindera melissifolia*) and the Kern mallow (*Eremalche kernensis*), selected for their differences in geographic extent and habitat requirements. Using species recovery and 5-year review documents published by the U.S. Fish and Wildlife Service, we were able to identify additional spatial data (e.g., National Wetlands Inventory, SSURGO soils database, etc.) to refine the ranges. While the total suitable range for the pondberry was reduced by 80%, the total suitable range for the Kern mallow was increased by 155%. A subsequent comparative overlap analysis using the 2022 Cropland Data Layer (CDL) showed the refined pondberry range had a decrease of 93% in overlap with agriculture compared to the existing range. For the Kern mallow, despite the increase in the refined range, the overlap with the CDL decreased by 82% and only ~27% of the current Kern mallow range has suitable habitat. These approaches demonstrate that options are available to refine ranges of listed species, thereby reducing proposed PULA sizes without compromising species protection and allowing growers greater access to crop protection tools.

5.02.B.T-05 Developing Localized Solutions for Diverse Cropping Systems in Washington and Oregon: Learnings from a Bottom-Up Approach to Endangered Species Act Pesticide Mitigations

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Agriculture in Oregon and Washington is exceptionally diverse, producing over 200 specialty crops. The diversity of the agricultural landscape provides unique challenges for EPA's development of pesticide mitigation measures for protecting species under the Endangered Species Act (ESA). In 2022, EPA developed a workplan to comply with ESA requirements by implementing new approaches and requiring measures to reduce off-field movement of pesticides for protecting non-target species, including ESA-listed species. These required measures largely focus on conservation practices and activities that physically alter the landscape to reduce runoff and erosion. Landscape changes typically require land ownership, substantial investment in new equipment and are not easily adaptable to all cropping systems or non-crop scenarios. As EPA continues to produce new strategies to fulfill its ESA obligations, there is growing concern among stakeholders on how proposed mitigations will be implemented on a local level. Oregon State University, Washington State Department of Agriculture, Oregon Department of Agriculture, National Marine Fisheries Service, and Compliance Services International, on behalf of the FIFRA Endangered Species Task Force (FESTF), hosted four local workshops across Oregon and Washington to engage local agriculture, conservation and regulatory stakeholders in identifying state implementation challenges and needs at the local level. Stakeholders were brought together in a fifth and two day long workshop to review the findings and outline solutions for local implementation of ESA pesticide mitigations. This presentation will cover learnings and updates from these workshop discussions.

5.02.B.T-06 Washington and Oregon Development of a Regional ESA Pesticide Program While Working with Agriculture to Develop Localized Mitigation Systems

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The pesticide state lead agencies (SLAs) in Oregon and Washington work to regulate the sale and use of pesticides according to coregulatory authority under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The Oregon Department of Agriculture (ODA) and Washington State Department of Agriculture (WSDA) conduct comprehensive work to certify and train applicators, regulate the sale and use of pesticides, conduct regulatory compliance efforts, conduct water quality monitoring, and implement pesticide and Endangered Species Act

(ESA) protection work. WSDA and ODA work to educate applicators and commodity groups, and conduct crop and pesticide usage assessments, and develop mitigation practices with partners. WSDA and ODA are working with federal agencies and other partners to develop a comprehensive state Pesticide Stewardship Program to establish a statewide and regional ESA and program. While WSDA and ODA have been holding ESA Pesticide workshops with growers and commodity groups, the agency staff have been working to develop a state-based ESA Pesticide approach. The state and regional approach could be utilized by EPA and the Services as system to implement interim ecological measures (IEMs) through EPA approved state level conservation programs (SLCPs). The approach will be utilized by SLAs in Oregon and Washington and also could be a model for SLAs throughout the nation. The proposed programmatic structure and approach for Oregon and Washington could also be utilized in the development of SLA roles outlined in the next EPA FIFRA Cooperative Agreement Guidance for the next grant cycle. Staff from WSDA and Compliance Services International (CSI) produced a spring 2024 issue paper related to the future roles of SLAs in the ESA Pesticide approaches, for the Council for Agricultural Science and Technology (CAST) series related to FIFRA and ESA. ODA and WSDA are utilizing information from the ESA Pesticide workshops and the concepts outlined in the CAST issue paper for informing the future processes for SLA involvement and leadership for state, regional and national implementation of the ESA Pesticide Programs.

5.02.P-We All Things Related to Endangered Species Assessment

5.02.P-We-182 Non-Agricultural Use Data Layers: Options and Methodologies for Supporting ESA Overlap Analysis

Jillian LaRoe[1]*, Matthew E Kern*[2] *and Christopher M Holmes*[3]*, (1)Applied Analysis Solutions, United States, (2)Balance EcoSolutions, (3)Applied Analysis Solutions*

Exposure assessments for Endangered Species have benefitted from quality spatial data on use sites for agricultural applications of pesticides. There is less development of available data for assessments in non-agricultural settings where pesticides are applied and need to be assessed, like forests, railroads, golf courses, parks, and other commercially managed vegetation. The emphasis on agricultural use sites has produced refined methodologies as well as defined and delineated data sets (e.g., Use Data Layers). However, there is ambiguity within non-agricultural uses in terms of delineating potential use sites and developing robust and comprehensive spatial datasets, particularly at national scales. Recent advancements in data processing/platforms along with the scale and extent of available spatial data present an opportunity to develop more comprehensive datasets for non-agricultural overlap analyses (relationship of use sites to species habitats). We have created customized approaches leveraging detailed datasets, like Open Street Map, to help fill this data gap. We incorporated additional information from other sources, such as historical high resolution aerial imagery, to support boundary editing and refinement of application areas. We present a combination of data sources and methods that can be tailored to curate data for specific objectives, tools and strategies to refine potential exposure for non-agricultural scenarios, as well as applied examples using golf courses, railroad vegetation management, and forests.

5.02.P-We-183 Effectiveness of Tree Lines as Pesticide Drift Barrier in Agricultural Landscapes

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The most efficient pollinators are insects including keystone bees that sustain biodiversity, conserve habitat, and promote healthy ecosystems. The recent US EPA draft Herbicide Strategy identifies potential impacts to the listed endangered species, analyzes the extent of restrictions according to the location, crop type and potential of herbicide exposure and finally determines mitigation measures for protection. Some of the recommendations commonly encourage establishing pollinator habitats around agricultural areas. However, established pollinator habitats can inadvertently become contaminated from agrochemicals moving off crop fields. Of concern are systemic neonicotinoid seed treatment insecticides. Dust laden with seed treatments may drift off fields in spring during planting which can contaminate nearby flowers. This project examined the role of existing tree lines as potential drift barriers to reduce non-target pesticide exposure. Neonicotinoid residues were collected using sticky traps within pollinator habitats with and without drift barriers to determine the field-relevant exposure levels to foraging pollinators. Transect surveys were also conducted to assess biodiversity and abundance of beneficial insects in habitats. Results will improve landscape management recommendations and promote the use of existing landscape features that provide more sustainable solutions for promoting pollinators in agricultural . Drift barriers were effective in reducing the drift up to 96% and 51% and 55% in floral abundance and diversity respectively and similarly 87% and 15% increase in insect abundance and diversity in three years after the establishment of the pollinator habitats.

5.02.P-We-184 AMMPS: A Web-Based Tool for Managing Endangered Species Data to Support Pesticide Risk Assessment

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In January 2022, the US Environmental Protection Agency (EPA) announced a policy that requires Endangered Species Act (ESA) evaluations to be completed for all new conventional active ingredient pesticide registrations. The intent of the policy is to ensure that pesticide registrations do not jeopardize the continued existence of species listed under the ESA. To aid their assessment, EPA has asked registrants to provide “upfront mitigations” to “minimize the effects of incidental take to listed species that could result from use of a pesticide” and in December 2023, EPA released final guidance for pesticide submissions that require ESA reviews. While the guidance provides various activities that registrants can take to improve efficiency of ESA evaluations, where or when mitigations may be needed for a given pesticide action has yet to be defined by policy or guidance. This presentation will discuss a data-focused approach to determining when and where mitigations may be needed based on pesticide risk profile, labeled uses of the pesticide, extent of potential use, location of ESA-listed species, and species attributes. Examples will be provided using AMMPS, a tool to help guide discussions on mitigations and other measures for pesticides and ESA. As there are unknowns surrounding the implementation of the policy, the presentation will also include potential improvements for future data applications and strategies.

5.02.P-We-185 Uncertainties in Non-Target Terrestrial Plant Risk Assessments

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While there are uncertainties associated with all ecological risk assessments, people unfamiliar with the EPA non-target terrestrial plant (NTP) testing requirements may not be aware of how these data are used for risk assessment purposes. NTP risk assessments are based on the results of two EPA or OECD guideline studies: seedling emergence and vegetative vigor. These studies are generally conducted using annual agricultural crop species, consisting of six dicot species and four monocot species. These studies are short duration tests, 14 to 21 days. These results are then extrapolated to all types of terrestrial plants, including perennials, woody species, wetland species, and emergent aquatic species. In these tests, the crop species are exposed to pesticides either as planted seeds in a low organic content sandy soil or as very young seedlings (2-4 true leaf stage). These studies have either three (vegetative vigor) or four (seedling emergence) quantitative endpoints that can be used for NTP risk assessments. Endpoints include seedling emergence, plant survival, plant height, and above-ground plant weight; plant weight is often the most sensitive endpoint and therefore is the endpoint most often used for NTP risk assessments. These endpoints are reported as EC25, EC50, and NOECs, with the EC25 values for the most sensitive monocot and dicot species being used for non-endangered species risk assessments and the NOECs for the most sensitive species being used for endangered species risk assessments Drift and runoff estimates are used in the TerrPlant model in conjunction with the NTP testing results to calculate RQs used for NTP risk assessments. However, under field use conditions, plants treated with or exposed to herbicides often outgrow effects of sublethal herbicide treatments, so it is not clear how relevant these sublethal NTP testing endpoints for height and weight are with respect to overall plant survival and seed production. This presentation will explore effects on weedy and/or woody plants exposed to herbicides in the field and compare those results, including any regrowth, to reported EC25 or NOEC values that EPA uses for NTP risk assessments.

5.02.P-We-187 Sensitivity of Alabama Freshwater Gastropod Species to Nickel Exposure

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Snails are effective bioindicators due to their prolific distribution, high level of endemism, and capacity to accumulate contaminants. Freshwater snails have unique ecological niches which are imperiled by land-use change and the introduction of hazardous chemicals. To assess how environmental alterations affect gastropods, lab-based studies are needed to characterize the toxicity of specific stressors. This can help guide policy decisions and remediation efforts. The aim of this research was to characterize acute toxicity of nickel (Ni) towards endemic snails (*Somatogyrous georgianus*, *Elimia cahawbensis,* and *Elimia spp.*) and measure the bioaccumulation of Ni and mineral elements including calcium, magnesium, potassium, and sodium. Snails were exposed to six concentrations (25-800 μg/L) of Ni for 96h. Additionally, a local species of least concern, *Physella acuta,* was exposed to nickel concentrations (125-2000 μg/L) for comparison to the endemic species. Among the studied snail species, *E. cahawbensis* was the most sensitive to Ni with the lowest lethal concentration (LC) where 50% of the organisms died at 88.88 μg/L Ni. The LC50 for *Somatogyrous georgianus* was 167.78 μg/L Ni.

Elimia spp.'s LC50 was 393.13 µg/L Ni. The LC50 for *Physella acuta* was 436.56 µg/L Ni. Except for *Elimia spp*., mortality of the other two endemic snail species corresponded to the whole-body uptake of Ni. Ni exposure also influenced sodium and calcium uptake for *Elimia* spp. All three endemic species are potential candidate species for characterizing localized effects of human activities, and this study provides a first step in characterizing how snails would be affected by environmental alterations. More research is needed to further characterize potential effects of other human stressors on these endemic snail species. Additionally, research into subindividual responses and routes of exposure can further elucidate variations in species sensitivity.

5.03.P-Th Bayesian Networks in Environmental Risk Assessment and Management

5.03.P-Th-060 Hierarchical Clustering and Bayesian Networks for Assessing Chemical Mixture Risks to Community-Level Endpoints

John F Carriger[1] *and Michael Brooks*[2]*, (1)U.S. Environmental Protection Agency, Gaithersburg, United States, (2)U.S. Environmental Protection Agency, United States*

Risk assessments often consider multiple hierarchical levels of an ecological community from individual to population to community to landscape. Each of these levels contain emergent properties; however, their measurements often rely on incomplete or indirect data such as effects from individual chemicals on individuals of a species. One feature that may facilitate the utilization of advanced data on multiple stressors to community-level endpoints is Bayesian network clustering. Clustering with Bayesian networks can condense multiple variables into fewer variables that succinctly group salient features yet retain the distributions of the original variables for inferences. If the cluster groupings are meaningful and representative, the clusters can then be utilized along with the distributions of the individual variables to characterize risks. Bayesian network clusters can be machine learned from multiple statistical approaches but can be combined with expert knowledge for their development. We explore the possibilities in a hypothetical risk assessment for an aquatic ecological community in a lake system facing multiple effluent constituents from both runoff and groundwater discharge. The community clusters are developed to represent potential phase shifts based on abundance and presence of different species. Taxa-specific clusters are used to assess overall risks to similar species groupings, but meta-clusters provide overall summaries of status and condition across all taxa. Likewise, clusters for groupings of similar compounds (e.g., herbicides) and latent factors for runoff and groundwater effluent input to the lake systems can be developed and linked to the assessment endpoints. Mixture loadings representing event-based and longer-term discharges to the system can also be differentiated for acute and chronic exposure time-periods. The potential benefits, drawbacks, knowledge/data requirements, and research needs for this approach will be explored considering practical requirements for future applications. *The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

5.03.P-Th-061 A Demonstration of Counterfactual Analysis of Wildfire Outcomes: Risk Reduction of a Catastrophic Fire from Fuels Treatment Performance

John F Carriger[1] *and Matthew Thompson*[2]*, (1)U.S. Environmental Protection Agency, Gaithersburg, United States, (2)Pyrologix*

Counterfactuals provide valuable tools for assessing the added benefits of management measures and are becoming more utilized by the wildfire risk assessment community. Periodic wildfires are critical to the health of fire-adapted ecosystems but increasing severity and frequency of wildfires may be occurring from past fuels buildup and climate change conditions for many areas where wildfires can occur. One management technique for reducing the risk of a catastrophic wildfire is from removing or treating fuels buildup in the understory of forests. This may occur through mechanical removal or prescribed burns. However, even with these management efforts a risk of a catastrophic wildfire still exists. Counterfactual analysis is the highest rung on Judea Pearl's causal ladder and provides powerful tools for examining whether past events that occurred (e.g., a catastrophic wildfire) might not have occurred under different causal conditions (e.g., a fuels treatment program). This presentation will show how counterfactual analysis may be performed using Bayesian networks through the salient lens of wildfire risk assessment. Scenarios will be analyzed that demonstrate the calculation of necessary, sufficient, and necessary and sufficient causal calculations with Bayesian networks for hypothetical wildfire scenarios. Bayesian networks make powerful tools of probabilistic risk assessment tractable in a causal inference framework. Preliminary work suggests the possible utility of causal counterfactual analysis with Bayesian networks for retrodictively assessing performance of past mitigation investments and prospectively assessing the return on investment. *The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

5.03.P-Th-062 Implementing a Socio-Environmental Cluster Analysis Using Bayesian Profile Regression to Assess Community Vulnerability to Per- and Polyfluoroalkyl Substances (PFAS) Exposure

Katherine Y Deliz Quiñones[1]*, Eric Coker*[2]*,* ***Sanneri Elibet Santiago Borrés***[3]*, Yusuf Jamal*[4]*, Kayan Clarke*[4]*, Karen Sem*[4]*, Devon M Nenon*[4]*, John A Bowden*[4]*, Antarpreet Jutla*[1] *and Jean-Claude Bonzongo*[1]*, (1)Environmental Engineering Sciences, University of Florida, (2)Environmental and Global Health, University of Florida, BCCDC, (3)University of Florida, Gainesville, United States, (4)University of Florida*

Per- and polyfluoroalkyl substances (PFAS) are globally pervasive, persistent, and toxic, posing significant health risks across diverse environmental settings. Marginalized communities experience disproportionate exposure to harmful PFAS levels due to the uneven distribution of pollution sources, further exacerbated by climate change. We introduce a quantitative vulnerability assessment framework, employing model-based clustering of socioeconomic, environmental, and flood inundation indicators, to identify vulnerable subgroups with similar socio-environmental characteristics. Our research establishes that PFAS spatial distribution in soils is primarily regulated by a site proximity to a contamination source and its flood risk, both of which increase communities' exposure risks. Utilizing Bayesian profile regression (BPR), we investigate clustering of correlated variables and the ecological relationship between social and environmental covariate profiles and soil PFAS levels in Brevard County, Florida. With BPR, we are able to investigate the spatial patterning of clusters of socio-environmental profiles in the study area and identify vulnerable sub-regions most susceptible to an adverse outcome such as exposure to environmental contamination. Therefore, our objectives include identifying clusters of socio-environmental profiles and modeling associations between soil PFAS levels and these clusters. The BPR analysis revealed five clusters of socio-demographic and environmental profiles, with three characterized by elevated flood risk levels. Clusters with heightened flood risk exhibit the highest soil PFAS concentrations. The median census tract-level total PFAS concentration in soil for the study area is 1,480 (with an interquartile range of 1,005 to 1,930) parts per trillion. Further statistical analyses, including linear regression, demonstrate associations between socio-environmental clusters and PFAS levels, highlighting the significance of pollution source proximity and flood risk. This study facilitates identification of communities facing high combined social and environmental vulnerability, with disproportionately elevated soil PFAS levels. Moreover, it advances understanding of PFAS distribution dynamics and underscores the imperative of addressing environmental injustices for equitable protection.

5.03.T Bayesian Networks in Environmental Risk Assessment and Management

5.03.T-01 Amphibian Ecological Risk Assessment Using Bayesian Networks

Jill Awkerman *and Jackson Reimer, U.S. Environmental Protection Agency*

Probabilistic risk assessment approaches are often used to integrate available datasets and expert knowledge when robust empirical evidence is lacking. Standard toxicological data for amphibians are sparse, to the extent that common risk assessment methods often rely on available exposure data from other aquatic or terrestrial species as representative of different amphibian life stages. Statistical approaches to compensate for lacking data are warranted, particularly as efforts to minimize animal testing are increasingly advocated. Bayesian belief networks provide flexible modeling strategies that address known uncertainties and allow a means of interpreting minimal data in the appropriate context. Here we use a library of amphibian species data to categorize life history traits and ecological habits that influence vulnerability to pesticide exposure. We build on previous exploration of population simulations to probabilistically determine ecological risk associated with pesticide applications for representative amphibian species to different types of agrichemicals. Assessment procedures that leverage known causal inferences to accommodate data gaps offer a screening level approach to anticipating potential risk to wildlife populations. The flexible modeling strategies outlined herein demonstrate a versatile tool to incorporate relevant data while accounting for uncertainty.

5.03.T-02 Utilizing Bayesian Networks for Safe-by-Design of Chemicals in Consumer Products: Quantitative Adverse Outcome Pathway-Based Approach

Jaeseong Jeong *and Jinhee Choi, School of Environmental Engineering, University of Seoul, Korea, Republic of (South)*

As consumer products increasingly incorporate a wide range of chemicals, comprehensive toxicity assessments are not keeping pace, highlighting the need for robust early-stage evaluation methods. Traditional toxicity assessment methods often fall short in preemptively identifying potential health risks due to the extensive variety of chemicals used. This study introduces a Bayesian Network (BN) modeling approach to assess and compare the toxicity of consumer product chemicals, with a focus on variations in physicochemical properties. We first developed an adverse outcome pathway (AOP) for these chemicals by integrating existing experimental data and insights from toxicological literature. Utilizing BN, we modeled the probabilistic dependencies between chemical properties and their toxicological outcomes. This allowed us to identify and compare the influence of specific physicochemical properties, such as molecular structure and descriptors, on toxicity. Our findings indicate that the impact of these properties varies significantly across different types of consumer products. By incorporating changes in toxicity pathways in a quantitative AOP framework, our approach enhances the reliability of toxicity predictions and supports the implementation of the Safe-by-Design concept. This approach not only aids in understanding the causal relationships within chemical toxicity but also facilitates the development of safer consumer products through informed design choices.

5.03.T-03 Multiple Prenatal Outdoor Exposures are Associated with the Infant Gut Microbiota: An Investigation with Bayesian Networks

Yidan Zhang[1,2] *and Boyi Yang*[2]*, (1)Department of Earth and Environmental Science, (2)Department of Occupational and Environmental Health, Sun Yat-sen University, China*

Outdoor environmental exposure such as air pollution and greenspace has been linked to modify human gut microbiota. However, research on the associations of prenatal outdoor environmental exposures with infants’ gut microbiota is limited. This study, performed in a birth cohort of 765 mother-infant pairs living in Maoming in Guangdong province, China, aims to assess the associations of multiple prenatal outdoor environmental exposures with infants' gut microbiota. We assessed the infant gut microbial diversity and composition using 16S rRNA gene amplicons sequencing in meconium samples collected from infants in our study. We measured 12 exposures at each mother' s residential address during their pregnancy, including six air pollutants (i.e., particulate matter with an aerodynamic diameter of 1 µm or less (PM_1), 2.5 µm or less ($PM_{2.5}$), and 10 µm or less (PM_{10}), nitrogen dioxide, sulfur dioxide, and ozone), air temperature, relative humidity, greenspace measured by Normalized Difference Vegetation Index, blue space measured by Normalized Difference Water Index (NDWI), population density, and artificial light at night. An exposome-wide association approach was used to estimate associations between exposures and infant’s gut microbial alpha-diversity. Graph-Guided Fused Lasso (GFLasso) models were applied to identify associations between exposures and microbial genus. Bayesian network was used to construct the network structure of the associations of exposures with microbial alpha-diversity and genus. We found higher prenatal exposure to PM_1, $PM_{2.5}$, and ozone were significantly associated with infants' lower gut microbial alpha-diversity, whereas air temperature and relative humidity were associated with higher gut microbial alpha-diversity. GFLasso model showed higher air pollution, lower relative humidity and NDWI were associated with higher relative abundance of potential pathogens (e.g., *Mycoplasma, Prevotella*) and lower relative abundance of beneficial genera (e.g., *Lactobacillus*, *Ruminococcus*). Bayesian networks showed NDWI was indirectly associated with higher gut microbial alpha-diversity and lower abundance of *Prevotella* by changing the level of air pollution, air temperature, and relative humidity. Our findings suggest that multiple prenatal outdoor environmental exposures were associated with infants' gut microbiota. Residential blue space may indirectly improve infants' gut microbiota by reducing air pollution and modifying the local climate.

5.03.T-04 Classifying Metal Soil Concentrations with Bayesian Networks: Urban Background in the Southeastern United States

John F Carriger[1], *Robert Ford*[2], *Tim Frederick*[2], *Sydney Chan*[2] *and Yuen-Chang Fung*[3], *(1)U.S. Environmental Protection Agency, Gaithersburg, United States, (2)U.S. Environmental Protection Agency, (3)Tetra Tech*

Metal concentrations in urban soil samples frequently contain levels that are not attributable to a specific site. These concentrations are from diffuse sources and distinguishing them from site contaminants can better inform remediation decisions. To understand urban background concentrations for contaminated site remediation and risk assessment, a previous effort by the U.S. Environmental Protection Agency collected and analyzed urban soils in five states in the Southeastern U.S. In the present work, data from seven cities (Gainesville, FL; Lexington, KY; Louisville, KY; Raleigh, NC; Winston-Salem, NC; Columbia, SC; and Memphis, TN) in these five states were used for a Bayesian network cluster analysis to explore the differences in urban soil metal concentrations. Initially, the influence of non-detected data on discretization thresholds was examined and metals data that produced a bias in threshold detection for all discretization methods were excluded. For the remaining metals, data clustering with expectation-maximization was used to create a new factor variable with cluster states based on the metals data. A naïve Bayesian network was the basis for the clustering where each of the analyzed metals was a child node of the latent factor variable that contains the clusters as variable states. A posterior probabilistic analysis with the resulting network was used to interpret the clusters in terms of metal concentration levels and relationships to cities, soil texture and land use characteristics. This work demonstrates how data clustering with Bayesian networks can help understand key characteristics of soil sampling data for urban regions. *The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

5.03.T-05 A Bayesian Network Modeling Application for Prescribed Fire Spatial Planning in the Southeastern United States

Sandhya Nepal, *Lars Y Pomara and Nicholas P Gould, U.S. Department of Agriculture Forest Service, Southern Research Station, Asheville, North Carolina*

Prescribed fire is a primary tool for promoting diverse and healthy ecosystems, improving climate change resilience, and reducing wildfire risks by removing hazardous fuels. In addition to risk management goals, the USDA Forest Service has shortleaf pine and white oak forest restoration goals in the Interior Highlands subregion of the southeastern US to promote resilient landscapes with high conservation value. Given these multiple objectives, there is a need for data-driven planning tools to strategize forest management investments tailored to existing landscape conditions, social realities, and management capabilities. We collaborated with a team of scientists, planners, and managers to co-develop spatial prioritization models that provide decision support for prescribed fire planning in the Interior Highlands subregion. We developed and applied an expert-driven Bayesian network model that integrates biophysical, ecological, and socioeconomic information at the landscape level to quantify the likely influence of prescribed fire on ecological restoration and wildfire risk reduction objectives. Further, we integrated network model results into a spatial optimization analysis under plausible management scenarios (representing partner-informed priorities and constraints) to identify high-opportunity landscapes where prescribed fire investments will be effective and efficient for advancing goals. Spatial data products from this study will provide planning options under a range of scenarios to allow for flexibility, given future management uncertainties.

5.03.T-06 Eliminating the Barriers to the Broader Adoption of Bayesian Networks in Environmental Assessment and Management

Wayne G Landis, *Western Washington University*

The use of Bayesian networks (BNs) and related tools has grown in many fields including ecological risk assessment. However, there appears to be resistance to exploiting the many advantages of using these robust probabilistic tools. These barriers to wider adoption appear to be: an adherence to the methods and assumptions of the risk assessment paradigms of the initial formulation of ecological risk assessment for the US EPA, the learning curve necessary to build and parameterize an appropriate BN, a general lack of experience in interpreting the outputs, misunderstandings of the nature of conditional probability, and a lack of appreciation of the flexibility of BN models to incorporate data from field studies and other modelling forms. BNs also forgo the calculation of hazard quotients and do not rely on the estimation of point values or hazard concentrations (an HC5 from a species sensitivity distribution for example). Statements such as, there is too much uncertainty to calculate a quantitative risk assessment and therefore a qualitative risk assessment was performed, belies an unawareness of the innate ability of BNs to accurately describe uncertainty and incorporate it into a risk assessment. Two other items also appear to be inhibiting the adoption of these methods. Experiments and field studies are not yet designed to take advantage of tools that can incorporate a large number of variables and data describing their distributions over space and over time. It is possible to calculate the distribution of risk over landscapes of the data exists for a location. The lack of toxicity testing that is designed to describe an entire exposure-response unnecessarily adds to uncertainty. Experiments that capture the interactions within mixtures and a range of environmental conditions are key to increasing certainty in estimating toxicity. Perhaps the greatest roadblock is the lack of understanding among managers and decision makers on the strengths of these newer approaches over the archaic quotient values and similar tools. Dismantling these barriers requires a concerted effort to translate the outputs of BNs and similar tools to the more efficient adaptive management frameworks that will be possible.

5.04.P-Mo Between the Guidelines: Common Issues, Pitfalls, and Unwritten Considerations in Ecotoxicology Data Packages

5.04.P-Mo-135 Toxicity of Formulated Plant Protection Products to Rats as a Predictor of Their Toxicity to Birds

*Stephanie Plautz[1] and **John Marton**[2], (1)Corteva Agriscience, Moon Township, (2)Corteva Agriscience*

Some regulatory authorities require acute bird testing on formulated plant protection products for product registration. To evaluate whether acute bird testing on formulated products adds useful information to the product toxicity dataset, an analysis will be conducted using acute bird and rat toxicity data extracted from Corteva's archives. The acute toxicity of formulated products to rats and birds will be first compared to the acute toxicity of the active ingredients they contain using LD_{50} values (the dose at which 50% of the tested animals died). For formulated products containing only one active ingredient, the measured product LD_{50} will be converted to active ingredient units using the active ingredient content of the formulation. For formulated products containing ≥2 active ingredients, model deviation ratios will be used to compare formulated product and active ingredient toxicity. Formulated products with LD_{50} values >3 times lower than predicted based on active ingredient LD_{50} values will be considered more toxic than expected. Data will then be evaluated to determine whether a lack of higher-than-expected formulated product toxicity in rats is predictive of the same in birds.

5.05.P-Tu Bridging the Gap Between Science Development and Policy, Regulatory, and Technology (PRT) Needs for Complex Substances - Supporting Data-Driven Decision-Making in Heath & Environmental Risk Assessment and Management

5.05.P-Tu-164 Development of a Tiered Approach to Screen Mixture Effects in Consumer Products: A Case Study of Cleaning Products in Korea

Yongmin Jung, Keon Kang, ***Hyunwoo Kim*** *and Jinhee Choi, School of Environmental Engineering, University of Seoul, Korea, Republic of (South)*

In consumer products, diverse individual chemicals are mixed and co-exposed to consumers, underscoring the imperative for comprehending the mixture toxicity in the varied components employed across these products. Therefore, we developed a tiered approach to screen the systemic toxicity of mixtures in consumer products using *in vitro* assays. In Step 1, we collected information on consumer products from Ecolife, the Korean consumer product database, and selected cleaning products as a case study. In Step 2, we categorized the functional uses of cleaning products and identified binary combinations among 92 biocides known to be active chemicals. In Step 3, we curated *in vivo* data from the European Chemicals Agency (ECHA) database to assess the potential systemic toxicity induced by individual substances within the mixtures. In Step 4, we screened the cytotoxicity of individual substances using human cell lines to determine the mixture composition ratio based on an equitoxic approach. In Step 5, we analyzed the effects of mixtures in consumer products by comparing the observed cytotoxicity with the predictions from the Concentration Addition (CA) and Independent Action (IA) models. Synergistic effects on cytotoxicity were observed with 1,2-Benzisothiazol-3(2H)-one and triclosan co-exposed at concentrations below the regulatory limit, raising concerns about the mixture effects in consumer products. In this study, we proposed a tiered approach for identifying binary mixtures co-existed within consumer products and screening their mixture toxicity. This study highlights the blinding point and persistent challenges concerning the mixture toxicity contained in products. Our tiered approach will offer valuable insight for navigating these complexities and facilitating the effective evaluation of mixture toxicity. *This work was supported by Korea Environmental Industry & Technology Institute through 'Core Technology Development Project for Environmental Diseases Prevention and Management' (2021003310005) and through Technology Development Project for Safety Management of Household Chemical Products (RS-2023-00215309), funded by Korea Ministry of Environment.*

5.05.P-Tu-165 Plastic Additives and the Regulation of Chemicals of Concern

Jessica G Lambert[1]*, Kathlyn MacDonald*[1]*, Griffin Miller*[1] *and Natalia Vinas*[2]*, (1)Noblis, (2)Midsouth Regional Chapter*

Synthetic plastic production and use has grown exponentially since it was initially developed in the early twentieth century. Plastics play a critical role across nearly every sector, including packaging, construction, textiles, transportation, and electronics. Plastic additives and processing aids are employed to modify the characteristics of plastic polymers and ultimately achieve the

performance characteristics required for these varied purposes. Some of these technical capabilities include heat and chemical resistance, antioxidants, stabilizers, low-outgassing and low coefficient of friction. Due to this flexibility in performance, plastics have become ubiquitous. Chemical additives and processing aids, used in plastic production, may be released throughout the life cycle of the plastic and many have properties that potentially pose a risk to human health and/or the environment. However, a limited number of these substances have been studied. There is a growing push to expand the boundaries of current regulatory instruments to limit or eliminate the use of chemicals of concern as additives in plastics. Approximately half of the known additives found in plastics have been identified as substances of potential concern and are already covered by at least one chemical prioritization list at the supranational, national, and subnational levels in a number of jurisdictions. The intent of these policies is to reduce, restrict, and eventually eliminate the use of potentially harmful plastic additives. These policies will aid in the transition to more sustainable product development including safer plastic additive alternatives. However, some applications lack plastic additive alternatives that would allow a product to meet existing technical requirements and/or performance specifications. For products that do, these alternatives may not be drop-in replacements and alternative design changes may be required. For the Department of Defense (DoD), these additives provide plastic with unique physical, thermal, and chemical properties that provide essential and critical functionalities essential for mission readiness. This presentation defines existing and proposed regulatory instruments to limit or eliminate the use of chemicals of concern as additives in plastics; identifies how these regulatory drivers interface with the DoD; and elucidates the critical functionalities of plastic for the DoD.

5.05.P-Tu-166 Automating the Review of Data in the ECOTOXicology Knowledgebase to Support Regulatory Decision-Making

Janine Fetke[1], *Kellie A Fay*[2], *Jennifer Olker*[3], *Rachel Dean*[1], *Stephanie Griffin*[1], *David Turk*[1], *Devin Jones*[1], *Jennifer C Brennan*[1], *Patricia Fontenot*[2] *and Tyler Amrine*[1], *(1)U.S. Environmental Protection Agency, (2)U.S. Environmental Protection Agency, (3)U.S. Environmental Protection Agency, Great Lakes Toxicology and Ecology Division*

The ECOTOXicology Knowledgebase (ECOTOX) contains ecological toxicity and bioaccumulation data curated from public literature on nearly 13,000 chemicals. Data can be extracted from ECOTOX for a wide range of activities, such as regulatory risk assessment. However, this process can be labor intensive and, given the frequent additions of new data to ECOTOX, there is a need to perform the extraction and analysis of the available data on a periodic basis. To this end, an automated pipeline was developed using R to allow for efficient and accurate identification of chemicals that fit search criteria. This presentation describes the function and output of the automated pipeline, illustrated by two case examples. The first example applies acute aquatic toxicity and bioaccumulation criteria to a per and polyfluoroalkyl substances (PFAS) dataset. In the second example, screening criteria are applied to generate a dataset for trichloroethylene (TCE) and its analogues to support potential read across and compute taxa-specific hazard toxicity values. This pipeline can (1) identify chemicals in ECOTOX that meet specific toxicity and bioaccumulation criteria, (2) filter on applicable data fields (e.g., species group, chemical concentration, specific endpoints, bioaccumulation, and duration), and (3) compute acute or chronic hazard values, thereby producing a synthesized dataset for further evaluation. The pipeline can potentially be used to efficiently streamline the evaluation of large datasets. *The views expressed in this abstract are those of the authors and do* *not necessarily represent the views or the policies of the U.S. Environmental Protection Agency. Any mention of trade names, manufacturers or products does not imply an endorsement by the United States Government or the U.S. Environmental Protection Agency.*

5.05.P-Tu-167 Effect Driven Prioritization of Contaminants in Wastewater Treatment Plants Across China: A Data Mining-Based Toxicity Screening Approach

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A diversity of contaminants of emerging concern (CECs) present in wastewater effluent, posing potential threats to receiving waters. It is urgent for a holistic assessment of the occurrence, distribution, and risk of CECs related to wastewater treatment plants (WWTP) on national and regional scales. A data mining-based risk prioritization method was developed to collect the reported contaminants and their respective concentrations in municipal and industrial WWTPs and their receiving waters across China over 20 years. A total of 10,781 chemicals have been reported in 8,336 publications, of which 1,037 contaminants were reported with environmental concentrations. While contaminant categories varied across WWTP types (municipal vs. industrial) and regions, pharmaceuticals and cyclic hydrocarbons were the most studied CECs. Contaminant composition in receiving water was closer to that in municipal than industrial WWTPs. Publications on legacy pesticides and polycyclic aromatic hydrocarbons in WWTP decreased recently compared to the past, while pharmaceuticals and perfluorochemicals have received increasing attention, showing a changing concern over time. Detection frequency, concentration, removal efficiency, and toxicity data were integrated for assessing potential risks and prioritizing CECs on national and regional scales using an environmental health prioritization index (EHPi) approach. Among 666 contaminants in municipal WWTP effluent, trichlorfon and perfluorooctanesulfonic acid were with the highest EHPi scores, while 17α-ethinylestradiol and bisphenol A had the highest EHPi scores among 304 contaminants in industrial WWTPs. The prioritized contaminants varied across regions, suggesting a need for tailoring regional measures of wastewater treatment and control.

5.05.P-Tu-168 The Value of Employing Zebrafish Embryos Applying Different Toxicity Assessments and QSAR Models to Predict Toxicity of Fragrance Constituents

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Fish acute toxicity data are part of the compulsory data in the process of chemicals' registrations worldwide and are employed for two purposes environmental hazard and risk assessments. Preceding the regulatory process, chemicals establishments screen new substances in the phase of development to comprehend their toxicity profiles. For this matter, cost effective but reliable tools to predict toxicity, employing New Alternative Models, should be assessed. Fish acute toxicity results from test (zebrafish, OECD TG 203) used for regulatory submission of fragrances ingredients were used to verify the predictivity of the outcome of QSAR models (ECOSAR and iSafeRat) and the results from the Fish Embryo Test (OECD TG 236). The simplified assays based on the OECD TG 236 were steered in > 30 fragrance ingredients belonging to several chemical classes and with a wide physico-chemical profile. In the case of volatile substances precautions were taken to evade losses and cross contamination. The 96 hours LC50 were attained from the simplified assays (8 concentrations, 15 embryos per experimental condition, 5 embryos per well in 24 well-plates). The 96 hours LC50 were based on lethality pointers. When comparing the results from both embryotoxicity tests (the mini-assay and OECD TG 236), it was clear that the toxicity tendencies were similar, with an average factor of 2 difference in the 96-hour LC50 values. Most cases, the OECD TG 236 was more conservative. There was slight difference between the zebrafish juveniles LC50 values (OECD TG 203) based on measured and nominal concentrations, reflecting that the exposure concentrations had been well maintained, except for the substances with the uppermost vapour pressure. This had also an impact on the embryotoxicity simplified assays where the toxicity was largely underestimated (Vp from 10 to 400 Pa). The juvenile fish toxicity predictions by ECOSAR are more conservative than the predictions by iSafeRat, while the estimations of the embryotoxicity was under-estimated in 1/3 of the cases, especially when it comes to very volatile substances. Whether this is also related to a particular chemical class or Mechanism of Action is currently being explored. After the complete scrutiny of this unique dataset, we aim at finding a recommendation for an appropriate approach for reliably screening new chemicals for their fish acute toxicity and potentially to use that information in a regulatory context.

5.05.P-Tu-169 Development of a Pilot Database of Chemical Characterization Information for Substances of Unknown or Variable Composition, Complex Reaction Products or Biological Materials (UVCBs)

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UVCBs pose challenges to risk assessors, registrants, scientists and regulators. To address these challenges, the tripartite UVCB Committee of the Health and Environmental Sciences Institute (HESI), has been developing an exposure-based, tiered approach for UVCB prioritization and risk assessment. The first step of this approach (aka Tier 0) is highly dependent on the availability of baseline chemical characterization data or “Tier 0 information” that can be used to prioritize UVCBs and streamline their safety evaluation. Tier 0 information includes exposure, compositional, and hazard data such as use profiles, production volumes, physicochemical properties, and (eco)toxicological data. Despite the prevalence of UVCBs in the chemical space, there is currently no public-facing database of relevant chemical characterization data for these substances. Thus, there is an opportunity to develop a database addressing this need.

To assess feasibility and develop robust and efficient methodologies for data collation, a subgroup of HESI’s UVCB committee is developing a pilot database of Tier 0 information for UVCBs. For ~100 commercially relevant UVCBs, data were collected from scientific publications, databases, and reports. This information includes substance identifiers (i.e., CAS Registry Numbers, EC Numbers, names), structural descriptors, registration data, physicochemical properties, use and exposure profiles, and hazard data for whole substances and representative constituents. Methods and challenges encountered have been documented to allow for reproducibility and improvement in future data collection efforts. Steps to automate the data mining processes are being investigated to aid in database maintenance and future expansion efforts. This effort demonstrates the feasibility of developing a database of information for UVCBs and highlights associated challenges, including with scaling up the number of included substances. It also drives forward the implementation of a UVCB risk assessment framework, providing information that can be utilized by registrants, risk assessors, and regulators for prioritization, risk assessment, and read across. Ideally, obtaining buy-in from industrial and regulatory partners will be key to facilitate the data collection process, and ultimately, realize of the full potential of such a database. *The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the US EPA.*

5.05.P-Tu-171 Applying the Principles of Grouping and Read-Across to Different Lines of Evidence to Support the Development of an Ecotoxicity Testing Strategy for Hydrocarbon UVCBs

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Substance grouping and read-across are commonly employed to reduce the amount of testing required for chemical registrations under the EU REACH regulation (EC 1907/2006). These approaches use relevant information from analogous (‘source') substances to predict the properties of ‘target' substances. While available guidance tends to be tailored for mono-constituent substances, similar concepts can be applied for ecotoxicity testing of more complex UVCB substances (substances of Unknown, Variable composition, Complex reaction products, or Biological origin). In the present effort, we apply multiple lines of evidence, aligned with read-across principles, to design a testing program for hydrocarbon UVCBs. Petroleum substances (PS) are comprised complex combinations of hydrocarbons for which the precise identity of all constituents is usually unknown, and the composition is often variable. Despite these uncertainties, PS may be categorized according to their manufacturing process and their broadly similar physical-chemical properties. Using a comprehensive GCxGC analytical program, the composition of each hydrocarbon UVCB substance can be broken down into relative concentrations of groups defined by carbon number and chemical class, termed hydrocarbon blocks (HCB). Using non-polar narcosis as the common primary mechanism of toxic action for hydrocarbons, toxicity would be correlated with bioavailability. It is then possible to use representative constituents for each of the relevant hydrocarbon blocks to estimate the toxicity of the whole substance. For the testing strategy, we determine the “worst-case”, or most toxic, sample using two independent methods: (i) an experimental biomimetic extraction-solid phase microextraction (BE-SPME) approach which mimics aqueous exposure to PS in the environment; and (ii) the PetroTox QSAR model that utilizes two sub models (i.e., target lipid model and an oil dissolution model) to estimate PS toxicity to ecological receptors. The outcomes of these two exercises allow for the selection of a “worst-case” PS sample for testing and account for the range in compositional variability across samples. The concepts and various lines of evidence applied here will support testing strategies for multiple PS categories and could inform chemical prioritization initiatives or other regulatory assessments of UVCBs.

5.05.P-Tu-172 Application of MOSH/MOAH GCxGC Methods to Support Bioaccumulation Testing of Hydrocarbon UVCBs in Fish

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The assessment of chemical bioaccumulation in fish is an important component of global chemical assessment and regulatory programs. Bioaccumulation assessments for petroleum substances remain difficult because (i) they are comprised of complex and variable combinations of hydrocarbons (i.e., UVCBs) and (ii) there are limited test data for the thousands of possible hydrocarbon constituents in petroleum substances, due to limited commercial availability of the individual constituents and their incompatibility with standard test systems. To deal with these issues, we are running whole substance OECD 305 dietary bioaccumulation test paired with supporting two-dimensional gas chromatography (GC×GC) analysis to analytically track individual constituents, or groups of constituents, over time. This approach of generating constituent-level information in a whole substance test design has been successfully applied in seawater biodegradation tests. However, the increasing complexity of biological sample matrices (e.g., due to lipids and other biogenic compounds) can present challenges for adequate peak separation and identification. In the present study we aim to optimize and test analytical methods needed to To improve peak separation and recovery, fish samples were subject to alkaline digestion and epoxidation steps to remove residual lipids and polyenes which thereby reduced analyte interference and carryover between chromatogram runs. Addition of an HPLC pre-fractionation step to separate saturated (MOSH) and aromatic (MOAH) hydrocarbon fractions also greatly reduced peak overlap and interference. Preliminary analyses revealed the presence of MOSH contaminants in control fish, possibly from the prepared fish food or sample homogenization procedures. Food-derived oils may naturally contain MOSH/MOAH and are commonly added to fish feeds in bioaccumulation studies. Caution should therefore be taken in sample preparation to avoid MOSH/MOAH cross-contamination. Following a blank-correction, apparent detection of C10-C15 hydrocarbons in exposed trout was observed. These data are being used to optimize the sample processing and analytical methods so that additional analyses can be performed to determine the bioaccumulation and biotransformation behaviour of these compounds.

5.05.P-Tu-173 Develop a Community-Based Participatory Approach to Evaluate the Dietary Exposure of Per- and Polyfluoroalkyl Substances (PFAS) in an Underrepresented Community

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Due to the great concerns about the health risks of per- and polyfluoroalkyl substances (PFAS) and the presence of multiple sources, this project aims to develop a community-based participatory research (CBPR) approach for evaluating PFAS exposure in the summer of 2024. Specifically, the focus will be on non-volatile long-chain PFAS compounds, evaluating their presence through routine food consumption and water intake among residents in the underrepresented Central Savannah River Area (CSRA). We will recruit participants by establishing partnerships among the studied community (i.e., Richmond County in CSRA), the scientific researchers, the outreach team, and the regulatory agencies. A family-based survey tool will be developed to quantify food consumption information and collect food items and drinking

water for PFAS measurement. Utilizing probabilistic exposure modeling, specifically Monte Carlo Simulation, we will calculate community-based daily PFAS intake rates and compare them to recommended safety thresholds. This comprehensive approach aims to generate a better understanding of PFAS exposure within the studied community. This study will promote collaborations among scientific researchers, local communities, stakeholders, and regulatory agencies by employing a CBPR approach. Doing so fills critical knowledge gaps about PFAS exposure in high-risk, underserved areas such as the CSRA. Anticipated outcomes include an enhanced understanding of PFAS exposure from various sources and pathways, leading to increased awareness of geospatial variations that help identify susceptible populations and areas. This study highlights environmental justice concerns and contributes to risk management and decision-making processes. An accurate exposure modeling and risk assessment can pinpoint specific environmental issues, offer detailed health advisories, and ultimately promote environmental and human health. This project will also provide students with valuable research opportunities on contaminants of emerging concern (CECs) and help students understand environmental issues, develop critical thinking abilities, and apply problem-solving skills.

5.05.T Bridging the Gap Between Science Development and Policy, Regulatory, and Technology (PRT) Needs for Complex Substances - Supporting Data-Driven Decision-Making in Heath & Environmental Risk Assessment and Management

5.05.T-01 Tripartite Perspectives on Challenges and Opportunities for the Testing and Assessment of Substances of Unknown or Variable Composition, Complex Reaction Products, or Biological Materials (UVCBs)

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Complex substances such as multi-constituent substances and UVCBs usually result from either industrial processing, natural substances, or chemical reactions, and make up approximately 20-25% of chemicals registered globally (e.g., under TSCA or REACH). Because of the variable and complex nature of source materials, and variability inherent to production processes, these substances can contain many, sometimes uncharacterized, constituents whose concentrations may vary between batches. To identify and advance the various challenges associated with UVCB testing and assessment, the Health and Environmental Sciences Institute (HESI) organized an international workshop *Exploring the complexities of UVCB testing and risk assessment* that took place on September 18th and 19th, 2023, in Reykjavik, Iceland. The 25 workshop participants represented academia, governments and regulatory agencies, as well as, the private sector, and originated from Belgium, Canada, Denmark, France, Germany, the Netherlands, Norway, the United Kingdom, and the United States of America. The HESI UVCB workshop aimed at initiating multi-sectoral, tripartite discussions on the advantages and disadvantages of whole substance vs. representative constituent testing and assessment, and how

to best combine both approaches. Workshop attendees also identified further research needs, and attempted to establish potential consensus solutions for environmental risk assessment.
We will present the insight from the workshop, which contributed to reinforcing the exposure-centric tiered approach previously developed by the Committee for the environmental risk assessment of UVCBs and multi-constituent substances. The case-study based discussions held during the workshop highlighted critical questions that ultimately helped provide some guidance to strategically test different aspects of whole UVCB substances and their representative constituents. Altogether the discussions and case-studies from the workshop generated information that will serve as a foundation to robust risk assessments.

5.05.T-02 EU's PMT/vPvM Framework: Prioritization, Regulation, and Industry Responsibility

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The prioritization process of chemical substances under the classification of Persistent, Mobile, and Toxic (PMT/vPvM) in the European Union (EU) has significant implications for safeguarding drinking water resources from contamination. The European Commission's Delegated Regulation, amending the CLP Regulation, introduces new hazard classes and criteria for the classification, labelling, and packaging of substances and mixtures. A framework has been developed to streamline the prioritization of existing PMT/vPvM substances, necessitating immediate attention from REACH registrants, regulators, researchers, and the water sector. This framework, informed by stakeholder engagement, encompasses five prioritization categories: PMT/vPvM hazard, REACH emission likelihood, analytical and monitoring gaps, remediation gaps, and exposure levels. Selection criteria include adherence to PMT/vPvM criteria, identification of data gaps, presence in drinking water resources, and detection methods. From the chemical industry, expectations arise regarding responsible practices, compliance with prioritization and classification criteria, transparent reporting, and addressing data gaps related to hazard assessments. Active participation in monitoring campaigns, support for laboratory investigations, and responsiveness to remediation efforts are also crucial. Overall, the chemical industry is expected to play a proactive and responsible role, aligning with the shared goal of safeguarding drinking water resources against contamination. The presentation will describe the regulatory landscape designed by the European Commission's amendments to the CLP Regulation, specifically targeting PMT/vPvM substances. It will meticulously navigate through the prioritization and classification procedures, shedding light on the pivotal tests and endpoints crucial for hazard assessment, while also addressing the inherent challenges in conducting such tests. Moreover, it will underscore the impacts of test outcomes on the chemical industry, using a case study to illustrate how initial inclusion of a substance in the PMT list can shift upon further persistency data analysis, highlighting the importance of adhering to criteria, transparent reporting, and active engagement in monitoring and remediation endeavors to uphold the integrity of drinking water resources.

5.05.T-03 A Revised Strategy for Monitoring to Inform Management of Emerging Contaminants in San Francisco Bay

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Tens of thousands of chemicals and mixtures of chemicals have been registered for production and use worldwide. Many may be considered contaminants of emerging concern (CECs), broadly defined as chemicals that are un- or under-regulated, not commonly monitored in environmental media, and have the potential to enter the environment and cause adverse ecological or human health impacts. Limited resources and frequent lack of occurrence, persistence, and/or toxicity data to establish exposure and risk thresholds that protect the beneficial uses of aquatic ecosystems make prioritization of CECs for monitoring and management challenging. In addition, lack of information on sources or uses of CECs often limits the ability of policymakers to identify and prioritize control measures that effectively prevent pollution. The Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) is an innovative collaboration between regulatory agencies, the regulated community, and scientists working to identify and inform priority questions faced by Bay water quality managers. The RMP has monitored CECs since 2001. Thanks to the region's sustained focus, San Francisco Bay is one of the most well-studied aquatic ecosystems for CECs, and has become a hub of CECs science and management. The RMP has developed a multi-faceted strategy for cost-effective CEC identification and monitoring prioritization to support management efforts and minimize impacts to the Bay. First, for CECs known to occur in the Bay, the RMP establishes priorities using a tiered risk-based framework that guides future monitoring studies for these contaminants. Findings from these studies, in turn, provide key data to update evaluations of potential risk for each contaminant or class. Second, the RMP focuses on monitoring and modeling in contaminant pathways, such as municipal wastewater effluent and urban stormwater runoff, to inform source identification and control strategies. Third, the RMP uses novel approaches, including non-targeted analysis and new approach methodologies for hazard assessment, to identify additional CECs to examine. Finally, RMP staff review findings from other regions to identify new CECs for which no Bay occurrence data yet exist. This strategy has been newly revised to best support stakeholder needs, and may act as a case study on successful design and prioritization of CECs science to inform management.

5.05.T-04 Assessing Chemical Pollution in Surface Waters Arising from Human Consumption: HydroFATE as a User-Friendly Tool

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Chemical contaminants in waterbodies pose significant risks to both environmental and human health. In addition to extensive monitoring not being feasible worldwide, existing models for assessing contamination risk often require technical expertise and high computing power, limiting their accessibility and applicability. We address this challenge by introducing HydroFATE, a global contaminant fate model that we redesigned to serve as an easy-to-use tool to screen for chemical contamination risks arising from human consumption in surface waters globally. By leveraging HydroFATE, stakeholders such as the chemical/pharmaceutical industry, wastewater treatment facility operators, health authorities, policy makers, and environmental consultants can efficiently prioritize monitoring, regulatory compliance, and management efforts and strategies. HydroFATE is user-friendly, fast, and scientifically robust. Initial applications of HydroFATE have demonstrated its efficacy, with recent peer-reviewed studies showcasing its ability to identify antibiotic pollution. Furthermore, ongoing efforts to expand the model to cover various contaminants aim to enhance its utility and impact. By promoting collaboration with government agencies, non-profit organizations, and industry stakeholders, we aim to advance the adoption of HydroFATE for informed decision-making and policy development, ultimately contributing to the preservation of global water quality.

5.05.T-05 Continued Development of Screening Level Models for Metals and Organics in Rivers

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Screening-level models have been applied to generalized environmental settings (i.e., “unit worlds”) to develop integrated rankings of chemical hazard by assessment of critical chemical loads. The Tableau Input Coupled Kinetics Equilibrium Transport Unit World Model (TICKET-UWM) was developed originally as a screening-level model for a generalized flow through lake environment with a water column and a sediment. The model simulates both organics and metals. For metals, TICKET-UWM simulates speciation and bioavailability using WHAM and the Biotic Ligand Model (BLM), which play integral roles in metal risk assessments and in criteria development/application both in Europe and the United States. Further TICKET-UWM development has allowed it to assess risk on a site-specific basis or for set of prototypical site types under various loading scenarios/time scales by considering the unique transport and chemical features of the waterbody. Recent model development work has focused on leveraging the model’s comprehensive treatment of metal speciation to assess metal transport and fate in flow water systems such as rivers, streams, and canals. A 2-dimensional model, TICKET-2D, was developed as an extension of TICKET-UWM to specifically consider metal transport, fate, and effects in advection-dominated (i.e., riverine) systems. This flexible tool was used to quantify copper concentrations in the water column and sediment resulting from release from sheet pilings in a canal. Most recently, a new module for the TICKET-UWM has been developed that focuses on quantifying concentrations in rivers and streams downstream of a point source discharge containing metals and/or organic chemicals. The module allows the user to quantify concentrations in the near field (prior to complete lateral mixing) and in the far field. The model includes a river and stream database that allows for easy specification of the receiving water physical characteristics (flow, mixing parameters, depth). It also includes a water quality database to facilitate assessment of speciation and bioavailability in the receiving water. The module can be applied for screening level assessments for effluent discharges from a variety of industrial, mining, and oil and gas production processes.

5.05.T-06 Target Lipid Model Update and Proposed Refinement of HC5 Calculation Procedure

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For more than two decades, the Target Lipid Model (TLM) has been used to estimate the aquatic toxicity of hydrocarbons and other non-ionic organic chemicals across a wide range of organisms and is the fundamental model used to predict the toxicity of petroleum products. The TLM has also been applied to derive hazard concentrations intended to protect 95% of aquatic species (i.e. HC_5). Given the growing body of aquatic toxicity studies in recent years, there is an opportunity to update the acute and chronic aquatic toxicity database used for TLM calibration/validation and re-evaluate aspects of the original model formulation. The primary focus of the new modeling work was reconsideration of octanol as the target lipid surrogate. A total of eight different partitioning models were considered: log K_{ow} estimated by EpiSuite, via SPARC, Goss (2005), and Abraham & Acree (2010); membrane-lipid water by Endo (2011), storage lipid-water by Geisler (2012); various lipid-water by Kuo (2013); and the poly-parameter Target Site Model (pp-TSM) by Kipka (2009) and Boone (2019). Each model was evaluated utilizing an updated database of critically evaluated and reliable single-chemical aquatic toxicity data for acute endpoints. The ability of each partitioning model to predict acute effects was evaluated using goodness-of-fit descriptions such as root-mean square log error and prediction bias. Previously, the HC_5 was calculated using a statistical extrapolation procedure first presented in McGrath (2004). This approach requires that the underlying acute critical target lipid body burden (CTLBB) and Acute-to-Chronic ratio (ACR) data both conform to a normal or log-normal distribution. Based on the new updated database obtained in this work, TLM derived acute CTLBBs are log-normally distributed, however, ACR data failed log normality tests. To overcome the distribution constraints imposed with this HC_5 calculation procedure an alternative approach is proposed. This method relies on boot-strapped error estimation of the TLM calibration and a convoluted distribution analysis of the CTLBBs and ACRs to yield a model for HC_5 estimation which is protective of 95% of species based on comparison to measured chronic data. The pp-TSM has been selected as the preferred target lipid surrogate. This approach has the advantage that it is free from distributional assumptions, is easy to use and can be readily applied in future updates as additional aquatic toxicity data become available.

5.06.P-Mo Bridging the Gap from Risk Assessment to Risk Management

5.06.P-Mo-136 Risk Based Volume Delineation for Remediation

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It is customary to have either default regulatory cleanup goals or risk-based goals applied for consideration in remediation planning. However, application of these goals on a “point by point” basis can result in unnecessary volume removal and associated costs since cleanup volumes and subsequent residual levels at a Site can be calculated using a risk-based alternative approach. A risk-based volume delineation considers what residual levels for “risk drivers” can remain at a Site following cleanup such that a level of No Significant Risk can be achieved and demonstrated. This type of protective, end-game oriented, approach can be used in regulatory programs that permit “risk-based” closure including state programs such as in Massachusetts, Connecticut, or Pennsylvania, and Federal Programs including USEPA CERCLA, RCRA or TSCA. In this case study, we will explore the specifics of: selecting cleanup levels for the constituents of concern; characterizing and classifying remedial “areas;” identifying which locations (and depths) in a remedial area required removal to achieve an acceptable (residual) exposure point concentration; and ultimately, calculating the associated, required volume to attain those objectives. For this case study, the residual EPC calculations were based on 95% UCL of the mean and relied on use of ProUCL and other statistical methods to evaluate data distributions and appropriate upper bound EPCs. The application of this risk-based volume delineation resulted in roughly a 30-40% percent reduction in volume compared to a “point-by point” approach, and increased sustainability by decreasing the need for additional trucking and disposal of generated remediation waste. Additionally, the costs for the proposed remedy were reduced by roughly 40% since a significant portion of the remediation costs are driven by transport and disposal.

5.06.P-Mo-138 Don't Over-Estimate PFAS - Importance of Appropriate Use of Dermal Absorption Factors

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The lack of an appropriate model for assessing per- and polyfluoroalkyl substances (PFAS) dermal absorption (ABS_d) directly impacts toxicity and risk assessment in several significant ways, including health guidelines and regulations, environmental and occupational exposure, and consumer product safety. Addressing the lack of an appropriate model for PFAS ABS_d is crucial for improving toxicity assessments and ultimately protecting public health. This requires continued research efforts to develop more accurate and reliable methods for evaluating dermal absorption and incorporating these findings into risk assessment and regulatory decision-making processes. Dermal exposure to chemicals is assessed differently depending on whether that exposure is applied to skin as a solid or in liquid. In accordance with USEPA guidance, a chemical in water can be eliminated from the residential exposure pathway (i.e., tap water ingestion and showering/bathing) screening process as contributing significantly to the risk for the dermal route if the chemical contributes less than 10 percent of the exposure derived from the oral pathway. For non-residential water exposures (i.e., swimming or wading), organic chemicals with log K_{ow} (octanol–water partition coefficient) and molecular weights outside of the effective predictive domain (EPD) would decrease the overall amount of chemical available for absorption after the exposure event. The log K_{ow} values used by USEPA to calculate the EPD for PFAS in liquids (i.e., measure of dermal absorption) sometimes uses the predictive range and other times uses surrogates to determine if a PFAS compound is within or outside the EPD. However, it does not seem to follow a consistent process, nor does it employ the first principles approach from the Soil Screening Guidance of relying on measured values when they exist. This value is important for the swimming/wading exposure scenario because the EPD establishes the applicability of the dermal exposure route. This presentation is intended to provide an overview of the importance of using appropriate PFAS ABS_d values and how using the range of available inputs may underestimate or overestimate actual exposure levels. Dermal exposure screening levels developed using USEPA dermal absorption assumptions listed in the Regional Screening Level chemical specific parameters table for PFAS can provide a perspective but may not be appropriate in all evaluations.

5.06.P-Mo-139 ToxiRiskOptimizer: Automating Endpoint Value Calculations for Comprehensive Early-Stage Ecotoxic Screening

Yang Li, *Melissa Harris, Steve Hicks, Gyan Harwood and Tamara Lunsman, Corteva Agriscience*

Early-stage discovery of plant protection products (PPP) requires high-throughput toxicity screening of 1,000s of compounds annually. Our study introduces a model-as-a-service framework which enables the analysis of data from high-throughput assays in early-stage discovery process. This approach aids in de-risking the PPP development pipeline, ensuring that resources are effectively allocated towards compounds with the most promising safety profiles. Here, we automated the calculation of endpoint values for three high-throughput assays -

including an EC50 for acute *Daphnia magna,* and LD50s for acute contact and oral honey bees. Automating the data acquisition process enabled rapid screening and informed decision-making, dramatically increasing data throughput and process efficiency. Incorporating model averaging techniques alongside advanced analytical methodologies - such as decision trees, statistical models, and dose-response analyses provided accurate estimate of EC50 and LD50 values across a variety of assays and paved the way for use in risk assessments with little turnaround time for 1,000 of compounds annually. We validated this approach against a dataset of over 380 compounds to demonstrate the framework's robustness. This methodology optimizes the calculation of endpoint values for use in early-stage risk assessment during the development of new PPPs, ensuring that only the most environmentally sustainable compounds progress to comprehensive and time-intensive Good Laboratory Practice studies.

5.07.P-Th Evolving Safety Assessments of Biological-Based Crop Protection Products: Progress of the OECD's Expert Group Biopesticides

5.07.P-Th-063 Adaptation of the *Daphnia magna* Reproduction Test: Experience Gained from Testing Bacterial Biocontrol Agents

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In the context of aquatic risk assessment of microbial pesticides, ecotoxicity tests with the water flea *Daphnia magna* are frequently submitted to provide information on potential toxicity, infectiveness and pathogenicity of the Microbial Pest Control Agent (MPCA). However, there are some general considerations to take into account when testing microbial pesticides. Since MPCAs behave as particles that do not dissolve in test solutions, testing at high microbial densities (e.g., at the Maximum Hazard Concentration according to tiered testing approaches) can lead to elevated turbidity in the test vessels, often in connection with physical effects (e.g., by interfering with the daphnids' filtering system) and water quality changes (e.g., drop of oxygen). Actual exposure in test suspensions can be variable and may depend on whether the MPCA remains suspended, or settles to the bottom of the test vessel. Given the lack of appropriate test guidelines or guidance, study results from microbial ecotoxicity tests are often difficult to interpret or they lack consistency. In the current study we have performed *Daphnia* tests with bacterial biocontrol agents approved as microbial active substances in the EU. Pre-tests focused on preparation of stable suspensions, visual observations and plate counting methods. Acute and chronic *Daphnia* tests were performed on the basis of Organisation for Economic Co-operation and Development (OECD) Test Guidelines with necessary adaptations for MPCAs such as inclusion of non-infectious controls (attenuated controls) along with sterile filtrate controls. The aim of the study was to obtain a better understanding of the cause of effects in *Daphnia* tests and to provide insight into potential improvements for microbial testing that could be considered in the development of test guidelines and guidance documents.

5.07.P-Th-064 Considerations for Analytical Verification of Microbial Products in Different Test Systems

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As interest and demand grow for microbial crop protection products worldwide, there is a growing need for proper risk assessment and guideline-based testing to gauge potential environmental impacts. Most robust studies conducted under either OECD and/or OCSPP guidelines call for verification of dosing and assessment of stability of the test item in the test system. Conventional chemicals can be assessed with traditional chemistry methods, however, microbes require a different approach, whether through plating or molecular methods. Much like conventional chemical analysis, different test systems and matrices pose unique challenges when quantifying the test item. The purpose of this work is to investigate and identify current and future considerations for plating and conducting CFU enumeration of microbial test materials in various test matrices. Examples of test designs and their corresponding matrices that will be evaluated include: freshwater (used in Daphina magna Life cycle toxicity and pathogenicity test, OECD 211), avian diet (used in avian oral pathogenicity and toxicity test, OPPTS 885.4050), honeybee diet (used in honey bee dietary pathogenicity and toxicity test OCSPP 885.4380), artificial soil (used in earthworm toxicity testing, OCED 207 and 222), as well as artificial algal media (used in algal toxicity test, OECD 201). Considerations that will be addressed in this work will include but are not limited to: sterility of the test matrix, feasibility of test matrix analysis, and utilization of abiotic replicates. By identifying and addressing potential issues with plating various matrices, we can produce reliable CFU enumeration data and properly assess risk in this growing field of products.

5.07.T Evolving Safety Assessments of Biological-Based Crop Protection Products: Progress of the OECD's Expert Group Biopesticides

5.07.T-01 Importance of Biopesticides in Modern Agriculture and the Role of Natural Products

***Charles Cantrell**, United States Department of Agriculture, Agricultural Research Service*

The biopesticide (biocontrol) market was valued at US 4.9 billion in 2022 and is expected to grow by 15% from 2023 to 2029 reaching a value of 15 billion. This significant growth is due in part to a rise in concern by consumers about synthetic pesticide hazards to the human body and to the environment as well as a rising incidence of pest resistance to conventional pesticides, among other reasons. Biopesticides often provide pest specific solutions that avoid unnecessary harm to non-target species. Biopesticides have also evolved alongside pests which necessitates environmental pathways available for rapid degradation resulting in shorter environmental half-lives. This talk will explore the traditional usage of natural product as pesticides in the 19th and early 20th centuries, the transition to conventional synthetic derived pesticides, and finally the current shift back to more natural product-based solutions for pest management using both microbial biopesticides and biochemical biopesticides. The role that natural products play in this transition and the impact they continue to have on pesticide discovery will be discussed.

5.07.T-02 Progress of the OECD Expert Group on Biopesticides in Identifying and Addressing the Testing and Risk Assessment Challenges of Biological-Based Crop Protection Products

***Shannon Borges**[1] and Lisa S Ortego[2], (1)Environmental Protection Agency, (2)Bayer CropScience*

The interest and use of biological materials in crop production is increasing globally at a rapid pace, experiencing a 15% cumulative annual growth rate. Part of the interest is that these technologies are viewed as safer alternatives to conventional chemicals. The Organization for Economic Co-operation and Development (OECD) Expert Group Biopesticides (EGBP) was established by the Working Party on Pesticides in part to harmonize methods and approaches used to assess biological pesticides. Each year, the EGBP hosts a day-long seminar highlighting a topic that presents challenges in the testing and assessment of biopesticides. In 2022, the EGBP held a conference on Innovating Microbial Pesticide Testing to focus specifically on these issues. Based on the outcomes of the seminars and the conference, the EGBP has begun an intensive workplan to investigate the challenges and improve the testing and assessment of this unique category of plant protection products. The following are the focus areas for the EGBP. 1. Develop guidance document on best practices for ecological testing 2. Revise bee testing guidance and organize a ring-test to optimize pathogenicity evaluation 3. Adapt US EPA mammalian toxicity/pathogenicity guidelines to an OECD guideline 4. Explore new approach methodologies (NAMs) for sensitization and adverse outcome pathway screening tools and their applicability to biologicals 5. Develop guidance document to determine when an *in vivo* test is needed 6. Revise aquatic invertebrate guidance to address technical issues related to particulate test substances, attenuated controls, dose confirmation 7. Develop a list of secondary metabolites of concern 8. Develop a list of non-target insect species amenable to laboratory testing. The work of the EGBP involves a multi-stakeholder approach including participants from government, academia, non-governmental organizations, and the business sector providing a comprehensive set of experiences to tackle the issues in this area. The origin, objectives and activities of the EGBP will be presented along with the EGBP seminar program. The focus of the presentation will be the rationale for the key focus areas, the approaches taken to advance the science and the progress to date.

5.07.T-03 Re-evaluating Pathogenicity and Infectivity for MCPA's in Ecotoxicity Testing

Henry O Krueger[1] *and Jeffrey C Wolf*[2]*, (1)Eurofins Agroscience Services, Easton, (2)EPL*

The USEPA's ecotoxicity test guidelines for microbial pest control agents (MCPA's) were released in 1996 and largely based on testing of conventional chemicals. There has been a large increase in the registration of MCPA's over time because they are considered to have reduced risk and are effective at targeting pest species with low risk to nontarget species. There is worldwide recognition that MCPA guidelines need to be improved and updated based on decades of work, the increasing number of products being registered and the benefit they can provide. Initiatives are currently underway in the Europe EU, the US and Canada to update and improve guidelines for the evaluation of risk. There are some marked differences in testing MCPA's compared to conventional chemicals. Ecotoxicity testing with conventional chemicals look for toxic effects on survival, growth, and reproduction, while MCPA's add infectivity and pathogenicity as endpoints. Pathogenicity can be simply defined as the potential for a microbe to cause disease, while infectivity refers to the capability of a disease-causing microorganism to infect a host. Clear definitions and standardization of the methods for pathogenicity and infectivity need to be developed. One way to preliminarily screen for potential pathogenicity is to assess taxonomic relatedness and bioinformatics (gene sequencing) to predetermine any potential for pathogenicity and infectivity. Such data can be used during problem formulation to help determine what types of tests need to be conducted and which ones are not relevant. Such an approach is analogous to using Quantitative Structure-Activity Relationships (QSAR's) and read-across to predict how conventional chemicals will react in the environment or within organisms, but in this case, we are using taxonomy and genetics instead of molecular structures to predict outcomes and possibly waive studies based on scientific rational. GMO's have used such techniques in the past (e.g., toxicity, allergenicity). There is typically a lot of information on known pathogens, especially for bees, birds, and fish. By comparing how closely a microbe is related to known pathogens can be extremely useful information in building a weight of evidence in determining the potential for toxicity or pathogenicity. Potential activity can be further confirmed and refined by comparing sequences of whole organism or specific traits between MCPA's and known closely related pathogens.

5.07.T-04 Review of Non-Target Insect (Honey Bees) Test Methods for Assessing Effects of Microbial Pesticides; Methods What Works and What Are the Challenges

Michael Patnaude[1]*, Ashlee Kirkwood*[2] *and Arthur Putt*[3]*, (1)Smithers, (2)Ecotoxicology, Smithers, (3)Smithers - Environmental Risk Sciences, United States*

With the increasing growth of developing and producing microbial pesticides, fit-for-purpose test methods are needed for review and assessment of these products, particularly with non-target organisms such as honey bees. Microbial pesticide evaluation presents a challenge for testing as the microorganisms are diverse in type (i.e., bacteria, fungi, protozoans, viruses), grow and thrive under different conditions than those of the non-target test organisms, and they are not soluble in the typical sense compared to conventional pesticides. Test methods exist for honey bee (*Apis mellifera*), Pink-Spotted Lady Beetle (*Coleomegilla maculata*), and Green Lacewing (*Chrysoperla carnea or Chrysoperla rufilabris*). These existing test methods or guidance documents are outdated, lacking technical detail, not sufficient in duration or clear on route of exposure of the products, and not appropriate to determine endpoints which evaluate infectivity and pathogenicity to honeybees. Most, if not all, of the current methods are designed to address and evaluate conventional pesticides. We review existing honey bee test methods and guidelines and highlight the benefits and weaknesses of these existing methods and how methods can be improved to address many of the challenges and align the testing/results with risk assessment for these novel products. The updated methodologies focus on optimized test conditions and diet to extend test duration, methods of dosing diets, confirmation of exposure to microbes, test designs, addition of attenuated control groups, biological observations, and endpoints collected during the studies. The progress and results of these updates for testing of microbial products will be presented, along with challenges that will need to be addressed in future work.

5.07.T-05 Difficulties in Effective Microbial-Based Product Testing on *Apis mellifera (L.)* in the Laboratory

Joshua Zuber[1]*, Daniel R Schmehl*[2] *and Lisa S Ortego*[2]*, (1)Bayer AG, Research Triangle Park, United States, (2)Bayer CropScience*

The increased interest in the production of microbial-based plant protection products has resulted in the necessity for novel risk assessment protocols to observe potential effects. Microbial pesticides are required to be evaluated for pathogenicity, which requires an extended observation from the standard honey bee 10-day test (OECD 245). The current EPA guideline for assessing pathogenicity of a microbial pesticide (OCSPP 885.4380) requires a 30-day observation to properly evaluate safety, but this test duration is typically difficult to achieve with adult honey bees in laboratory bioassay cages due to control performance. Furthermore, homogeneity in test diets is difficult to achieve due to the physical properties of a microbial test substance. Larval testing with A. mellifera (L.) also presents challenges due to the innate anti-microbial activity of royal jelly that is used to create the diet used the testing, which can inhibit the active microbe in a diet assay and may result in a false negative. Here we present some of our recent experiences in developing novel experimental approaches for honey bees to overcome the challenges of microbial product testing. We have evaluated factors that may increase adult honey bee longevity

in the laboratory. Additionally, assessment based on genomic evaluation comparing microbial genetic sequences to known honey bee pathogens may be a method to overcome the technical barriers for evaluating the safety of a microbial product to honey bee larvae. It is also critical that we develop approaches for microbial products that can separate mortality from pathogenicity and infectivity. Our goal is to contribute to internationally standardized testing approaches to strengthen the effective evaluation for microbial-based products.

5.07.T-06 Improving Aquatic Toxicity Tests with MCPA - How, When, and at What Levels to Test

Henry O Krueger[1] *and Sarah McCoy*[2]*, (1)Eurofins Agroscience Services, Easton, (2)Eurofins Agroscience Services*

An environmental risk assessment is a function of hazard and exposure. There is currently a lack of modeling and studies to characterize an aquatic exposure via MCPAs, which are vastly different than conventional chemicals. Preliminary work for exposure assessments would be useful and informative when setting test levels. Current guidelines set upper limits of test concentrations in water at unrealistically high levels. These concentrations may be relevant for products that are directly sprayed to water for mosquito control or invasive aquatic plants, but are not realistic for products directly sprayed to fields, soil incorporated, in-furrow applications, or seed treatments. Testing such high concentrations in water can result in opaque test solutions and excessive particles suspended in water that can interfere in the feeding of invertebrates and clog gills in fish. Aquatic testing of conventional chemicals has well defined upper limits for testing; 100ppm or the limit of water solubility. Alternatively, MCPA's are often particles suspended in water and solubility limits are not applicable. Assessing particle distribution in water is more relevant; are they floating, sinking, or in suspension in the water column. Consideration of cell viability, dormancy and metabolite expression in water is also important. We suggest several shorter and less expensive trials to address some of these questions. Preliminary testing more appropriate for MCPA's would be; distribution of particles in the water column, cell viability and expression, as well as effects of light, temperature, pH and salinity. These types of preliminary trials can be useful tools for better understanding exposure that then can be used to design better aquatic tests. The information is also useful in providing data that can be used to support waiver requests.

5.08.P-Mo Examining Causation in Risk Assessment, Site Management and Damage Assessments for Contaminated Sediment Sites

5.08.P-Mo-140 Comparison of Empirically and Mechanistically Derived Sediment Quality Guidelines for Use as Screening Levels in Risk Assessment

***Joy Mcgrath**, GHD*

Sediment quality guidelines (SQGs) are derived using empirical or mechanistic approaches. Empirically based guidelines are derived using databases of paired sediment chemistry and biological responses and relate sediment concentration to the frequency of an adverse response. Mechanistically based SQGs are derived by considering the inherent aqueous toxicity of the chemical to different biota coupled with site-specific sediment characteristics known to influence bioavailability such as organic carbon for organic constituents and pH and hardness for metals. Additionally, SQGs are derived to be either protective or predictive of adverse effects in benthic organisms. A critical review evaluating SQGs for use in screening-level risk assessments to identify sediments that may pose a risk to the benthic community was undertaken using polycyclic aromatic hydrocarbons (PAHs). Sediment PAHs can pose harm to the benthic community. Numerous SGQs for the protection of benthic life are available to assess the risk of individual PAHs and PAH mixtures in sediments. SQGs for PAHs were compiled and compared, and performance evaluated for predicting the presence or absence of toxicity using an extensive field data set. Recommendations for the use of SQGs in screening evaluations and enhancements to current approaches are discussed.

5.08.P-Mo-141 Pragmatic Approaches to Causality-Based Refined Screening at Legacy Contaminated Sediment Sites

*Phyllis Fuchsman[1], **Kyle Fetters**[2], Sydney Kruse[1] and Shuo Yu[1], (1)Ramboll, (2)Ramboll, Beachwood, United States*

The limitations of association-based sediment quality guidelines (SQGs) can pose challenges when assessing and managing legacy contaminated sediment sites, particularly when those limitations are not well recognized by stakeholders and regulators. Default SQGs can indicate an overall level of contamination (by chemical mixtures) that may be associated with toxicity in urban waterways, but the same guidelines are typically much less useful in predicting effects caused by specific contaminants at legacy industrial sites. As such, overprediction of sediment toxicity and misattribution of its causes are common. A wide range of tools is available for site-specific sediment assessments, but we have also found it useful to draw upon additional lines of evidence from the scientific literature to inform refined screening assessments and support causation analyses. Depending on the chemical of interest, lines of evidence relevant to understanding cause-effect, concentration-response relationships include spiked sediment toxicity test results, equilibrium partitioning analyses, and case studies of sites where the chemical of interest is the predominant sediment contaminant. We illustrate this approach with examples for several metals and organic chemicals, including arsenic, lead, mercury, zinc, PCBs, and DDT. Results of this type of structured and robust literature review and meta-analysis can help to focus sediment assessments on the most important chemicals of interest, provide context for site-specific analyses of chemical bioavailability and toxicity, and support development of sediment cleanup goals.

5.08.P-Mo-142 Using Sediment Toxicity Tests to Develop Remediation Goals for Polycyclic Aromatic Hydrocarbons

***Susan B Kane Driscoll**[1], Jason James[2], Sean Ryan[3] and Frank Dombrowski[4], (1)Exponent, (2)Exponent, (3)Exponent, (4)WEC Energy Group*

Polycyclic aromatic hydrocarbons (PAHs) are often contaminants of concern at sediment sites. Laboratory toxicity tests can be used to develop remediation goals for the protection of highly exposed and potentially sensitive ecological receptors such as benthic invertebrates. Data from 14 sites will be used to examine the relationship between the reponse of Hyalella azteca in laboratory toxicity tests to various measures of exposure, including concentrations of PAHs in bulk sediment, concentrations of PAHs in porewater measured with solid phase microextraction (SPME), and predictions of toxicity based ont eh U.S. Environmental Protection Agency's equlibrium partitioning sediment benchmarks (U.S. EPA 2003). Issues related to the development of remediation goals from exposure-response models, including treatment of outliers and the influence of non-aqueous phase liquid in the sediments will be discussed. An alternative approach to the development of remediation goals, the Receiver Operating Curve

(ROC) method, which focuses on minimizing the rate of false positives (samples classifed and toxic that are not toxic) and false negative (samples classified as non-toxic that are toxic) will also be discussed.

5.08.P-Mo-143 Guidelines for Selecting Bioaccumulation Models for Nonionic Organic Contaminants when Assessing Risk at Contaminated Sediment Sites

Robert M Burgess[1]*, Ashley N Parks*[2]*, Susan B Kane Driscoll*[3] *and Michael J Kravitz*[4]*,*
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Over the last 30 years, several models have been developed to predict bioaccumulation for assessing and managing the risks associated with contaminated sediment sites. These models include simple ratios of biota to sediment concentrations (i.e., biota-sediment accumulation factor (BSAF)), use of passive sampling polymers as a surrogate for organism lipid, and complex food web models that consider metabolic biotransformation and excretion as well as spatial and/or temporal changes. Bioaccumulation models have many applications including: (i) predicting concentrations of contaminants in organisms from concentrations in environmental media, (ii) developing site-specific conceptual models and risk assessments, (iii) back-calculating risk-based sediment remedial goals, and (iv) predicting past or future tissue concentrations before or after remediation. However, selecting which bioaccumulation model to use at a given site involves balancing several critical decision considerations including (1) investigation objectives such as which contaminants are being monitored and the nature of the decision being made, (2) the level of accuracy and precision needed for the modelled estimate(s), (3) the types of data available, and (4) resources available for applying a given model. The U.S. Environmental Protection Agency is developing guidelines that provide an overview of bioaccumulation models for nonionic organic contaminants and an approach for selecting the appropriate bioaccumulation model(s) for use at contaminated sediment sites. This presentation and the document it describes will assist site managers and environmental scientists to consistently select the optimum model(s) for bioaccumulation-related applications at their contaminated sediment sites.

5.08.P-Mo-145 Influence of Sediment Organic Matter on the Stabilization of Heavy Metals in Sediments Using Activated Carbon

Seoyeon Park[1] *and Yongju Choi*[2]*, (1)Seoul National University, Seoul, Korea, Republic of, (2)Seoul National University, Korea, Republic of (South)*

The accumulation of heavy metals in sediments can lead to potential problems, such as toxicity to plants, benthic organisms, and ecosystem imbalances. In-situ stabilization is a promising technology for the remediation of heavy metal-contaminated sediments, serving as a cost-effective and less disruptive alternative to conventional methods. For successful sediment amendment, it is crucial to demonstrate the adsorbents' capabilities for heavy metal adsorption in the presence of sediment organic matter. Sediment organics can influence adsorption in various ways—by blocking adsorbent pores, preventing adsorption, binding with heavy metals to form complexes, or serving as a bridge to enhance adsorption. This study explores how sediment organic matter affects the heavy metal adsorption properties of activated carbon, the most commonly used adsorbent in in-situ stabilization. Sediment samples from two distinct river environments—a forested lake and an urban industrial area—were analyzed. The organic matter in the water extract of these samples exhibited different properties: the forest lake organic matter

(FL-OM) had higher levels of humic substances, whereas the urban industrial organic matter (UI-OM) was richer in proteinaceous materials. Tests showed that FL-OM binds more effectively with heavy metals (especially Zn, Cd, and Pb) and activated carbon, enhancing adsorption capacity compared to UI-OM. This indicates that OM with humic properties acts as a more efficient bridge in the adsorption process than does proteinaceous OM, thus potentially improving the efficiency of the sediment remediation by activated carbon amendment. These findings emphasize the significance of the characteristics of sediment organic matter in remediation and its careful consideration in in-situ stabilization applications.

5.09.P-Mo General: Environmental Risk Assessment

5.09.P-Mo-147 Proof of Concept of a Combine Strategy for Endocrine Disruption Evaluation

Andrea Weiner, Ander Arbelaiz, Christoph Rummel, Mikel Queralt and ***Arantza Muriana****, BBD BioPhenix S.L. - Biobide, Spain*

In recent years there has been a collective awareness of the impact chemicals can have on the environment and the potential effects on the endocrine system. It has become relevant that numerous endocrine-related disorders are associated to the estrogen pathway. Endocrine-isrupting Compounds (EDCs) are frequently found in the environment and may have a profound impact on the development and important physiological processes including sexual development and reproduction, neurodevelopment, and immune system of vertebrate organisms, especially in aquatic models. The negative impact of these EDCs is becoming a public health issue. Therefore, the requirement for adequate test systems to assess the potential Endocrine-Disrupting (ED) risk of new chemicals whether they affect the thyroid and/or estrogen pathways as well before they are placed on the market should be of major relevance for humans and wildlife. The advantages of using zebrafish embryos as new alternative methods (NAMs) are vastly known, such as complying with the 3R Principle of Replacement, Reduction, and Refinement generating fewer ethical impediments. The EASZY assay is an OECD TG 250 designed to detect endocrine active chemicals acting as agonists through estrogen receptors (ERs), by inducing the expression of the green fluorescent protein (GFP). The EASZY assay allows the detection of estrogenic activity of chemicals on transgenic Tg(*cyp19a1b*:GFP). Complementary, Biobide has developed, validated, and published a fluorescence-based image assay for thyroid inhibition analysis of chemicals, proved to be applicable for the classification of EDCs, employing another transgenic zebrafish line (Tg(*tg*:mCherry)). We have evaluated how the reference compounds of the thyroid inhibition assay had agonist activity to the ER or vice-versa. To this end, we tested positive and negative compounds of both pathways to confirm their main mechanism of action. As positive reference compounds we tested Potassium perchlorate, Propylthiouracil, Benzophenone-2, Estradiol, Bisphenol A, and Norethindrone, while as negative reference compounds we tested Acetaminophen and Dexamethasone. Therefore, we can conclude the use of two zebrafish assays for detecting EDCs acting through different pathways is quintessential to obtain a comprehensive toxic potential of the EDCs.

5.09.P-Mo-148 Use of Site-Specific Bioaccumulation Factors and Models to Estimate Contaminant Levels in Insectivorous Wildlife Food

Vickie Reat*, WSP , Burnaby, TX, Canada*

A Screening Level Ecological Risk Assessment (SLERA), Baseline Ecological Risk Assessment

(BERA) Problem Formulation (PF), and BERA were prepared for a Superfund site to evaluate creek and soil contamination. Sediments in the creek contain elevated levels of polychlorinated biphenyls (PCBs), semi-volatile organic compounds (SVOCs), and metals resulting from historical industrial activities. The SLERA and BERA PF concluded that ecological risks were possible for wildlife that consume sediment invertebrates. The final BERA used site-specific bioaccumulation factors and equations, based on co-located emergent aquatic insect tissue and sediment concentration data, to better define exposure estimates for insectivorous wildlife species (e.g., tree swallow and little brown bat). These two wildlife species feed almost exclusively on flying insects, often foraging over water. The emergent aquatic insects were collected using various methods, including light traps, Malaise traps, and/or sweep nets from three exposure areas in the creek. Dominant insects in the tissue samples included damselflies, dragonflies, caddisflies, mosquitoes, and midges. Geometric mean biota-sediment accumulation factors for high molecular weight polycyclic aromatic hydrocarbons (PAHs), barium, copper, lead, selenium, and vanadium and a log-linear regression equation for total PCBs, were used in the BERA to model contaminant concentrations in the diet of the swallow and bat. The use of site-specific bioaccumulation models and equations substantially reduced estimated contaminant levels in wildlife food items compared with literature-based models, in some cases reducing hazard quotients by 44 to 99% and thereby improving the accuracy of exposure and risk estimates for wildlife that feed on emergent aquatic insects.

5.09.P-Mo-149 Determining the Exposure Point Concentration for Human and Ecological Exposure: From the Field to the Lab to the Desktop

Donna J Getty[1] *and Julie Rothrock*[2]*, (1)SRC, (2)SRC, Portland, ME*

Exposure point concentrations (EPCs) are applied in both human health and ecological risk assessments as an estimate of the concentration of a chemical in a site medium (e.g., soil, sediment, water) averaged over the area to which a receptor is exposed (the exposure area or exposure unit [EU]). Because the true arithmetic mean concentration cannot be calculated with certainty from a limited number of measurements, EPA recommends that an estimate of the 95 percent (%) upper confidence limit of the arithmetic mean (95UCL) be used as the EPC for most chemicals for quantifying exposure and potential risk to human and ecological receptors. The approach to calculate a 95UCL depends on the number of data points available, the shape of the data distribution, and the degree of censoring (i.e., samples below the detection limit). Data collected within each EU need to be of sufficient quantity to capture likely maximum exposure concentrations and to develop EPCs based on the 95UCL that, with a reasonable degree of certainty, represent a high-end concentration that receptors will be exposed to in the EU. Data also need to be of sufficient quality to provide a reasonably accurate measure of concentrations of chemicals of potential concern (COPCs) or chemicals of potential ecological concern (COPECs) in environmental media to which human and/or ecological receptors will be exposed. The more accurate and precise the EPC is at representing the concentrations of contaminants in the EU, the less uncertainty associated with exposure and risk calculations, increasing their representativeness. Many factors, from field collection of environmental samples to sample processing in the laboratory and data evaluation in the office, influence the defensibility and uncertainty associated with EPCs derived for use in human health and ecological risk calculations. These include field sampling design (random versus biased), sampling methodology (e.g., discrete versus composite sampling, such as incremental sampling methodology [ISM]), laboratory sample processing methods (e.g., homogenization, sieving), achievable laboratory reporting limits and their relation to risk-based screening levels, and methods used to calculate 95UCLs (e.g., ProUCL software, ISM Calculator). This poster will examine the influence of these variables on the derivation of EPCs using site-specific data, as well as factors to consider when using the same EPCs for both human health and ecological risk assessment.

5.09.P-Mo-150 Reviewing Cyanide Aquatic Toxicity Data to Revise National Recommended Ambient Water Quality Criteria

***Annie Philip Jacob**[1], Amanda Jarvis[2] and Kathryn Gallagher[2], (1)U.S. Environmental Protection Agency, (2)U.S. Environmental Protection Agency*

The EPA is currently updating the cyanide aquatic life ambient water quality criteria to reflect the latest scientific knowledge. Cyanide is present in a wide variety of forms, and the form of cyanide present in the environment plays an important role in its fate and transport. In aquatic environments, cyanide is found in both free and complexed forms. Free cyanide is the most toxic form to aquatic life. The EPA has been reviewing the scientific literature published to date to identify relevant new information on cyanide chemistry and toxicity, which will form the basis of the revisions to the existing cyanide criteria. Data were obtained from published literature reporting acute and chronic exposures of cyanide that are associated with mortality, growth, and reproduction. This set of published literature was primarily identified using the ECOTOXicology database (ECOTOX) maintained by the U.S. EPA, Office of Research and Development. All studies are being evaluated for data quality as described by EPA Office of Water's (OW's) standard operating procedure. OW is completing Data Evaluation Record for each species from the cyanide studies identified by ECOTOX. This in-depth review will ensure the studies used to derive the criteria result in robust, scientifically defensible revised criteria for cyanide. This presentation will provide an overview of current toxicity literature evaluated for data quality to support the revision of cyanide ambient water quality criteria.

5.09.P-Mo-154 PFAS Concentrations in Aqueous Environmental Media Unrelated to Releases (Background Conditions)

***Nadia Podpora**[1], Samantha Townsend[2], Tamara R House-Knight[2] and Francis Ramacciotti[2], (1)Contaminated Site Assess & Remediation, GHD, (2)GHD*

Awareness of concentrations of per- and polyfluorinated alkyl substances (PFAS) in environmental media have been on the rise globally. In parallel with this awareness are multiple emerging regulations, criteria, screening levels, etc. related to PFAS concentrations in water that may need to be addressed (e.g., cleaned up) in accordance with regulatory environmental programs. One of the areas where more data has become available are research studies and environmental monitoring programs that are measuring, and detecting, PFAS in rainwater and surface water at concentrations that could exceed screening levels/criteria and are from areas where there are no known or suspected releases. Concentrations of PFAS in these media may greatly impact the evaluation of results from sites where there are also no (or limited) known or suspected releases. They could also hamper or confuse site characterization or delineation of plumes. Therefore, having a thorough understanding of background levels of PFAS, and thus unrelated to a particular site, could mean the difference between closure and cleanup. This work will present the results of a meta-analysis of concentrations of five select PFAS (perfluorooctanoic acid [PFOA], perfluorooctanesulfonic acid [PFOS], perfluorononanoic acid [PFNA], perfluorohexanesulfonic acid [PFHxS] and Hexafluoropropylene oxide-dimer acid [HFPO-DA or GenX chemicals]) in rainwater and surface water to quantify potential background conditions. Data are sourced from available research studies and environmental monitoring programs in the Great Lakes region. Results will be presented to illustrate the comprehensive statistics among these datasets. Comparisons of relevant statistics to the April 10, 2024 U.S. EPA maximum contaminant levels (MCLs) will also be completed to show whether or not these waters without a known or suspected release would exceed, and thus could by themselves show elevated background concentrations that could migrate onto a site. The implications these concentrations have on properly assessing background conditions, conducting PFAS sampling events and the evaluation of results in a contaminated site context will also be included in the discussion.

5.09.P-Mo-155 Per- and Polyfluoroalkyl Substances in Fish Tissues from Downstream Locations of a Department of Energy Site in New Mexico

Shannon Marie Gaukler[1]*, Justin Clements*[2]*, Jenna Stanek*[2]*, Zachary Jones*[2]*, Jessica Celmer*[3]*, Hanna Mora*[2] *and Jesse Berryhill*[2]*, (1)Environmental Stewardship, (2)Environmental Stewardship, Los Alamos National Laboratory, (3)Environmental Stewardship, Los Alamos National Laboratory*

Los Alamos National Laboratory (LANL) located in Los Alamos, New Mexico, was established during World War II to develop atomic weapons as part of the U.S. Army's Manhattan Project. Today, LANL operates as a multidisciplinary institution that focuses on solving national security challenges. LANL is actively investigating both current and historic uses of per- and polyfluoroalkyl substances (PFAS) at the site, for example, the use of aqueous film forming foams. Here we investigate PFAS in fish muscle and liver tissues from the Rio Grande and a reservoir located downstream of LANL to assess for potential LANL-impacts. The objectives of our study were to 1) evaluate PFAS loads in fish from locations downstream of LANL and compare them to fish from upstream control sites, 2) compare overall PFAS composition in fish between the sites, and 3) compare PFAS observations between muscle and liver tissues. We observed that most PFAS compounds were not detected in fish. Perfluorooctanesulfonic acid (PFOS) was the most commonly detected compound in fish from all locations and observed muscle concentrations were below levels considered safe for human consumption. Liver samples contained higher levels of PFAS relative to muscle samples. There were no differences in PFAS concentrations between upstream and downstream locations. These data suggest that while there are potential uses of PFAS at LANL, there are no adverse impacts to fish due to LANL activities in the surrounding region.

5.09.P-Mo-158 Assessing the Cyclic Siloxanes Monitoring Data in Sediments and Biota from Tokyo Bay and Lake Kasumigaura, Japan

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The cyclic siloxanes known as octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) are categorized as volatile methyl siloxane (cVMS) compounds. These substances are not naturally found and are synthesized for various industrial applications, spanning construction, automotive, electronics, pulp and paper, oil and gas, medical, and aerospace sectors. The widespread usage of cVMS has attracted attention due to concerns regarding their potential environmental impact, prompting inquiries into their presence and levels in environmental mediums such as water, sediment, and biota. Regulatory scrutiny of cVMS has intensified in recent years due to environmental apprehensions. Despite their low solubility in water, cVMS exhibit strong adsorption to organic matter found in sewage sludge, sediment, and soil, raising concerns about their persistence in ecosystems. Research on cVMS in aquatic environments in Japan is limited, and there is insufficient understanding of their prevalence within the country. This study aims to investigate the occurrence of cVMS in water environments, with a focus on analyzing their distribution and temporal trends over a 10-year period in sediments and biota from Tokyo Bay. Additionally, the presence of cVMS in water, sediments, and biota in Lake Kasumigaseki was quantified. The presentation will discuss the prevalence and concentrations of these compounds in Tokyo Bay and Lake Kasumigaseki, along with the bioaccumulative potential and ecological risk of cVMSs based on monitoring data.

5.09.P-Mo-159 A Bitcoin Mining Computer Coolant Fluid Spill - An Emerging "Contaminant"?

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Bitcoin, and other cryptocurrencies, are created by using powerful computers to solve computationally difficult problems. As the cryptocurrencies became more expensive, "Bitcoin miners" started using more powerful computers that generated significant heat that can slow down or damage the computers. The fans and air conditioners used to cool cryptocurrency mining machines may use up to 20% of the total power consumption, produce up to 90 decibels of noise, draw in dust and other contaminants, and the vibration of the fans causes wear-and-tear on the computer components. One common solution to this problem is immersion cooling the machines in nonconducting dielectric fluid with higher heat transfer capacity than air or water. A confidential client experienced multiple spills of several hundred gallons of BitCool BC-888® dielectric coolant at a cryptocurrency mining facility in the southwest US, approximately 350 meters away from a man-made lake. Fortunately, the spill was contained well in advance of it reaching the lake, but there was not much information available about the coolant. The goal of this poster is to introduce environmental professionals to where and how computer coolants are used, their contents and chemistry, potential human health and ecological effects from spills, and some strategies for spill management. This includes creating a theoretical conceptual site model. The demand for even more intense computing will only grow greater, and the frequency and magnitude of coolant spills are likely to increase.

5.09.P-Th Late Breaking Science: Environmental Risk Assessment

5.09.P-Th-177 Nationwide Monitoring of Legacy and Emerging per- and Polyfluoroalkyl Substances (PFASs) in Multiple Environmental Samples From Korean Coastal Waters

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Per- and polyfluoroalkyl substances (PFASs) have been used in diverse applications, such as textiles, paper materials, and semi-conductors. Regulations of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) led to concerns the introduction of emerging PFASs, including alternatives and precursors in global markets. In this study, legacy and emerging

PFASs were measured in multiple coastal matrices, such as seawater, sediment, and bivalves, collected in 50 locations along the Korean coasts from 2019 to 2023 to investigate occurrence, spatial distribution, and temporal trends. Almost all legacy PFASs were detectable in all environmental samples, indicating ubiquitous characters. C8-based PFASs, such as PFOA and PFOS, were major PFASs in all environmental samples. Perfluorooctane sulfonamide (FOSA) and perfluoro-2-propoxypropanoic potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (F-53B), an alternative to PFOS, were detected in sediment or bivalve samples, implying a shift in consumption patterns from legacy to emerging PFASs. Matrix-dependent contamination and profiles of PFASs were observed. Seawater was dominated by PFOA and short-chained PFASs, whereas the sediment and bivalves were dominated by long-chained PFASs. Carbon-chained length was a crucial factor governing environmental behavior and bioaccumulation of PFASs. Decreasing time trends were observed in concentrations of long-chained sulfonates, including PFOS, and PFHxS during 2019–2023, whereas concentrations of carboxylates, and short-chained PFASs, such as PFBS and PFBA, increased. This is likely due to a time gap for regulatory actions between sulfonates and carboxylates.

5.09.P-Th-178 Occurrence of Legacy Phthalate Esters and Alternative Plasticizers in Indoor Dust From Korea and Malaysia

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Plasticizers are a category of chemicals extensively used in consumer and industrial products, which resulted in ubiquitous contamination in the indoor environment. Phthalate esters (PAEs) are used as a representative plasticizer and their exposure is associated with human health outcomes. Along with the restrictions of legacy PAEs (e.g., DEHP), a variety of alternative plasticizers (APs) have been introduced into industrial markets. Limited studies have been conducted on the occurrence and human exposure of APs in indoor environments. In the present study, an analytical method was developed for the quantification of 37 plasticizers (14 legacy PAEs and 23 APs) in indoor dust using an ultrasonic extraction method and GC–MS/MS analysis. Dust samples (n=100) were collected from Korea and Malaysia to investigate the concentrations, contamination profiles, and exposure risks of all plasticizers. Almost all PAEs and APs were detected in indoor dust from both countries, indicating ubiquitous contaminants in indoor environments. Among APs, bis(2-ethylhexyl) terephthalate (DEHPT), acetyl tributyl citrate (ATBC), bis(2-propyl heptyl) phthalate (DPHP), di-isononylcyclohexane-1,2-dicarboxylate (DINCH), 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB), and bis(2-ethylhexyl) adipate (DEHA) were predominant in all dust samples. Concentrations of plasticizers in Korean dust were significantly higher than those observed in Malaysia due to different consumption. The contribution of APs was higher than PAEs in Korea, whereas the contribution of APs was lower than for PAEs in Malaysia. This implies the rapid change in plasticizers in Korea due to global and domestic regulations. Although the current exposure levels of plasticizers were lower to cause significant health risks, the assessment of plasticizers with multiple exposure pathways is required to protect human health.

5.09.P-Th-179 Predictive Performance Evaluation of Ecotoxicity Prediction Model "KATE2020" Using Newly Announced Chemical Substances As Validation Set

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Expectations on the use of *in silico* methods such as quantitative structure activity relationships (QSAR) have been mounting with the increase in the consciousness of animal welfare. National Institute for Environmental Studies, Japan has been developing QSAR model "KATE2020" that predicts toxicity of organic chemicals to fish, daphnid and algae by classification based on chemical structure and linear regression with octanol-water partition coefficient as a descriptor. In addition, KATE2020 judges whether a target chemical is predicted within applicability domain (AD) for structure and descriptor. In this presentation, we will report the predictive performance of KATE2020 for fish acute toxicity (96 h LC50) and daphnid acute toxicity (48 h EC50) using newly announced chemical substances under Chemical Substances Control Law of Japan (CSCL new chemicals) as a validation set. Measured toxicity data were collected from the web site (www.nite.go.jp/chem/jcheck/list7.action?category=240), where testing results of CSCL new chemicals are published for which five years elapsed since premanufacture notification. As a result, 96 and 121 data were obtained for fish acute and daphnid acute, respectively. In addition to KATE2020, US EPA's ECOSAR2.2 was used as comparison which predicts ecotoxicity by classification and regression as in KATE2020. Toxicity values of dataset were predicted using both models, and (i) how many chemicals were predicted within AD, (ii) for those within AD how many predictions fell within a factor of 10 (between one tenth and tenfold) against measured toxicity values, were computed. (i) 60% and 55% of CSCL new chemicals were predicted within AD by KATE2020, and 81% and 87% by ECOSAR2.2 for fish acute and daphnid acute, respectively. (ii) 91% of predictions fell within a factor of 10 against measured toxicity values for both fish and daphnid acute, and the percentage by ECOSAR2.2 was 78% for both organisms. These results imply that relatively new chemicals are more likely to be judged as out of AD but it is not necessarily difficult to predict correct toxicity values of them once predicted within AD in comparison to the previous results evaluated using OECD high production volume chemicals as a validation set. KATE2020's predictivity for CSCL new chemicals was demonstrated and it would be expected that including these chemicals in KATE2020's training set could expand the model's AD.

5.09.P-Th-180 Comparing the Sensitivities of Mollusks and Standard Aquatic Species to Pesticide Active Ingredients

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To support registration of active ingredients for plant protection products globally, testing on three taxonomic groups of aquatic organisms (fish, daphnia, algae) covering various trophic levels is generally required. However, there are differences in ecotoxicity data requirements based on geography. For example, a marine mollusk (e.g. oyster) test is currently a data requirement for pesticide registration in the United States, but the test guideline and endpoints are not aligned with data requirements in the European Union. These discordances may lead to inappropriate use of data by European authorities, resulting in potentially significant impacts on the aquatic risk assessment. Therefore, the goal of this project was to compare sensitivities of mollusks to standard aquatic test species to better understand if mollusks are covered in the existing risk assessment framework for pesticide active ingredients in Europe. Oyster acute toxicity data for 142 pesticide active ingredients was obtained by searching the National Pesticide Information Retrieval System, US EPA's ECOTOX database, and regulatory databases in March 2024. Acute toxicity data for fish and invertebrates were then acquired from reliable regulatory sources for each active ingredient to match with the oyster data. Results demonstrate that for ~1/3 of the active ingredients, oysters were more sensitive compared to fish and/or invertebrates. Calculated comparison factors (fish or invertebrate endpoint / oyster endpoint) for these active ingredients ranged from 1.13 to 96.5. Only two compounds had an oyster endpoint that was > 100 times lower (comparison factors of 119 and 900) than the acute fish or invertebrate endpoint. Considering the standard assessment factor of 100 used in the risk assessment in Europe for these species, the RAC of 140/142 compounds assessed in this study would cover the acute risk for mollusks. A more detailed assessment of the compiled dataset is currently being conducted to look for additional trends. Overall, these data will help to better inform regulatory authorities on how to address potential risks to taxa (e.g. mollusks) not currently covered in risk assessments.

5.09.P-Th-181 Bioaccumulation and Health Risk Assessment of Heavy Metals in Soil and Plantain Fruits Obtained From Selected Mechanic Workshops in Obio/Akpor Local Government Area, Rivers State

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Automobile workshops litter every available space in Port Harcourt. Waste generated from these workshops is poorly managed and form substantial components of the soil in and around these places. This obviously brings up toxicological risks for those who consume plant products from around the areas, leading to environmental issues related to public health. This study evaluated the bioaccumulation and health risk assessment of heavy metals in soil and plantain fruits obtained from selected mechanic workshops in Obio/Akpor LGA, Rivers State. Soil and plantain fruit samples were randomly collected from Choba (Site A), Rumuigbo (Site B), Rumuolumeni (Site C) and University of Port Harcourt Agricultural Farm (Control Site). Physicochemical and proximate concentration was analyzed with standard analytical techniques by AOAC, while heavy metals lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni) and cobalt (Co) composition were analyzed with Atomic Absorption Spectrophotometer (S4–71096, Solar thermos-elemental, China). Physicochemical results for soil samples in the test sites were above that of the control site. Pb (0.96 ± 0.06 – 47.04 ± 0.08), Cd (0.03 ± 0.00 – 2.37 ± 0.03), Cr (0.87 ± 0.08 – 19.40 ± 0.18), Ni (0.06 ± 0.02 – 10.49 ± 0.21) and Co (0.12 ± 0.01 – 6.60 ± 0.22) levels in soil from the test sites were above that of the control site and below WHO/FAO permissible limit for soil. However, Cd (0.01 ± 0.00 – 0.40 ± 0.01) and Pb (0.03 ± 0.00 – 7.26 ± 1.15) levels in plantain fruit biomass from test sites were above World Health Organization/Food and Agriculture Organization (WHO/FAO) permissible limit. Bioaccumulation factor ranged between 0.0023 – 0.292, with Nickel in site A having the highest value. Average daily dose (ADD) result suggests that ingestion was the major pathway of heavy metal exposure, followed by dermal and inhalation. Hazard index (HI) of soil for children population was above that of the control site and below 1 Person-1 United State Environmental Protection Agency (USEPA) standard. Total Life Cancer Risk (TLCR) for soil and plantain in the test sites were above that of the control site samples, and lower than the permissible lifetime risk of 10-6–10-4 set by USEPA for carcinogens. The study revealed the possible Bioaccumulation of Pb, Cd, Cr, Ni and Co in plantain fruit biomass and no possible risk associated with heavy metal and exposure in auto-mobile workshops and consumption of plantain grown around auto-mobile workshops.

5.09.P-Th-182 Assessment of Risks to Early Life Stage White Sturgeon From Exposure to Metals Associated With Slag in the Upper Columbia River, WA. Part 2: Estimating Exposure Based on Life History

*Jennifer Holder and **Derek Pelletier**, ERM International Group*

Due to concerns around recruitment failure of white sturgeon in the Upper Columbia River, there has been special focus on understanding the effect of potential stressors on the early life stage (ELS) white sturgeon. While spawning has been widely documented and first-feeding larvae have been captured at numerous sites within this reach, recruitment of juveniles has been negligible. Upon hatching, larval sturgeon hide in interstitial spaces in preferred substrate (e.g., gravel and cobble) until they absorb their yolk sac. While hiding, they may be exposed to metals in bottom water and porewater in the interstitial spaces. Therefore, as part of the baseline ecological risk assessment for metals associated with slag in the Upper Columbia River, risks to ELS white sturgeon from exposures to metals in porewater were assessed. The porewater effects concentrations for cadmium, copper, lead, and zinc were developed based on a review of surface water toxicity studies on early life stage white sturgeon (see accompanying DeForest et al. poster). Exposure was estimated based on measured concentrations of metals in porewater collected from potentially preferred substrate in the upper reach of the Upper Columbia River. Porewater samples from locations that could potentially provide suitable hiding habitat were identified based on sediment facies mapped in the reach in 2018 and 2019. Substrate that is more than 50% fine grained material (i.e., sand, silt, and clay) was assumed to provide insufficient hiding habitat for ELS white sturgeon so porewater samples from those substrates are not included in the exposure assessment. To understand regional exposures, potential exposures to were also compared to potential exposures from upstream reference areas.

5.09.P-Th-183 Ecological Risk Assessment Using a Bayesian Network Relative Risk Model (BN-RRM) for Microplastics and Their Leachates in the San Francisco Bay Region

***Cynthia C. Kuhn**[1], Emma Sharpe[2] and Wayne G Landis[3], (1)Institute of Environmental Toxicology and Chemistry, Western Washington University, (2)Environment Science, Western Washington University, (3)Western Washington University*

Ecological Risk Assessment Using a Bayesian Network Relative Risk Model (BN-RRM) for Microplastics and Their Leachates in the San Francisco Bay Region

***Cynthia C. Kuhn**[1], Emma Sharpe[2] and Wayne G Landis[3], (1)Institute of Environmental Toxicology and Chemistry, Western Washington University, (2)Environment Science, Western Washington University, (3)Western Washington University*

This project conducted an ecological risk assessment for microplastic leachates in the San Francisco Bay area using a Bayesian Network Relative Risk Model (BN-RRM). Studies in recent years have shown that micro- and nano- plastics are widespread in the environment, and that exposure to them and the chemical leachates they produce can have toxicological and physiological effects. Because of this, there is an increased interest in understanding how they transport through an ecosystem and what risk they pose to the organisms living there, specifically for chinook salmon, northern anchovy, and macroinvertebrate community structure. This project includes new data on microplastic toxicity. Other contaminants including mercury and bifenthrin are included, allowing a comparison of risk between these two contaminants and microplastics. This project will show the risk posed by microplastics to aquatic organisms and place that risk in context with two contaminants already subject to regulations. The BN-RRM is a successful framework for regional scale ecological risk assessments of multi-stressor systems, allowing for the creation of a model with predictive capability and adaptive potential as new data become available. Plastic particle and leachate toxicity data generated by Oregon State University and Western Washington University, environmental sampling data from the San Francisco Estuary Institute and site-specific water quality, chemical, and land use data from regional databases. *This study was funded by the National Science Foundation Growing Convergence Research Grant (1935018) program.*

5.09.P-Th-184 Assessment of Risks to Benthic Invertebrates From Exposure to Metals in the Upper Columbia River, WA. Part 1: Identifying Potential Causes of Toxicity to *H. azteca* in Sediment Bioassays

Derek Pelletier[1], *Jennifer Holder*[1] *and Robert Santore*[2], *(1)ERM International Group, (2)Windward Environmental*

Multiple lines of evidence (LOEs) were used to assess risks to benthic invertebrate communities exposed to metals associated with slag present in sediment in the Upper Columbia River in northeastern Washington State. This poster presents the results for one of those LOEs – the evaluation of sediment bioassay response as it relates to exposure of benthic invertebrates to metals in sediment and porewater. Consideration of LOEs associated with in-situ benthic invertebrate community metrics are presented in a companion poster by Pelletier et al**.** The analyses are conducted with data from multiple investigations where sediment and porewater chemistry data are available from samples collected from the Upper Columbia River, north of Marcus Flats (OU1), and upstream reference areas that were also used in sediment bioassays with the freshwater amphipod, *Hyalella azteca*. Because sediment samples from OU1 with substrate comprised primarily of medium, coarse, and very coarse sand capture those samples that are most likely to contain slag-related metals, the concentration-response analyses are conducted with two sets – one with samples that are primarily medium, coarse, and very coarse sand and one with all other samples - to reduce potential noise in the concentration-response models due to differences in stressors. For each data set, the laboratory exposure metrics evaluated include solid-phase sediment concentrations for 23 metals, bioavailability-adjusted solid-phase exposure metrics (i.e., simultaneously extracted metals and acid volatile sulfide), dissolved concentrations of 15 metals in porewater, and bioavailability-adjusted metrics consistent with EPA’s ambient water quality criteria for five metals – cadmium, copper, lead, nickel, and zinc. The biological response results from the four bioassay studies that were integrated include 28-day survival and growth and 42-day survival, growth, and reproduction. Multiple concentration-response model types were fit to each data set, and a robust, decision framework was used to identify models that best characterize relationships and meet minimum standards for validity, goodness of fit, and plausibility. This presentation identifies the metals exposure metrics that are found to be most reliably associated with biological response for *H.*

azteca in the sediment samples from OU1 where slag-related metals are more likely to be present.

5.09.P-Th-185 Assessment of Risks to Benthic Invertebrates From Exposure to Metals in the Upper Columbia River, WA. Part 2: Consideration of In Situ Benthic Invertebrate Community Metrics

Derek Pelletier[1]*, Katherine Palmquist*[2]*, Chad Wiseman*[3] *and Jennifer Holder*[1]*, (1)ERM International Group, (2)Exponent, (3)HDR*

Multiple lines of evidence (LOEs) are used to assess risks to benthic invertebrate communities exposed to metals associated with slag present in the sediments of the Upper Reach of the Upper Columbia River (OU1) in northeastern Washington State. The LOEs include those commonly associated with a sediment quality triad approach: sediment and porewater chemistry compared to benchmarks, sediment bioassay data, and benthic invertebrate community metrics. In addition, three integrative LOEs were developed to capture correspondence between the triad LOEs: concentration-response models for media chemistry and bioassay data, concentration-response models for media chemistry and community metrics data, and the relationship of bioassay data and community metrics from co-located sediment samples. The results from the concentration-response models with bioassay data are presented in a companion poster by Pelletier et al. This presentation focuses on the three LOEs that incorporate benthic invertebrate community metrics reported as part of a sediment quality triad study conducted in 2019. For the first LOE, benthic invertebrate community metrics are categorized according to the environmental values of community abundance, production, and composition and compared to those in samples from similar habitat in upstream reference areas using multiple statistical techniques. For the second LOE, the community metrics are correlated with metals exposure metrics and habitat factors in the co-located sediment and porewater samples to determine whether any of the metrics that differ between upstream and OU1 samples are correlated with potential exposure to metals or other habitat factors. For the third LOE, the community metrics are correlated with responses from bioassays conducted with co-located sediment samples. These LOEs are combined with the other LOEs for benthic invertebrate communities listed above to arrive at a common finding for benthic invertebrate communities in OU1.

5.09.P-Th-186 Assessment of Risks to Early Life Stage White Sturgeon From Exposure to Metals Associated With Slag in the Upper Columbia River, WA. Part 1: Development of Bioavailability-Based Toxicity Benchmarks

David K. DeForest*, Kelly E Croteau and Robert Santore, Windward Environmental*

Toxicity benchmarks were developed to evaluate exposures of early life stages of white sturgeon (*Acipenser transmontanus*) to cadmium, copper, lead, and zinc in porewater of the Upper Columbia River. Although only early life stages of white sturgeon were considered to have potential porewater exposures in the Upper Columbia River reaches being evaluated, acute and chronic toxicity data from tests that encompassed a variety of life stages and exposure durations were initially compiled. Because these toxicity tests were conducted over varying water chemistry conditions, the toxicity data were first adjusted to a common bioavailability condition to determine the relative sensitivities of the tests compiled. For copper, the biotic ligand model (BLM) that provides the basis for the US Environmental Protection Agency's (USEPA's) ambient water quality criteria for copper was used to convert water-based toxicity thresholds to thresholds expressed as the copper concentration on the biotic ligand (i.e., BL-Cu). For cadmium, lead, and zinc, the USEPA's hardness-based models were used to adjust the toxicity thresholds to a common hardness concentration. As expected, chronic tests were more sensitive than acute tests for all four metals. These chronic tests were either initiated with embryos or 1-2 day post-hatch (dph) larvae. However, for copper, a 96-h acute test with 2 dph larvae had a toxicity threshold comparable to the USEPA's chronic copper criterion, suggesting that short-term exposures by this life stage are comparable to long-term exposures in chronic studies encompassing multiple life stages. Overall, white sturgeon are more sensitive to copper and zinc relative to their respective ambient water quality criteria than to cadmium and lead, the latter of which had sensitivities that were more than seven-times and ten-times greater than their chronic ambient water quality criteria, respectively. The resulting sturgeon-specific toxicity benchmarks for copper (based on the BLM) and cadmium, lead, and zinc (based on hardness models) were subsequently integrated with early life stage exposure data for the Upper Columbia River as discussed in the companion poster by Holder and colleagues.

5.09.P-Th-187 Current Frameworks for Risk Assessment of Hydrocarbon Streams and Products Are Flexible and Ready for Alternative Non Crude Oil-Based Feeds

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Hazard and risk assessment of complex petroleum-derived substances has been in a state of continuous improvement since the 1970s, with the development of approaches that continue to be applied and refined. Alternative feeds are defined here as those coming into a refinery or chemical plant that are not hydrocarbons from oil and gas extraction such as biologically derived oils, pyrolysis oil from biomass or other, and recycled materials. These feeds are increasingly being used for production of liquid hydrocarbon streams, and hence, there is a need to assess these alternatives, subsequent manufacturing and refining processes and end products for potential risk to humans and the environment. Here we propose a tiered, problem formulation-driven framework for assessing the safety of hydrocarbon streams and products derived from alternative feedstocks in use. The scope of this work is only focused on petrochemical safety assessment, though the principles may be applicable to other chemistries. The framework integrates combinations of analytical chemistry, *in silico* and *in vitro* tools, and targeted testing together with conservative assumptions/approaches to leverage existing health, environmental, and exposure data, where applicable. The framework enables the identification of scenarios where new hazard and/or exposure assessments may be needed and incorporates tiered approaches to do so. It can be applied to enable decisions efficiently and transparently and can encompass a wide range of compositional space in both feedstocks and finished products, with the objective of ensuring safety in manufacturing and use.

5.09.P-Th-188 Examining Hazards of Cleaning Products With and Without Green Product Labeling

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Empirical toxicology data is not available for most of the chemicals in commerce. Our previous research has examined health hazards of cleaning product ingredients, and employed probabilistic approaches to identify thresholds of toxicological concern, derive uncertainty factors, and examine hazards of chemical classes. In the present study, we selected three cleaning products each for laundry care, hard surface cleaning and dish care, and then selected three additional cleaning products each in these three categories that are marketed as “greener” or received the Safer Choice label from the US Environmental Protection Agency. We then developed a framework for considering chemical complexity, inclusion of GRAS (generally recognized as safe) chemicals, and diverse toxicology data for ingredients within these for laundry care, hard surface cleaning and dish care to examine whether “greener” marketed and / or Safer Choice labeled products are less hazardous. We subsequently propose a unique probability based approach using chemical toxicity distributions to examine health hazards within and among specific consumer products, and further anticipate this approach can be extended to other products in commerce.

5.09.P-Th-189 Per- and Polyfluoroalkyl Substances (PFAS) Analysis in Plankton Samples From the Gulf of Maine

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Per- and polyfluoroalkyl substances (PFAS) are a diverse group of synthetic chemicals commonly used in various industrial applications for their water- and grease-resistant properties. PFAS are persistent in the environment, undergo long-range transport, and have been linked to adverse health effects in both wildlife and humans. PFAS enter the environment through various sources such as the use of firefighting foams and the discharge of industrial and municipal wastewater, with the oceans ultimately serving as their final sink. Our understanding of PFAS transfer within the marine trophic food web is limited, particularly regarding whether and how PFAS bioaccumulate in lower trophic levels, such as plankton. To understand PFAS bioaccumulation in plankton, we collected nine plankton samples from the Gulf of Maine in October 2022. All samples were collected during the Endeavor Cruise 692 using a 200-micrometer mesh-size plankton net. The net was towed at 1 knot for 15 minutes at depths between 7 and 30 meters, collecting a total volume of 1 liter. Samples were freeze-dried and extracted using established protocols for biota based on the EPA 1633 method. Final extracts were analyzed for 56 PFAS using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). We detected 16 PFAS in plankton at all sites with PFOS being the most abundant compound, detected between 0.06 ng/g d.w. and 0.89 ng/g d.w. Generally, the higher PFAS concentration was found at sites closer to the cost ($\sum$16 PFAS between 1.75 ng/g d.w. and 2.00 ng/g d.w.). However, the highest total PFAS concentration (2.01 ng/g d.w.) was found in plankton collected offshore between the Jordan and Georges Basins, where waters from various regions mix due to major surface currents. This finding can provide a better understanding of why offshore marine organisms at higher trophic levels were recently found to be contaminated with PFAS.

5.09.P-Th-190 Studies To Evaluate Mobility and Persistence of Wild-Type and Gene Edited Microbial-Based Agricultural Products

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Global interest in biological-based products for agriculture is growing rapidly, including products based on micro-organisms. This is an expanding market globally worth $12 billion with a 15% compound annual growth rate, and conservative estimates put this market worth $27 billion by 2027. These products cover a range of functions such as the following. Biofertilizers are typically microbes that increase the amount or availability of nutrients e.g., nitrogen fixing bacteria. Biostimulants improve crop yields by affecting nutrient uptake, modifying the microbiome, or stimulating root growth for water management among other modes of action. Biocontrol products (biopesticides) are primarily fungicides and insecticides and can be biochemicals or microbials. These groups of agricultural products are considered a safer alternative to conventional chemicals for a number of reasons including the efficacy may or may not rely on the chemistry that they produce, and aspects of pesticide regulation like food residues may be irrelevant (no crop residues). However, such products are still evaluated for safety, and there are a number of technical challenges associated with testing microbial-based products. The US EPA has published testing guidelines specific for microbial-based products. They included a guideline for environmental fate type testing, or environmental expression, and while the testing guideline describes the type of information that is sought, it does not describe how to conduct the test. In order to address questions related to environmental fate, a study was conducted to investigate the potential for persistence and mobility of both wild-type and gene edited micro-organisms from a potential corn seed treatment use. A method with proper specificity and sensitivity is required to be able to detect microbes of interest in a complex environment. The microbes in this study were quantified using qPCR technology in water, soil, root and plant tissue at various corn growth stages and time points after planting. Results demonstrated that mobility and persistence were limited from this use pattern, and the microbes did not migrate to the phyllosphere.

5.10.P-Tu New Approaches and Data to Evaluate Environmental Risks of Sunscreens

5.10.P-Tu-174 An Acute Ecotoxicological Test Coupled with Transcriptome Analysis of a Sunscreen Ingredient, Oxybenzone Using Coral Acropora tenuis for Ecological Risk Assessment and Molecular Epidemiological Survey

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Coral reefs are highly biodiverse and productive ecosystems but are threatened by global and local stresses. In addition to rising seawater temperature, the effect of organic ultraviolet (UV) filters on corals has recently attracted attention. However, due to the lack of standardized toxicity tests for corals, limited studies and effects have been reported to date. Moreover, there is little understanding of differences in the mechanisms of action between UV filters and other environmental stresses. To address these knowledge gap and expand the scientific basis, an acute toxicity test coupled with transcriptome analysis on an adult reef-building coral *Acropora tenuis* using an UV filter oxybenzone was conducted. Comparative transcriptome analysis was performed to analyze differences in gene expression between corals exposed to oxybenzone and high seawater temperature using RNA-seq. *A. tenuis* was exposed to oxybenzone in five concentrations, or high seawater temperature condition (31°C) for 96 hours in a test system constructed with reference to OECD test guidelines for aquatic toxicity test. Multiple biological endpoints were measured including the coral color evaluated quantitatively by a color chart in combination with image analysis software, maximum quantum yield (F_v/F_m) measurement of photosystem II and visual assessment, and the lethal concentration 50% (LC50) was calculated as 3.9 mg/L. Risk assessment was conducted using previously reported measured environmental concentrations (MEC) and predicted environmental concentrations (PEC) derived from original box models constructed based on tourism statistics and regional geographic information in Japan, and MAMPEC that is originally developed for runoff simulations of antifoulants. Comparative transcriptome analysis revealed that 2,400 genes and 861 genes were detected as differentially expressed genes by oxybenzone exposure and high seawater temperature group, respectively, and 522 genes were in common. In this presentation, we provide an overview of the differences in the mechanisms of coral response due to stress sources. The information on the gene expression signature found in this study will be a beneficial indicator for eco-epidemiological studies of corals in real-world environments.

5.10.P-Tu-175 Probabilistic Emissions Model for UV Filters Released to Recreational Waters During Swim Events

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A critical element in understanding measured concentrations and predicting exposure levels of UV filters (UVFs) in recreational waters relates to the mass of UVFs released during swimming and bathing. A probabilistic mass transfer model was developed for oxybenzone, avobenzone, octocrylene, homosalate, and octisalate that estimates distributions of UVF mass released during individual swim events with sunscreen. The model incorporates the ratio of child to adult female to adult male swimmers and distributions of total skin area for each group adjusted using distributions of skin areas covered by different types of swimwear to which sunscreen would not be applied, and levels of immersion (e.g., wading vs full submergence). The resulting distribution of skin areas is combined with distributions of sunscreen application rates and UVF levels in sunscreen products to generate a distribution of UVF masses on skin in contact with water. The fraction released to water from each UVF skin load is calculated using a first-order equation describing the kinetics of release of UVFs based on lab studies in which sunscreen products were applied to pig skin samples that were incubated in seawater. The parameters in this equation are maximum fraction released (Rmax), a release rate into water (k), and time (t) spent in the water. Distributions of skin loads are combined with three matched sets of Rmax and k values and distributions of swim duration to generate a distribution of masses released to water during swim events for the five UVFs. The predicted median mass released ranged from 8 to 112 mg for the five UVFs. The model was built in Excel using the Crystal Ball plug-in for Monte-Carlo analyses. During model runs, Crystal Ball conducts a sensitivity analysis reporting a rank correlation coefficient and a variance contribution to the output forecast by each input parameter. The top four rank correlation coefficients were for application rate, fraction of UVF in product, swim duration, and male skin area. The sensitivity analyses indicated that the factor explaining the greatest amount of variation (56-69%) within the loading distributions was the application rate of the sunscreen product. The resulting distributions of UVF mass loads released to water per swim event forms the basis to estimate UVF concentrations in larger recreational areas.

5.10.T New Approaches and Data to Evaluate Environmental Risks of Sunscreens

5.10.T-01 Defensible Assessments for Evaluating Impacts of UV Filters on the Environment: A Path Forward

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Recent concerns about the potential toxicity of sunscreen active ingredients (ultraviolet [UV] filters) on aquatic organisms led to a National Academy of Sciences, Engineering, and Medicine (NASEM) report evaluating the state of the science on the environmental impacts of UV filters. The NASEM report, released August 2022, recommended EPA conduct an ecological risk assessment (ERA) of marketed sunscreen UV filters in aquatic environments. Following a review of the NASEM report, researchers at the EPA have determined that robust, quality data are lacking for a defensible ERA. Much of the data published on the effects of UV filters on aquatic organisms are based on non-standard test designs, endpoints with limited interpretability, are not applicable within the USEPA ERA framework, and/or do not adhere to best practices nor follow EPA's data quality standards. This presentation will overview the challenges of conducting an ERA with the current state of the science and provide an overview of next steps in ensuring robust, quality-assured data will inform defensible assessments of UV filters in the environment.

5.10.T-02 Temporal Variation of UV Filters at a Recreational Beach in Florida, USA

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Ultraviolet (UV) filters are a diverse group of organic and inorganic compounds that have a wide range of commercial applications, including in sunscreens, due to their UV light-blocking properties. Because of their widespread use in outdoor recreation understanding the temporal and spatial prevalence of these compounds in marine systems is a required part of an environmental risk assessment and to provide data for the development of predictive exposure models. For this study, the concentrations of twelve organic UV filters were monitored over a period of twenty-four hours at a popular swimming beach in Fort Lauderdale, Florida. Composite samples were collected along three transects at two locations that were different distances from and running parallel to the shore (nearshore and further offshore) at five to eight time points over the sampling period. UV filters were extracted from the water samples via solid phase extraction, and then analyzed quantitatively by liquid chromatography-tandem mass spectrometry. Six of the target analytes- oxybenzone, octocrylene, octinoxate, octisalate, homosalate, and avobenzone were observed to sharply increase up to two orders of magnitude above the lowest (i.e., early morning) levels during the peak recreational time in the mid-afternoon, before returning to previous levels by early evening. This pattern was observed at both locations, although peak measured concentrations were uniformly higher in the nearshore location transects. Oxybenzone, octocrylene, octisalate, and homosalate had the highest measured concentrations, with peak concentrations between 1 and 5 µg/L. These results highlight that aquatic organisms in popular recreational areas may encounter UV filters in short-term intermittent pulses at higher concentrations, rather than a steady environmental concentration, and emphasizes the importance of sampling for UV filters at times of both high and low recreational activity.

5.10.T-03 Toxicity of the UV Filter Octocrylene to the Scleractinian Coral *Acropora cervicornis*

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Ecotoxicological studies have shown variable responses in several coral species following exposure to organic UV filters. The UV filter octocrylene has been detected in waters surrounding coral reefs and in coral tissues, however, limited studies and effects on corals have been reported to date. To address this knowledge gap we conducted range-finding and, definitive acute (i.e. 96 hour) and chronic (i.e. 21 day) toxicity tests, based on standard US EPA and OECD guidelines, with the Atlantic staghorn coral *Acropora cervicornis*. Multiple biological endpoints were employed to assess the response of *A. cervicornis* compared to other species, including those pertinent to risk assessments (i.e. mortality and growth) along with other coral specific assessments, such as visual bleaching, coral condition (i.e. mucus production, tissue swelling and/or thinning), polyp retraction and photosynthetic efficiency. Acute toxicity was not observed with octocrylene at concentrations in the mg/L range and far exceeding solubility estimates (i.e., 40 µg/L). Two chronic toxicity tests were conducted using concentrations below estimated solubility and at levels measured in seawater (i.e. 0.05 – 36 µg/L nominal; 0.01 – 15.51 µg/L measured). In the first test using higher concentrations no treatment effects were observed for photosynthetic efficiency but statistically significant treatment effects at 17 and 21 days were observed for coral condition index and reduction in growth rate. In the second chronic test using lower concentrations of octocrylene no statistically significant effects were observed for visual bleaching, photosynthetic efficiency or coral condition index at any concentration or time point during 21-days of exposure. However, at 21-days there was a significant reduction in growth rate at the highest dose of 1.47 µg/L (measured; 4.05 µg/L nominal), with a IC10 of 1.01 µg/L (measured; 1.95µg/L nominal) and NOEC of 0.427 µg/L (measured; 1.35 µg/L nominal). These values are similar to the 21-day reproduction NOEC (2.66 µg/L measured) in *Daphnia magna*. Further studies are required to investigate the impact of octocrylene on a range of morphologically diverse and geographically distributed coral species using both adult and early-life stages in addition to the extent of bioaccumulation and mechanism of action of octocrylene in coral species.

5.10.T-04 Estimation of the Short-Term Chronic Marine Toxicity of the Sunscreen Ingredient Octocrylene to the Sea Urchin Arbacia punctulata using the EPA 1008 Method

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In recent years the need to evaluate the risk of recreational sunscreen emissions in coastal marine environments has grown in prominence. In the European Union, a marine ERA initially follows the ECHA freshwater method with an assessment factor applied to account for the higher taxonomic diversity of the marine environment. Uncertainty cannot be reduced without the collection of toxicity data for marine-specific phyla such as mollusks, cnidarians or echinoderms. There is however a lack of reliable standard and non-standard marine chronic toxicity test methods to refine marine ERAs. As a step to help fill this gap, the EPA method 1008 (Sea Urchin, *Arbacia punctulata*, Fertilization Test) was used to estimate the short-term chronic toxicity of the sunscreen ingredient octocrylene (OC). Modifications were aimed at ensuring the method was suitably reliable for testing discrete difficult-to-test substances, rather than effluents, and responded as expected to the reference substance copper sulphate. The results indicated that all validity criteria were met and octocrylene concentrations could be reliably maintained with low variability in the test medium and the octocrylene EC10 was 47 µg/L. The sea urchin fertilization is a sensitive endpoint for OC compared to literature values, and the method is well-suited for testing difficult to work with substances. Sea urchin embryo growth is a comparable endpoint, although published data have certain reliability issues. The data suggest fertilization is more sensitive to OC. Subsequently, early life stages of sea urchins, while short, are sensitive. Adults are documented to be less sensitive and longer tests covering the adult stage are highly challenging/ impractical, and more expensive to run. Overall, the modified EPA method 1008 is a reliable and cost-effective test for underrepresented marine-specific phyla in ERA and is well suited for the difficult-to-test sunscreen ingredient octocrylene.

5.10.T-05 Sampling Design and Model Selection to Evaluate the Exposure of Sunscreen and Cosmetic Ingredients to Marine and Freshwater Ecosystems using the MERCI Modeling Framework

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Sampling design and model selection for environmental risk assessment are guided by the relevant fate and transport processes, and ultimately, by the specific study questions pertaining to exposure. A tiered modeling framework called MERCI (**M**odels to **E**valuate direct **R**elease of **C**osmetic **I**ngredients into natural waters) has been proposed and tested to evaluate the potential environmental exposure of marine and freshwater organisms to ultraviolet radiation filters (UV filters) and cosmetic ingredients. The framework consists of four levels of assessment, ranging from simple, dilution-based screening assessments to complex, 3-dimensional circulation models and a toolbox to address uncertainty and specific questions that may arise during environmental risk assessment. This tiered modeling system includes an initial conservative screen of potential risk with minimal effort and information, and progressive tiers that introduce additional processes, input requirements, and complexity to improve the accuracy of predictions. Specific models are identified for each tier based on the environmental fate processes represented by that model, the governing equations and transparency of model code, input parameter requirements, the availability of model support, acquisition cost, and established acceptance by regulatory agencies. The selection of a particular model and model tier within the MERCI framework depends on the problem statement as well as data availability, and the hydrodynamics of the system being modeled. This presentation provides a roadmap for appropriate sampling and study design, where guidance is missing, and model selection within the MERCI framework, based on the desired captured processes and specific study questions pertaining to risk assessment. Our recommendations are based on model testing for three hydrodynamically different study sites, and sensitivity analyses conducted to identify the relative importance of model inputs.

5.10.T-06 Progress and Challenges in Modelling UV Filter Exposure from Swimmers in Recreational Waters

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Measured concentrations of UV filters (UVFs) in the marine environment are critical to evaluate potential ecological effects and risk., These data span a wide range for any one UVF (from the low ng/L to mg/L) likely due to difficulties in analysis, but also in collecting samples representative of the recreational waters being monitored. While the currently available UVF monitoring studies help to frame potential exposure levels, many lack information on the duration and extent (spatial, volume) of exposures reducing their utility for risk assessment and for extrapolating across time and space. Given the number and diversity of recreational waters and the need to understand concentrations over time, we investigated approaches for estimating exposure based upon mass transfer principles. Analysis of data in published studies on UVF release in water from sunscreen applied to skin revealed that these data were described by a first-order release. This equation was combined with other data to estimate the highest potential exposure levels in small closed and open parcels of water. Subsequently, this model was expanded by combining statistical distributions of skin surface areas of children and adults, sunscreen application rates, swim duration, and other factors to develop a probabilistic emissions model for various UVFs with skin release data. As proof of concept, this model was combined with the fraction of products with a given UVF, number of swim events during the day, recreational area water volume and turnover rates to estimate concentrations of oxybenzone at two sites with monitoring studies that had sufficient supporting data. In both cases, the median predicted concentrations were within a factor of two of the average measured concentrations. Key challenges included finding sufficient data to parameterize the model and understanding the hydrodynamics at a site, which can be constantly changing. Moreover, since the model is based upon mass transfer, sorption to suspended solids needs to be reconciled in the analytical approaches and within the model. Regardless, it provides a framework to help understand the spatial and temporal heterogeneity associated with UVF exposure in recreational waters. It also can assist in identifying information that may help extend the utility of future monitoring studies and factors to consider when interpreting measured concentrations reported in the literature.

5.11.P-Th Probing Linkages at the Land-Water Interface to Quantify Contaminant Fluxes and Insectivore Exposure

5.11.P-Th-066 Insect-Mediated Mercury Flux at the Great Lakes AOC site - Muskegon Lake

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Muskegon Lake is a 4,149-acre drowned river mouth lake that flows into the eastern shore of Lake Michigan. Muskegon Lake was designated an AOC through the Great Lakes Water Quality

Agreement in 1987 due to ecological problems caused by historical industrial discharges of pollutants (including sawmill debris and foundries), shoreline development and localized groundwater contamination. These sources resulted in sediment contaminated with various pollutants, including mercury. As of 2021, the last management actions were completed. In this study, sampling locations were identified at various locations along the shoreline of Muskegon Lake corresponding to various legacy issues. Concentrations of total mercury in sediment and Tetragnathid spiders varied by location. Further analysis is needed to determine if historical land use was connected to the observed mercury concentrations.

5.11.P-Th-067 You are What and Where You Eat: Spatial Patterns in Spider Diets and MeHg Concentrations Around Human-Made Ponds

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Spiders have been proposed as sentinels for monitoring aquatic contaminants, including methylmercury (MeHg). Riparian spiders that feed on MeHg-contaminated aquatic insects can have MeHg concentrations that reflect the level of contamination in the aquatic food web. However, relatively few studies have assessed the biological and ecological factors that affect MeHg concentrations within spider tissues. The present study assessed how spider MeHg concentrations were related to both proximity to waterbody (ecological factor), and spider diet (biological factor). In June and July 2018, spiders were collected up to 20m away from 12 human-made ponds in North Texas, USA. We assessed patterns for the spider assemblage (combining all taxa) and for each of the 8 spider taxa collected (Araneidae, Oxyopidae, Salticidae, Tetragnathidae: *Tetragnatha* sp., Lycosidae: *Pardosa* sp., *Rabidosa* sp., *Schizocosa* sp., Pisauridae: *Dolomedes* sp.). We used a stable isotope mixing model to estimate proportions of aquatic and terrestrial prey in spider diets, and linear mixed-effects models to test the effect of distance inland from ponds on spider MeHg concentrations controlling for spider size. For the spider assemblage, we detected a decrease in average spider MeHg concentration ($p<0.05$) and the proportion of aquatic prey in their diet (ANCOVA: $p<0.05$) with distance inland. These results suggest terrestrial prey, with lower MeHg concentrations, contributed more to the diets of the spider community at increasing distance from the ponds. Notably, MeHg concentrations varied among spider taxa and the spider community composition changed with distance from ponds. MeHg concentrations and proportion of aquatic prey decreased with distance inland in Salticidae and *Rabidosa* sp. ($p<0.001$). No significant effects of distance on MeHg or diets were detected in the other 3 taxa. The present study indicates biological and ecological factors can explain part of the variation in MeHg across both the spider community and within some taxa. The factors related to spider MeHg concentration, however, may not be consistent within each spider taxa due to differences in taxa hunting strategies and habitat uses. This study provides an example of how sampling design and grouping of taxa could affect how spider contaminant concentrations are interpreted. Understanding sample-characteristics that affect MeHg concentrations in spiders is crucial for designing MeHg monitoring projects using spiders as sentinels.

5.11.P-Th-068 Effects of Body Size and Season on Total and Methyl Mercury Concentrations in Orb-Weaving Spiders

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Riparian spiders have been proposed as sentinels of bioaccumulative contaminants, like methylmercury (MeHg). Riparian spiders become contaminated with MeHg when they feed on emergent aquatic insects and concentrations of MeHg in riparian spiders reflect the level of contamination of the aquatic food web. Although previous studies suggest riparian spiders have potential as sentinels of MeHg, few studies have examined how MeHg concentrations in riparian spiders vary seasonally. We sampled two genera of orb-weaving spiders (Araneidae: *Larinioides* sp. and *Metazygia* sp.) monthly from May-September 2019 at a private boat dock on Eagle Mountain Lake, Texas. We collected 574 spiders and separated them by, genera, size class and month of collection into composite samples. The samples were then analyzed for total Hg (THg, inorganic+MeHg) and MeHg. We observed no difference in THg concentration between genera and so combined them for subsequent analyses. During most months we observed a positive correlation between THg concentration and spider size, indicative of age-dependent Hg bioaccumulation, but the slope of the relationship between THg and size increased throughout the summer. As a consequence, the highest concentrations of THg occurred in the largest spiders in late summer and fall. Unlike, total Hg, MeHg concentrations were low and remained constant over the course of the study, resulting in a steady decrease in %MeHg in spider tissues. We hypothesize that these patterns were caused by an increasing reliance on terrestrial insect prey (with low % of MeHg) through the summer.

5.11.P-Th-069 Seasonality Affects Mercury, Lipid, and Stable Isotope Concentrations: Implications for the Use of Tetragnathid Spiders as Biosentinels

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Riparian spiders are used in ecotoxicology as sentinels of bioavailable contaminants that are transferred from aquatic to terrestrial habitats via emergent aquatic insects. Spiders in the family *Tetragnathidae* are particularly of interest because a high proportion of their diet consists of emergent aquatic insects, and they reflect insect mediated transfer occurring. The objective of this study was to determine if seasonality affected size, carbon and nitrogen stable isotopes, polyunsaturated fatty acid biomarkers, mercury, and metals concentrations in tetragnathid spiders. Spiders were sampled every two weeks from a single river site in Tennessee, USA for an entire active season (April through November). Spider mass steadily increased from April to September to a maximum value of 0.127g, then decreased sharply in October. Seasonal trends were observed for carbon and nitrogen stable isotopes and ω3 and ω6 polyunsaturated fatty acids, with significantly different signatures occurring late the active season. Overall, methyl mercury concentrations (range: 12.1-134.4 ng/g) and the methyl:total mercury ratio (range: 49-98%) increased throughout the active season with higher variability observed at the beginning and end of the active season. Collectively, our results indicated that seasonality impacted multiple important endpoints and that spiders collected during the beginning and end of the

active season may not be representative of spiders during the majority of the active season in the middle.

5.11.P-Th-070 Mercury (Hg) Concentrations of Spiders from Greenland: Potential as Sentinels of Hg Contamination in High Arctic Lentic Systems and Risk to Arachnivorous Birds

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Mercury (Hg) emitted in temperate and tropical regions can be transported to the Arctic where it is disproportionately deposited across the landscape. In aquatic systems, inorganic forms of Hg can be methylated to the toxic and bioaccumulative species: methylmercury (MeHg). In temperate zones, riparian spiders that specialize in consuming adult emergent insects (e.g., Araneidae and Tetragnathidae) accumulate high concentrations of MeHg and have been used as sentinels of MeHg contamination and these taxa frequently accumulate concentrations of MeHg of potential risk to arachnivorous songbirds. Although these taxa are useful sentinels in risk assessment studies in the temperate zone, they are not present in the High Arctic. The purpose of the present study was to assess the potential of a generalist spider species, the Arctic wolf spider (*Pardosa glacialis*), to serve as a sentinel of Hg pollution in the Arctic. In summer 2022, we collected 1460 wolf spiders and 8090 emergent aquatic insects (Chironomidae) from six ponds in Northwest Greenland (centered around 76.5° N, 68.8° W). Spiders and insects were composited by body size and collection site. MeHg concentrations for spiders and insects ranged from 260 – 690 ng/g dry weight (dw) and 51 - 130 ng/g dw, respectively. Spider Hg concentrations were strongly correlated with insect Hg concentrations ($R^2 = 0.78$), suggesting that wolf spiders can be used as sentinels of Hg contamination in Arctic lentic systems, and had Hg concentrations exceeding risk thresholds for arachnivorous songbirds.

5.11.P-Th-071 Riparian Consumers Offer New Insights into Metal Exposure in the Mining Impacted Clark Fork River, Montana

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Mining contamination poses significant threats to water quality and fisheries throughout the world however impacts to riparian ecosystems are poorly understood. Riparian consumers such as spiders and songbirds are dependent on freshwater ecosystems for food and habitat. Insect communities that reside in riparian soils impacted by mine waste are unique from those that reside in corresponding stream channels, yet both can accumulate metals and transfer them to riparian consumers. The different assemblages of insects and metals from these terrestrial and aquatic habitats can drive distinct risks to consumers of metal laden insects. Here we will present

preliminary findings of three ongoing coordinated studies investigating potential metal exposures in riparian consumers of the upper Clark Fork River, Montana including riparian spider and songbird communities and tree swallow colonies occupying nesting boxes. Riparian consumers were found to have elevated metals in tissues (whole spider, blood for birds) that reflect metal exposure pathways associated with historic mining (arsenic and lead) and the target of remedial actions meant to protect aquatic life. However, riparian consumers were also elevated in metals (selenium, mercury) not included in the remedial activities. These preliminary findings support the idea that risks to aquatic life may not reflect risks to riparian consumers.

5.11.P-Th-072 Mixed Pesticide Exposure Results in Transport of Neonicotinoid Insecticides into Riparian Food Webs and Alterations to Insect and Spider Microbiome Communities

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Aquatic ecosystems within agricultural basins receive mixed pesticide inputs from surface and subsurface pathways, resulting in chronic exposure for aquatic insects. The AltEn bioenergy plant generated ethanol from feedstock by recycling roughly 95% of North America’s excess pesticide-treated seed corn, generating 85,000 tons of wet cake (spent grains) over six years. The pesticide-contaminated wet cake was stored on-site and then applied to surrounding farmland. Aquatic insects are critical links between aquatic and riparian food webs and simultaneously serve as a predominant exposure pathway for riparian insectivores. Here, we investigated the accumulation and fate of pesticides driven by bioaccumulation of pesticides in larval aquatic insects, the degree of pesticide retention in adult aquatic insects during metamorphosis, and trophic transfer of pesticides to riparian spiders eating adult aquatic insects. We found that non-seed treatment pesticides (e.g., atrazine) bioaccumulated in larval insects and were retained through metamorphosis in adult insects but were not found in spiders. In contrast, seed treatment pesticides (e.g., neonicotinoid insecticides) were retained through metamorphosis, present at relatively high concentrations in emerging adult insects, and biomagnified in riparian spiders. Interestingly, the microbial community composition of larval insects and riparian spiders followed a pattern consistent with pesticide exposure gradients. In contrast, adult insect microbiomes were determined by the taxonomy of the insect host. Our results demonstrate that aquatic-derived neonicotinoid exposure has a significant bioaccumulation and transport potential across the aquatic-riparian boundary via transport by adult insects, and pesticide exposure significantly shapes the microbiome community in larval insects and riparian spiders.

5.12.P-Mo The Intersection of Human Health and Environmental Risk Assessment: A One-Health Perspective

5.12.P-Mo-160 Human Health and Ecological Risk Assessment of the Herbicide Flumioxazin for the U.S. Forest Service

__Julie Rothrock__[1], Margaret Roy[2] and Dan Tekiela[3], (1)SRC, Portland, ME, (2)KTL, (3)U.S. Forest Service

The United States Department of Agriculture (USDA) Forest Service (FS) is responsible for protecting and managing natural resources on National Forest System lands. Forest management practices include the implementation of integrated pest and vegetation management programs to protect, restore, and maintain forest health. Pesticides are one tool used by the FS to prevent, control, or manage forest insects, diseases, and invasive plants. When considering the use of a pesticide on forest lands, the FS conducts a human health and ecological risk assessment (HHERA) to evaluate risk to FS personnel, the public, and the environment. FS forest managers and pesticide coordinators use the results of the HHERA to make decisions regarding specific applications of pesticides on forest lands throughout the U.S. Flumioxazin is a light-dependent, peroxidizing N-phenylphthalimide herbicide used for pre- and post-emergence control of terrestrial and aquatic weeds. The FS anticipates using flumioxazin to control vegetation in surface water bodies; control terrestrial weeds and invasive plants (e.g., in roadsides, utility corridors, hardscapes, and conifer and hardwood production areas); and for the restoration of desirable vegetation in wildlife management areas, recreational areas, fire rehabilitation areas, natural areas, prairies, and fire breaks. In 2020, the FS conducted an HHERA, with contractor support, to estimate the nature and degree of potential risks, both human health and environmental, associated with the use of flumioxazin in FS vegetation management programs. The HHERA focused on non-crop applications to soil, foliage, and waterbodies for representative flumioxazin products, characterizing risks to workers, the public, and terrestrial and aquatic environmental receptors. The FS obtained critical toxicity data from Data Evaluation Records (DERs) of studies submitted to the United States Environmental Protection Agency (EPA) and characterized risks for backpack, boom, and aerial spray methods for application of aqueous solutions to soil, foliage, and water bodies; instillation of aqueous solutions to water bodies; and soil applications of granular formulations. The risk assessors calculated non-cancer hazard quotients (HQs) using FS modeling tools for general and accidental exposures of workers; acute and chronic exposures of the general public, mammals, birds, fish, aquatic invertebrates, aquatic macrophytes, and algae; and exposure of terrestrial plants.

5.12.P-Mo-161 Behavior of Metal Ions Under Various Soil Watering Regimes

Marcus Bowersox[1], *Matt Ferguson*[2], *John Roberts*[1], *Kaleigh Mateleska*[1], *Allison Satterfield*[1] *and Theresa K Lopez*[1], *(1)Tetra Tech, (2)ATSDR - Region 7*

A better understanding on the implications of watering gardens/crops with water containing heavy metals and the potential long-term soil quality impacts is needed due to the sizable gap in the fields of soil science and environmental toxicology. The purpose of this study was to determine whether and to what extent metals from water may accumulate in soils. Specifically, we worked to determine whether metal-contaminated water, even at levels near the National Primary Drinking Water Regulations, pose additional human exposure pathways through soil. This study demonstrated that for arsenic and copper, soil metal concentrations were different between soil that was watered every other day, versus soil that was watered every day. Also, for these two metals, concentrations increased with increased water concentration and increased duration of watering. However, increased duration of watering did not always cause a significant increase in soil metal concentrations. These preliminary results suggest (depending on the metal type) that accumulation in soil through soil or gardens irrigated with metal-containing water (at the levels used in the study) may not pose an increased exposure pathway risk. For arsenic and copper, there was a positive relationship between water-metal concentrations and soil-metal concentrations. These results provide some indication on how various environmental parameters (e.g., organic carbon, soil source, water chemistry, etc.) may influence soil metal levels after repeated watering. Importantly, it is unknown how this may translate to the uptake of these metals by vegetation grown in these soils. Characteristics of soils, including organic carbon and pH, appeared to have some effect on the retention of metals from water, but it is unknown if they present a significant bioaccumulation potential in produce that would then be ingested by humans. A recommended next step would be to evaluate the uptake of metals by plants from soils that have been watered with metal-containing water. If the soils retain the metals and there is no accumulation in plants from the soil, and if proper gardening and housekeeping practices are followed, then there would be little risk of additional exposure to humans from the produce ingestion pathway. *The results in this presentation have not been formally disseminated by Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.*

5.12.P-Mo-162 Optimizing Chemical Hazard Assessment Through Integration of Environmental Mobility

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The Enhesa Toxicology Team manages a vast database of chemical hazard assessments (CHAs), encompassing more than 300,000 substances subjected to authoritative list screening and around 5,000 substances with verified chemical hazard profiles. The existing CHAs cover a spectrum of 23 human health endpoints, including Carcinogenicity, Mutagenicity-Genotoxicity, Developmental Toxicity, Reproductive Toxicity, Endocrine Activity, and various acute and chronic toxicity parameters such as Acute Toxicity (oral, dermal, and inhalation), Sensitization (Respiratory and Dermal), Irritation (Dermal, Eye, and Sensory), Systemic Toxicity, Neurotoxicity, and Aspiration Potential. Additionally, environmental fate endpoints like Persistence, Bioaccumulation, Aquatic Toxicity (Acute and Chronic), and Environmental Transformation Products, as well as physical endpoints such as Reactivity and Flammability, are considered. Each chemical receives an overall hazard categorization, employing a stoplight system, where high concern chemicals (e.g., carcinogens) are designated as red, low concern chemicals (e.g., water) as green, intermediate levels as yellow/yellow-green, and substances with uncertain hazard profiles as gray. The chemical hazard assessments consider both human and environmental health and the hazard category scores accordingly represent a one health approach to CHAs. Recent efforts identified overlaps between verified substances and prior research by Arp and Hale, yielding 1,287 common substances. McLoughlin et al. (2024) presented initial findings on chemicals with mobility classifications from Arp and Hale's work, At SETAC Europe 2024, proposing HC red definitions inclusive of mobility. Attendees received a QR code for feedback submission. This presentation will delve into further mobility classification results, outline plans for integrating mobility assessments into additional hazard categories, and extend an invitation to attendees for feedback submission via QR code.

5.12.P-Mo-163 Explainable Artificial Intelligence Models for Toxicity Prediction Using ToxCast Data in Cross-Species Adverse Outcome Pathway

Jaeseong Jeong*, Donghyeon Kim and Jinhee Choi, School of Environmental Engineering, University of Seoul, Korea, Republic of (South)*

Artificial intelligence (AI) models offer a new opportunity to assess the potential toxicity of a vast amount of environmental chemicals. However, many toxicity prediction models are inherently 'black boxes', making interpretation challenging for toxicologists and hindering the regulatory acceptance of these models. Inherently, the mechanisms leading to the onset of apical toxicity are complex, and in the absence of process evidence, it is difficult to trust the results, which may be, in the worst case, a mere coincidence. The adverse outcome pathway (AOP) framework holds promise in addressing this issue. AOPs can link molecular toxicity endpoint to human and ecotoxicity endpoint. In response, this study aims to develop explainable artificial intelligence models for predicting toxicity using ToxCast data within an cross-species AOP. Initially, we identified cross-species AOPs focusing on endocrine disrupting mechanisms. We then collected in vitro bioactivity data from ToxCast and in vivo toxicity data from eChemPortalDB and ECOTOX DB were gathered. These data were then integrated into cross-species AOPs and each assay-AOP pair underwent further assessment to determine relevance to taxa within ecosystems. Finally, machine learning models were developed to predict each mechanistic and regulatory endpoint-based toxicity. As a result, models for each key event and apical endpoint achieved acceptable performance. This study can serve as a guide for developing explainable machine learning models based on AOPs, addressing the current primary bottleneck in using AI models for chemical risk assessment.

5.12.P-Mo-164 Integrating One Health into Adverse Outcome Pathways: A Multifaceted Approach to Assessing Developmental and Neurotoxicity of Bisphenol A and Its Alternatives

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Due to the recognized health risks associated with Bisphenol A (BPA), there is a shift towards using alternative substances. However, the safety of these alternatives must also be assessed to ensure they do not present similar or unforeseen hazards. In this study, we developed a battery of assays based on the adverse outcome pathway (AOP) framework to evaluate developmental neurotoxicity (DNT) for BPA and its alternatives. We initially constructed an AOP network for DNT using AOP-Wiki and existing literature and linked specific *in vitro* and *in vivo* assays to this network to develop a comprehensive battery of assays for evaluating DNT. Screening with human embryonic and neural stem cells for BPA and its alternatives showed effects on intracellular calcium flux and oxidative stress. Notably, the alternatives demonstrated similar adverse effects to BPA, affecting key markers like IL-6 and BDNF expression, indicative of comparable neurotoxic profiles. Subsequent testing involved wild type and mutant strains of *Caenorhabditis elegans* (*ngl-1* and *nrx-1*), with BPA significantly affecting the behavior of both strains. To understand the broader ecological implications, we utilized SeqAPASS, cross-species extrapolation tools, to compare the target proteins corresponding to the genes involved in key events across species. This *in silico, in vitro*, and *in vivo* combined approach not only provides a rapid and efficient method for screening DNT but also offers critical insights into the ecological and health impacts of the alternatives.

5.12.P-Mo-165 Evaluation of Harmful Heavy Metal Levels in Sediments Accumulated on Urban Roads: Implications for Urban Health and Pollution Management Strategies

Muhammad Faisal *and Zai-Jin You, Dalian Maritime University, China*

Hazardous metals have a substantial negative impact on people's health. To explore the levels, pollution indexes, and source apportionment of threatening heavy metals that were found in the road-deposited sediments of a conventional urbanized region was main goal. By utilizing inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectroscopy (AFS), 116 samples were extracted and evaluated throughout the city. Findings demonstrated that the concentration of all heavy metals, except Cr and Ni, was significantly greater than their background levels, indicating severe urban pollution. Three pollution indices I_{geo}, PI_N, and health risk assessment has been calculated to find out the severity of pollution. Hg and Cd were dangerous to the city, according to I_{geo} and PI_N. Health risk assessment showed the ranking in order: $HQ_{ingestion} > HQ_{dermal} > CDI_{ingestion} > CDI_{dermal}$ for children as well as adults. The heavy metal's risk from road-deposited sediments was highest in children in the $HQ_{ingestion}$ and HQ_{dermal} groups. Pb and As found to be harmful to people's health as well. In the city, road-deposited sediments were contaminated for multiple reasons, which the UNMIX model identified. Factor 1, which constituted 9.85% of the pollution, was attributed to natural sources. Factor 2—copper mine tailing contamination—was 19.10%. Agricultural activity contributed 21.40% to Factor 3. Factor 4—air pollution—was accountable for 15.80% of the contamination. Factor 5, which deals with industrial activity, was the most significant at 34.8 percent. It is critical to immediately institute thorough pollution control in the town because metals are present in the dust from all of these factors.

5.12.P-Mo-166 Tandem Assessment of Human Exposure and Ecological Exposure Using the PROduction-To-EXposure (PROTEX) Model

Li Li[1]*, Dingsheng Li*[1]*, Alessandro Sangion*[2]*, Zhizhen Zhang*[1]*, Shenghong Wang*[1]*, Jon A. Arnot*[2] *and Frank Wania*[3]*, (1)School of Public Health, University of Nevada, Reno, (2)Arnot Research and Consulting, Canada, (3)Department of Physical and Environmental Sciences, University of Toronto, Canada*

Human and ecological exposures to synthetic chemicals involve chemical emissions from multiple lifecycle sources, multimedia environments at multiple scales, multiple sources of exposure, and multiple pathways of intake and elimination. These components are governed by the complicated interactions between chemical properties, environmental conditions, and human and ecological characteristics. Such multidimensionality and complexity warrant a systematic, mechanistic perspective in exposure modeling and necessitate a holistic approach to track a chemical's journey from the production line to its presence in both humans and ecological receptors. In this presentation, we introduce a state-of-the-science model named PROduction-To-EXposure (PROTEX), which supports characterizing the entire continuum from chemical production, lifecycle multi-source emissions, multi-scale multimedia environmental fate and transport, and multi-pathway exposure, to the presence in humans and other organisms. PROTEX builds on theories of substance flow analysis, multimedia fate and transport, bioaccumulation, and exposure and toxicokinetics, and it integrates mechanistic descriptions of various physical, chemical, biological, and socioeconomic processes. Its mechanistic nature allows PROTEX to be parameterized for a wide range of chemical substances (both neutral and ionizable chemicals) in a wide range of regional environments (e.g., the subtropical U.S., the Canadian side of the Lake Ontario basin, central China, and the western Baltic drainage basin) for a wide range of human populations (Whites, Blacks, Asians, etc.) and organisms (aquatic and terrestrial animals). When supplied with time-variant production or use data, PROTEX provides dynamic time-dependent estimates, allowing users to reconstruct the long-term history of chemical contamination. Outputs from PROTEX support science-based risk assessment of chemicals for both human health and ecosystem integrity. This presentation will demonstrate how PROTEX fulfills the simultaneous consideration of both human health and ecological health under the concept of One Health.

5.12.P-Mo-168 Optimization of Infrastructure Placement using a novel Disease Burden Reduction Model

***Theresa K Lopez**[1] and Sara MacClellan[2], (1)Tetra Tech, (2)Tetra Tech*

Diarrheal disease is a leading cause of malnutrition and stunting in children under 5 years of age, causing approximately 525,000 deaths per year in this age group worldwide. Diarrheal disease presents challenges to the economic well-being and growth of developing countries, as well as placing an economic burden on earners and to all households affected by diarrheal disease. To combat diarrheal disease in low socio-economic status urban settings, an exposure and risk-based model was constructed from in-country and literature-based information to determine current disease burden levels. The model was constructed also to predict disease averted by combinations of planned infrastructure improvements. Based on the Disease Burden Reduction (DBR) we developed and using an example location, a total of 120,000 annual diarrheal cases were estimated when accounting for non-reported cases, non-treatment-seeking cases, and mild/moderate cases. It is estimated that with proper water supply, sanitation, and drainage infrastructure for the whole city; and improved behavior regarding hygiene, up to 87 percent of the diarrheal disease can be averted, with planned improvements to water and sanitation infrastructure. Within areas receiving a concurrent planned infrastructure improvement, it is estimated that diarrheal disease could be decreased by a minimum of 30% from current rates, with additional reductions up to 80% over time as more households are joined to the water and sewer systems in this location. The reductions are due in part to reducing and eliminating the cultivation of crops within drainage channels that are used for sewage disposal, by providing a safe means of disposal for sewage waste. By using a risk-based model, the most beneficial placement of infrastructure could be selected, using diarrheal disease reduction as a measure of effectiveness. This cost-effective approach to evaluating disease data within a population is transferable to various situations and can incorporate human and ecological receptors. The approach is flexible and can be used in other countries as well as for other diseases, using location-specific data to optimize design for the greatest benefit. The inclusion of several different areas of practice (public health specialists, risk assessors, and engineers) provided a stronger solution to the problem.

5.12.T The Intersection of Human Health and Environmental Risk Assessment: A One-Health Perspective

5.12.T-01 Beach Monitoring: An Evolving Practice at the Intersection of Environmental and Public Health

*Shannon Briggs[1], **Ryan R Otter**[2], Mike Eslick[3], Alexis Porter[4], Brian Scull[4], Brendan May[4] and Leah Wilson[4], (1)Michigan EGLE, (2)Annis Water Resources Institute, Grand Valley State University, (3)Public Health Muskegon County, (4)Grand Valley State University*

Beaches are an important habitat that at the intersection of environmental and public health. Our research team has over 20 years of sampling data from Muskegon County, Michigan, USA including Lake Michigan and inland beaches. This project has been, and continues to be, a coordinated effort between academia, Federal, State government, and local health departments. In this presentation, past results and specific case studies will be highlighted as well as how new emerging issues (e.g., harmful algal blooms, PFAS) and new technology (e.g., ddPCR) have been integrated into the project over time to provide insights into both environmental and public health. Specific endpoints to be discussed include results for E. coli using two separate methods (Colilert-18 and qPCR) and microbial source tracking (MST) markers related to humans, dogs, cows, pigs, and gulls. This project directly aligns with principles of the One Health model and shows how productive coordination between disciplines can help take scientific results and turn them into actionable decisions.

5.12.T-02 Microplastics in Marine Mammals: A One-Health Perspective to Inform Human Health and Environmental Risk Assessment

Bonnie Ertel[1], *Wayne E McFee*[2] *and Austin D. Gray*[3], *(1)University of South Carolina, Columbia, United States, (2)Coastal Marine Mammals Assessment Program, National Oceanic and Atmospheric Administration National Centers for Coastal Ocean Science, (3)Department of Biological Sciences, Virginia Tech*

As long-lived apex predators living in the coastal zone, marine mammals can be studied as ecosystem sentinels to provide an early indication of risks to human health. A local population of Tamanend's bottlenose dolphins (*Tursiops erebennus*) resides within the urbanized estuary surrounding Charleston, South Carolina. This population of wild dolphins exhibits high site fidelity and has been well studied as ecosystem sentinels for other anthropogenic contaminants such as PCBs, PBDEs, and PFOS. This research aims to elucidate the exposure pathways and biological fate of microplastics, including tire wear particles, in the coastal region using local bottlenose dolphins as ecosystem sentinels. Dolphins' gastrointestinal tracts have been collected during routine necropsies of stranded animals since 2016 and processed to quantify microplastic particles, which are then identified using raman spectroscopy. Preliminary results (n=38) indicate high levels of microplastic ingestion, ranging from 76-3841 total microplastics per dolphin (1083 ± 153 mean ± SE), with polypropylene and nylon being the most common polymers. This ongoing research will continue to monitor microplastics in this population and elucidate any spatial, temporal, dietary, or demographic variables influencing microplastic exposure. Additionally, we are quantifying microplastics in the preferred fish prey of local dolphins: Atlantic Croaker, Silver Perch, Spot, Star Drum, and Striped Mullet (n=20 of each species) to understand the trophic transfer of these contaminants to local seafood consumers, including humans. By taking a One-Health perspective, this study will help inform risk assessments by determining environmentally relevant exposure levels and ecologically relevant transfer pathways of microplastics in the coastal environment.

5.12.T-03 Pathological Effects of Persistent Organic Pollutants in Sentinel Fish Sampled in an Arctic Site Influenced by Military Contamination

Maria Eugenia Sancho Santos[1], *Renee Jordan Ward*[2], *Frank von Hippel*[2], *Danielle Dillon*[3], *Amina Salamova*[4], *John H Postlethwait*[5], *Elise Contreras*[3], *Staci L. Capozzi*[6], *Parinya Panuwet*[7], *Ruth Bremiller*[5], *Pamela Miller*[8], *David Carpenter*[9] *and Charles Buck*[3], *(1)University of Arizona, United States, (2)Northern Arizona University, United States, (3)Northern Arizona University, (4)Indiana University, (5)University of Oregon, (6)Paul H. O'Neill School of Public and Environmental Affairs, Indiana University, Bloomington, (7)Emory University, (8)Alaska Community Action on Toxics, (9)University at Albany-State University of New York*

Formerly utilized defense (FUD) sites in Alaska are significant sources of pollution containing Persistent Organic Pollutants (POPs). These compounds are known for their bioaccumulative and biomagnifying properties as well as their potential for endocrine disruption. FUD sites are often neighboring arctic rural communities, reliant on subsistence food. The high incidence of negative health outcomes in these indigenous societies may be related to their exposure to POPs through their diet and environment. This study investigated the possible pathological impacts associated with POPs levels, utilizing two native fish species, ninespine stickleback (*Pungitus pungitus*) and Alaska blackfish (*Dallia pectoralis*), as sentinels. The fish were sampled upstream and downstream of the FUD site in Sivuqaq, St. Lawrence Island, Alaska. Tissue analyses revealed higher POPs concentrations in individuals from the contaminated sites compared to reference locations. Downstream Alaska blackfish had fewer thyroid follicles and less thoracic area covered by pigmented macrophage aggregates compared to the ones collected upstream. In ninespine stickleback, the number of thyroid follicles showed variation among the sampling years. Despite varied trends, the presence of thyroid disparities in both sentinel species suggests potential disruption of normal processes in this endocrine organ. Our results underline the need for attention about the possible health impacts in arctic residents.

5.12.T-04 The Behavior of Metal Ions Under Various Soil Watering Regimes and Next Steps

Matt Ferguson[1] *and Marcus Bowersox*[2]*, (1)Office of Community Health and Hazard Assessment, (2)Tetra Tech*

A better understanding on the implications of watering gardens/crops with water containing heavy metals and the potential long-term soil quality impacts is needed due to the sizable gap in the fields of soil science and environmental toxicology. The purpose of this study was to determine whether and to what extent metals from water may accumulate in soils. Specifically, we worked to determine whether metal-contaminated water, even at levels near the National Primary Drinking Water Regulations, pose additional human exposure pathways through soil. This study demonstrated that for arsenic and copper, soil metal concentrations were different between soil that was watered every other day, versus soil that was watered every day. Also, for these two metals, concentrations increased with increased water concentration and increased duration of watering. Organic carbon's effect on arsenic soil-metal concentrations had an inverse effect when compared to the other metals. These preliminary results suggest (depending on the metal type) that accumulation in soil, through soil or gardens irrigated with metal-containing water (at the levels used in the study) may not pose an increased exposure pathway risk. For arsenic and copper, there was a positive relationship between duration of watering and soil-metal concentrations, and between water-metal concentrations and soil-metal concentrations.
These results provide some indication of how various environmental parameters (e.g., organic carbon, soil source, water chemistry, etc.) may influence soil metal levels after repeated watering. Importantly, it is unknown how this may translate to the uptake of these metals by vegetation grown in these soils. Characteristics of soils, including organic carbon and pH, appeared to have some effect on the retention of metals from water, but it is unknown if they present a significant bioaccumulation potential in produce that would then be ingested by humans. Our next steps include use of a similar watering technique and soil types, but also includes additional soil types, evaluates plant (edible produce) uptake, and also includes the addition of other contaminants. *The findings and conclusions in this presentation have not been formally disseminated by Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.*

5.12.T-05 Increasing Understanding of Human Exposure to Polycyclic Aromatic Hydrocarbons by Analyzing Human Blood Serum

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Exposure to polycyclic aromatic hydrocarbons (PAHs) has long been considered a risk to human and environmental health. Known to be toxic, mutagenic, and/or carcinogenic, PAH exposure occurs due to emissions from biomass combustion such as wildfires, petrochemical extraction, processing and use, as well as from various industrial processes. Currently, state of the art PAH exposure is approximated by measuring several hydroxy substituted PAHs (OHPAHs) in human urine. The human body can metabolize PAHs into OHPAHs for removal, and several isomers of hydroxy naphthalenes, fluorenes, phenanthrenes, and pyrene are common targets to assess PAH exposure in humans. Over the last ten to fifteen years, these same OHPAHs (and many others) have been measured in air, water, and soil samples. The measurement of these compounds in environmental samples presents an issue of primary exposure in humans versus metabolism assumed in past human urinalysis for OHPAHs. In order to better understand exposure to PAHs, we have developed a method for PAH quantification from human blood serum which uses gas chromatography coupled with high efficiency tandem mass spectrometry (GC-QqQ). Our method can quantify 20 PAHs in 500 µL human blood serum using liquid / liquid extraction followed by automated solid phase extraction to cleanup lipid and proteins from extracts. The extraction method uses acetonitrile to denature proteins, and a mixture of hexane : dichloromethane (5:1) as extraction solvent. The preliminary average extraction efficiencies for 20 PAHs average 117% (± 20%). Surrogate recoveries, for the isotope dilution method, averaged 63% (± 23%) of the spiked concentrations. This method is currently being optimized and will be used on bio-banked human serum samples for validation. Once this method is finalized, we will use it to determine the extent of PAH exposures in Californians, identify vulnerable populations, and assess temporal/seasonal trends in exposure. *The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.*

5.12.T-06 Seasonal Variations in Fine Particulate Matter (PM2.5), Black Carbon, and Oxidative Potential: A Tennessee Study

Voke Tonia Aminone[1]*, Gao Rong Lin*[2]*, Emma Lee*[3] *and Courtney Roper*[4]*, (1)Biomolecular Sciences, University of Mississippi, (2)Department of Health, Exercise Science and Recreation Management, University of Mississippi, (3)Department of Chemistry, University of Mississippi, (4)BioMolecular Sciences, University of Mississippi*

Seasonal Variations in Fine Particulate Matter ($PM_{2.5}$), Black Carbon, and Oxidative Potential: A Tennessee Study

Voke Tonia Aminone[1]*, Gao Rong Lin*[2]*, Emma Lee*[2]*, Courtney Roper*[1] *University of Mississippi, MS, USA*

Exposure to air pollutants, including fine particulate matter ($PM_{2.5}$), is a major global health risk. Oxidative stress due to $PM_{2.5}$ exposure results in elevated reactive oxygen species (ROS) leading to various health effects associated with $PM_{2.5}$ exposure. The measure of the intrinsic ability of

$PM_{2.5}$ to cause oxidative stress is known as oxidative potential (OP); hence, it is a health-based metric. The goal of our study was to identify seasonal differences in $PM_{2.5}$ and its components across locations in Tennessee. Black carbon (BC) analysis was performed on $PM_{2.5}$ filters donated by the Tennessee Department of Environment and Conservation (TDEC). The filters were then extracted to obtain $PM_{2.5}$ in solution for oxidative potential analysis using the DTT assay. Preliminary findings showed variations for $PM_{2.5}$ and BC as it ranged from 2.0 to 21.5µg/m³ and 0.01 to 4.9µg/m³ in the fall of 2014 (23rd September to 20th December). The oxidative potential of $PM_{2.5}$ from fall ranged from 0 to 1.29 pmol/min/m³. While in the winter of 2015, $PM_{2.5}$ ranged from 1.5 to 20.7µg/m³ and BC ranged from 0 to 5.4 µg/m³. We observed no significant differences in $PM_{2.5}$ concentrations between the fall and winter seasons, but we saw significant differences in BC concentrations between locations, such as between Kingsport, Harriman, and 8 other locations throughout Tennessee (Two-way ANOVA, $p\text{-value} \leq 0.05$). $PM_{2.5}$, BC, and OP analysis for additional seasons is underway. We expect to observe variations in seasonal levels of $PM_{2.5}$, BC concentrations, and oxidative potentials across different locations and seasons. We saw that the daily $PM_{2.5}$ concentrations were below the Environmental Protection Agency (EPA) Daily Ambient Air Quality Standards of 35µg/m³; however, the BC concentrations, which are not regulated, were above the concentrations reported by epidemiological studies and have previously been demonstrated to have adverse health impacts. This research highlights the importance of oxidative potential as a potentially comprehensive indicator for assessing the toxicity of $PM_{2.5}$ exposure on human health.

5.12.V The Intersection of Human Health and Environmental Risk Assessment: A One-Health Perspective

5.12.V-01 Predicted No-effect Concentrations of Down-the-Drain Waterpipe Wastewater Chemicals of Toxicological Concern

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Waterpipe (WP) tobacco smoking has emerged as a global phenomenon with increasing use prevalence, raising concerns about environmental impacts from WP waste disposal. FDA is required to assess the environmental impacts of its tobacco regulatory actions per the National Environmental Policy Act. Understanding the environmental impacts of WP waste disposal is a crucial aspect of such environmental assessments. We calculated the predicted no-effect concentration (PNEC; µg/L) based on available experimental bio-assay data on aquatic toxicity endpoints from the EnviroTox database and tools (Version 2.0.0) for 26 selected hazardous chemicals found in WP wastewater. We also calculated the ecological toxicity threshold of concern (eco-TTC) to establish a concentration expected to have a de minimis effect on aquatic communities for these 26 hazardous chemicals using the estimated PNEC values through logistic distribution function. Results indicate that the concentrations of 17 out of 26 selected hazardous chemicals in the WP wastewater exceeded the PNEC values. The estimated eco-TTC value for the 26 hazardous chemicals was 0.32 (µg/L; 0.04 - 1.28), which represents the ecological exposure limit expected to have a *de minimis* probability of negative effects on aquatic communities. For four chemicals (antimony, acrolein, manganese, benzene), the concentrations in hookah wastewater exceeded EPA's drinking water quality criteria. Antimony and manganese are heavy metals that are toxic to aquatic organisms and can bioaccumulate in the ecological food chain. Additionally, exposures to acrolein through drinking water can cause gastrointestinal irritation and benzene is a known human carcinogen. There is no safe level of exposure to benzene. The outcomes of this study represent only the hazard characterization portion for a screening risk assessment of WP wastewater chemicals. Results indicate that the presence of 17 chemicals in WP wastewater with concentrations exceeding PNEC values for aquatic organisms has potential ecotoxicity and water quality effects. However, to better understand the ecological risks of ‘down the drain’ WP wastewater disposal more thoroughly, future efforts could focus on exposure characterization by modeling the fate and transport of these chemicals while incorporating wastewater treatment facility level filtering. Our results also highlight the importance of awareness on the proper disposal of WP wastewater in residential and retail settings.

5.13.P-Mo Treatment and Characterization of Permian Produced Water to Support Re-Use

5.13.P-Mo-169 Evaluating Multiple Whole Effluent Toxicity Test Species to Support Application of Treated Produced Water for Beneficial Use

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Energy extraction operations in the Permian basin result in substantial volumes of produced water (PW) that is managed using a range of treatment, recycle and disposal options. In addition to the need to manage these large volumes of PW, there is a need for water in the Permian region so there are generally aligned motives to support beneficial use of treated PW outside the oil field. As a result beneficial use such as discharge to surface water, or land, is being evaluated by the industry, regulators, and other stakeholders. This presentation will focus on the evaluation of a range of common and non-standard whole effluent toxicity (WET) test species, such as fish, and *daphnia* vs terrestrial species or relevant in vitro cell-based assays. The analysis of water chemistry of the treated PW indicates that ammonia is a potential major stressor in the treated PW. The available treatment systems appear to be effective at removing salt, organics, and other inorganics including ammonia. It has been observed that some amount of ammonia can be transferred through treatment systems that do not have targeted ammonia removal post-treatment units. Available toxicity data were compared to known toxicity thresholds for ammonia confirming that this is the major stressor in treated PW and that other constituent classes like hydrocarbons, and metals, are not contributors or are very minor contributors. The available *in vitro* toxicity data that focus on the impact of organics appears show low toxicity, consistent with this general finding. Lastly, the tested terrestrial species appear to be less sensitive to treated PW with elevated ammonia than the aquatic species evaluated. These results are being used to inform treatment systems (e.g., ammonia stripping), as well as end use evaluations (e.g., surface water discharge vs. land application).

5.13.P-Mo-170 Simulation of Mechanical Vapor Compression Desalination for Produced Water Treatment in the Permian Basin

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The Permian Basin, a major oil and gas producing region, faces significant challenges in managing high Total Dissolved Solids (TDS) produced water with beneficial uses becoming increasingly important to reduce deep well disposal. This study evaluates the performance of Mechanical Vapor Compression (MVC) desalination of hypersaline solutions. MVC desalination is a closed-loop process that operates at low temperatures and under partial vacuum conditions. In this study, we evaluated a typical Permian basin wastewater feed based upon the chemistry of 11,000 water samples from both the Midland and Delaware basins. Median Midland basin produced water contained 130,000 mg/L TDS while the median Delaware basin produced water containing 70,000 mg/L TDS. We compared the specific energy consumption (SEC) and product water quality as a function of recovery ratio (RR-product water/feed water) and feed composition. Key concerns were the fate of ammonia nitrogen, trace hydrocarbons and carbonates in the feed. The SEC ranged from 14 kWh/m^3 to 28 kWh/m^3 for Midland basin produced water and 12 kWh/m^3 to 18 kWh/m^3 for Delaware basin, with volatile elements such as CO2 and NH3 significantly influencing the results. Excluding CO2 from evaluations led to an underestimation of SEC by 7-10% for higher recovery operations. Results on the distribution of constituents found that approximately 80% of ammonia remained in the brine, while nearly all the carbonate concentration transitioned to the distillate as CO2 gas. Although much of the ammonia was isolated in the brine waste stream the product stream contained sufficient ammonia to limit beneficial uses of the water without further treatment. The loss of dissolved inorganic carbon to the vapor phase led to no calcium carbonate precipitation despite its presence at near saturation in the feed. Trace hydrocarbons distributed between the distillate and brine streams according to their volatility. This study provides valuable insights into MVC desalination processes for treating high-TDS wastewater, examining both specific energy consumption (SEC) and component distribution. The findings could have significant implications for design and operation of MVC systems in the Permian basin including pre- and post-treatment requirements.

5.13.P-Mo-171 Evaluating Potential Effects of Produced Water on Plants and a Nitrifying Microbial Community

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The life cycle of oil and gas production produces large volumes of wastewater, referred to as produced water (PW). These fluids are increasingly being considered as a resource for irrigation, particularly in water-stressed parts of the country. However, information is limited regarding potential environmental effects that could arise from such reuse strategies. In this study, we evaluated the effects of PW on seed germination and microbial nitrification. PWs from five oil wells in California were tested along with an equal mixture of PW from each well and a synthetic, laboratory-manufactured PW. Tested serial dilutions ranged from 0.125% to 100% PW dissolved in well water that was mixed with deionized water to a hardness of about 100 mg/L as CaCO3; treatment dilutions had conductivity values of 50 to 40,000 µS/cm, respectively. For

seed germination, three species were tested: lettuce (*Lactuca sativa*), wheat (*Triticum aestivum*), and alfalfa (*Medicago sativa*). Seeds were irrigated with PW dilutions and put in an environmental chamber in the dark at 22°C for 10 days. Germination progress was assessed every two days. Seed germination sensitivity to PW varied by species with lettuce being the most sensitive and wheat the least sensitive species tested; the 50% effect concentration (EC50) among tested waters for seed germination averaged 30-40% dilution for lettuce and alfalfa but wheat was so resilient that some seeds germinated in 100% PW and an EC50 could not be determined. For microbial nitrification, we exposed a diverse nitrifier-enriched microbial (NEM) community to the PW treatments in the presence of ammonia. Exposures occurred in an incubator in the dark at 22°C. We observed that PW dilutions ≥ 6.25% (2,500 µS/cm) fully inhibited nitrification; PW dilutions below 2.5% (940 µS/cm) did not inhibit nitrification but slowed it in a dose-dependent manner. Next steps will include assessing PW effects on plant growth and contaminant uptake, soil chemistry, microbial community composition, and field soil microbiomes.

5.13.P-Mo-172 Non-Targeted Organic Micropollutant Characterization of Permian Basin Produced Water Treated Via Membrane Distillation Processes

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Thermal and membrane-based treatment technologies have been widely used in oil and gas produced water (PW) treatment due to their tolerance to high salinity and high desalination capabilities. Membrane distillation (MD) incorporates a combined thermal and membrane contaminant separation technique, delivering high-quality treated water for potential beneficial reuse. Even though MD processes could achieve near complete removal of salts, they can be less effective on organic micropollutants (OMPs) depending on the physical-chemical properties of the pollutants. This study assessed an emergent photocatalytic MD process against a classic vacuum MD process for removing OMPs from pre-treated PW collected from the Permian Basin using liquid chromatography mass spectrometry-based non-targeted organic analysis method. Once the suspect OMPs are tentatively identified, the putative toxicity of the suspects was predicted using a state-of-the-art cheminformatics tool developed by the United State Environmental Protection Agency. Sixty-five different OMPs were tentatively identified in pre-treated PW. The distillate from the vacuum MD and photocatalytic MD contained 25 and 29 OMPs, respectively, confirming their ability to separate organic contaminants along with salts. Residual OMPs in the distillate samples were associated with certain human health-based toxicities and ecotoxicities. Results necessitate follow-up targeted chemical and toxicity assessments to quantify the concentrations of the identified OMPs and actual dose-based toxicity to ascertain the suitability of the treated water for reuse. Overall, non-targeted OMP analysis is a valuable tool to dispel misconceptions about treated PW by identifying unknown contaminants and their related toxicity effects, enabling educated decisions regarding PW reuse.

5.13.P-Mo-173 Evaluating Toxicity Induced by Treated Produced Water on Human Cell Lines

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The oil and gas extraction process generates large volumes of produced water (PW) due to hydraulic fracturing. PW has a complex composition and constitutes high levels of total dissolved solids, oil, grease, radionuclides, heavy metals, dissolved organic matter, salinity, and hydraulic fracturing chemicals such as biocides and surfactants. Even with the most sophisticated water treatment technologies, we cannot guarantee the safe discharge and reuse of PW without comprehensive toxicity assays, as the treated water may still contain organic compounds that cannot be identified and quantified. This study investigated the toxicity effects of untreated and treated PW from thermal desalination technology from the Permian basin. The impacts of produced water on cellular metabolic activities, plasma membrane damage, apoptosis, and endocrine-disrupting effects were assessed using human intestinal epithelial (Caco-2) cells, human breast cancer cells (MCF-7), and human embryonic kidney cells (HEK293). Our results indicate that exposure to PW thermal distillate showed no significant impact on cell viability and membrane integrity, whereas untreated PW showed a significant effect on human cells. No oxidative stress or apoptosis was found to be induced by the thermal distillate. However, our gene expression study found the residual organic compounds in thermal distillate induced the activation of aryl hydrocarbon receptors (AhR), which may elicit a toxic response. The distillate after granular activated carbon and zeolite post-treatment was ineffective in the activation of AhR genes, indicating the necessity of post-treatment after thermal distillation. Overall, we demonstrated that PW, after appropriate treatment, has good potential to be discharged or reused safely.

5.13.P-Mo-174 Evaluation of the Toxicity of Produced Water Chemicals Using Nematode-Based Bioassays

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Safe disposal of produced water (PW) poses a significant challenge due to presence of hydrocarbons, salts and heavy metals. Remediation technologies are being explored to improve the quality of produced water and minimize potential toxicity. However, there is a need for biological testing methods to perform risk assessment of remediated produced water. Thus, developing a robust methodology for multiple toxicity end points is vital for informed decision-making on disposal or reuse of produced water. In this study, we use an organism-on-chip technology that integrates the nematode *C. elegans*, microfluidics and visual AI. *C. elegans* is a soil-dwelling nematode that has been extensively used as a biological model for ecotoxicity due to its short 3-week lifespan and 60-80% similarity with the human genome. The organism-on-chip technology involves culturing *C. elegans* in microfluidic chambers, daily exposure of chemicals supplemented in their diet, acquiring thousands of images to capture their response and using visual AI to extract the toxicity data. We used ammonium chloride (NH_4Cl) and sodium chloride (NaCl) as test candidates to develop the methodology for toxicity testing with a focus on development, reproductive, and chronic toxicity. Ammonium chloride in the concentration range of 300 – 30,000 mg/L was tested at two pH environments (pH 6.2 and 7.5) for toxicity due to dissolved ammonia (NH_3). NaCl was tested for a dose range of 300 mM to 1 M. We find that at pH 6.2, for NH_4Cl concentrations below 3000 mg/L there were no developmental defects. In terms of reproductive toxicity, no significant effects on brood size were observed for concentrations below 10,000 mg/L. Likewise, in the 6-day chronic toxicity study, we did not observe any lethality or locomotory defects for concentrations below 10,000 mg/L. With regards to nematode studies at pH 7.5, we did not observe negative effect on development, reproductive and viability. In the study with NaCl, developmental defects were observed at and above 300 mM concentration. In terms of reproductive toxicity, no significant effect on brood size was observed below exposure concentration of 700 mM. While we did not observe any lethality below 1 M, we observed locomotory defects at all the tested exposure concentrations. In summary, our approach and findings are the first step to assess toxicity due to chemicals in produced water, using a biological model and evaluation of multiple tox end points.

5.13.P-Mo-175 Remediation of Produced Water Impacted Soils Using Enhanced Evaporative Flux

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Incidental release of produced waters at oil and gas sites can have potential detrimental impacts on surrounding plants and soils. The most common remediation practice, involving excavation and disposal of impacted soil and supply of backfilled soil, is disruptive and expensive. To alleviate this burden, an aqueous solution containing crystallization modifiers can be applied to the soil to delay onset of salt precipitation and enhance the natural evaporative flux process, allowing salt crystals to be harvested at the soil surface. Prior studies focused on remediation of sodium chloride solutions in controlled settings. This research examines the efficacy of ferrocyanide as a crystallization modifier for remediation of produced water impacted soils, investigating the influence of soil class, produced water chemistry, and environmental conditions using experimental and modeling techniques. Most importantly, we tackle the challenging problem of how to apply the remediation fluid in an automated fashion to achieve removal efficiencies > 80%. Results of this work can inform field application of enhanced evaporative flux treatment and potential need for co-treatments to overcome application challenges.

5.13.T Treatment and Characterization of Permian Produced Water to Support Re-Use

5.13.T-01 Challenges and Opportunities for Produced Water from Oil and Gas Operations in the Permian: Setting the Scene for Beneficial Reuse of Treated Produced Water

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Produced water (PW) is the formation water extracted with oil and gas resources being produced from the subsurface, and can include the flowback water from the operation. This presentation aims to provide context of the volumes, water quality characteristic of PW in the Permian area (western TX and southeast NM), regional reuse opportunities, implications for required treatment systems, and safety evaluation of beneficial uses outside the oil field. The Permian basin is an important global source of oil and gas energy responsible for about 50% of the total US oil production in 2022 and will continue to support the ongoing energy transition. The production operations cover more than twenty operators, multiple regulatory jurisdictions, and multiple public and private stakeholders. In the Permian area, PW is developed at higher volumes than the oil production, around 5: 1 water:oil, which results in substantial volumes on the order of 20-25 million barrels per day (MBPD). Current industry practice is to recycle the PW in its operations to limit freshwater use. Over 20% of PW is recycled within the industry, and the remaining water is disposed in salt water disposal wells. For perspective these volumes are comparable to the typical flow rates of the major local river systems (Rio Grande 49 MBPD, Pecos River 11 MBPD). A major driver for beneficial use of water outside of the oil field is that the substantial water demands in this water scarce region to support local commercial, industrial, and agricultural uses. An additional concern is that recent studies have identified associations between saltwater disposal operations and induced seismicity. A key challenge with treating PW is that it is three- to five-times saltier than typical seawater, with Total Dissolved Solids (TDS) that can exceed 100,000 mg/L. Therefore, treatment often involves membrane or thermal distillation to achieve water quality that would support freshwater aquatic life. The lead strategy using these technologies is to extract freshwater from the PW, leaving the dissolved salts and other impurities with the residual waste stream. With this strategy, only about 40%-50% of freshwater can be extracted to avoid complications with precipitation of NaCl (halite). Furthermore, the presence of geogenic contaminants such as hydrocarbons, ammonia, and metals need to be addressed to ensure acceptable quality that enables water reuse opportunities.

5.13.T-02 Reuse of Produced Water in Agriculture

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One alternative to conventional disposal practices is the reuse produced water (PW) in agriculture. However, PW has been reported to contain contaminants of concern from the irrigation standpoint. We designed a greenhouse irrigation study, conducting an in-depth characterization of the effects of PW irrigation on soil microbiome, soil health, crop agronomic traits, soil metagenome. We also assessed the water used for the study in terms of its chemistry, toxicity, techno-economics, and statewide volume. Various reuse options, including crop irrigation, were also evaluated. Water quality results for our conventional PW indicate that most water quality parameters were either near or slightly above suggested guidelines of the potential end users. This gives great promise for beneficial reuse with minimal treatment necessary.

For our crop irrigation study, an apogee spring wheat crop was used and received the following water treatments: tap water **(Control**), undiluted produced water (**PW100**), 50% diluted produced water (**PW50**). Following wheat harvest, Soil Management Assessment Framework (SMAF) was employed to assess soil health, determining soil chemical, biological, and physical and overall Soil Health Indices (**SHI**). Soil samples were also used for metagenomic sequencing to analyze changes in microbial communities' functional potential in response to PW irrigation. Above-ground biomass (seeds and stems) was collected for yield and biomass comparisons. Wheat kernels and leaf tissue underwent metabolome analysis utilizing liquid chromatography mass spectrometry and gas chromatography mass spectrometry techniques. Above-ground biomass response comparison showed no significant differences between treatments, while the comparison of grain yield indicated that the PW treatment produced the highest yield. The overall SHI for the PW treatments were lower than for both the tap water. The carbonate containing PW was found to increase the pH of the soil as compared to tap water. Soil metagenome analysis revealed that PW100 irrigation led to an increase in abundance of genes associated with Hydrocarbon degradation, CRISR-CAS, Denitrification, ABC transporters, Glycoside hydrolases. Collectively, our findings suggest that PW used in this study has the potential to serve as a non-traditional source for agricultural irrigation, however studies consisting of multiple growing cycles in field conditions are required to evaluate long term effects of PW on soil and crop health.

5.13.T-03 Beneficial Reuse of Conventional Produced Water: A Denver-Julesburg Case Study

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Water scarcity in the United States is increasingly impacting diverse end users, including agriculture. Therefore, urgent exploration of alternate water sources is needed to alleviate the current strain on freshwater resources. A potential solution is produced water (PW), a byproduct from oil and gas production, which is generated in high volumes, but is rich in salts and organic compounds that complicate its utilization as an alternative water source. PW management strategies include discarding through evaporation ponds or deep well injection, which can have negative impacts on the surrounding environment. However, studies show that PW treatment decreases the amount of contaminants and may allow for PW to be used to supplement existing water supplies. Therefore, we aimed to determine the level of treatment necessary for beneficial reuse of PW in Colorado. We investigated 18 conventionally drilled wells across the D and J Sands in the Colorado Denver-Julesburg Basin to understand compositional variation and potential impact of generated water on crop irrigation, cattle irrigation, and recreational use. Using high resolution mass spectrometry and chromatography, we gathered qualitative and quantitative information about common PW parameters, such as diesel and gasoline range organics, metals, and surfactants. To support beneficial reuse, standard toxicity tests (i.e. LC_{50} assessment with *daphnia magna*), were performed. Finally, economic analyses were conducted to establish the feasibility of PW reuse within the specified industries. This study highlights the potential for the oil & gas industry to lower its environmental footprint while easing local water stress in industries including agriculture and recreation.

5.13.T-04 Chemical and Toxicological Characterization of Produced Water and Surrounding Surface Water in the Permian Basin

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A comprehensive understanding of the chemical composition and toxicological behavior of produced water (PW) is critical to advance the knowledge and tools for effective PW management, treatment, risk assessment, and feasibility for beneficial reuse. This study provides

the first step to better understanding PW quality and developing beneficial reuse programs that protect human health and the environment. In total, 46 PW samples from unconventional operations in the Permian Basin and ten surface water samples from the Pecos River in New Mexico were collected for quantitative target analyses of more than 300 constituents. Water quality analyses of Pecos River samples could provide context and baseline information for the potential discharge and reuse of treated PW in this area. Temporal PW and river water quality changes were monitored for eight months in 2020. PW samples had total dissolved solids concentrations ranging from 100,800-201,500 mg/L. Various mineral salts, metals, oil and grease, volatile and semi-volatile organic compounds, radionuclides, ammonia, hydraulic fracturing additives, and per- and polyfluoroalkyl substances were detected at different concentrations. A suite of in vitro toxicity assays using various aquatic organisms revealed that high salinity was the foremost toxicological driver in PW, followed by the organic contaminants. In vitro toxicity assays indicated different sensitives of the aquatic organisms to the chemical components present in PW. This study outlines baseline analytical information on understanding PW quality and proposes a risk-based pathway for PW treatment and fit-for-purpose reuse.

5.13.T-05 Reuse-Oriented Treatment Train for the Beneficial Re-Use of Produced Water (PW): Chemical and Toxicological Characterization of the Effluents

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Reuse of produced water (PW) from oil and gas (O&G) operations can supplement water demand in arid regions while addressing critical PW management and environmental issues. Given the complex and variable composition of PW, it is imperative to design and evaluate treatment trains through comprehensive chemical and toxicological testing. This study comprehensively evaluated PW quality throughout an integrated treatment train consisting of mechanical vapor recompression (MVR) distillation, granular activated carbon (GAC), and zeolite. Over 400 target analytes, including volatile and semivolatile organics (VOCs, SVOCs respectively), ions, heavy metals, dioxins and furans, per- and polyfluoroalkyl substances (PFAS) and radionuclides, among others, were chemically profiled. Acute and chronic whole effluent toxicity (WET) tests encompassed four trophic levels, including algae (*Pseudokirchneriella subcapitata*), invertebrates (*Ceriodaphnia dubia*), fish (*Danio rerio*), and bacteria (*Vibrio fischeri*). In total, 3 effluents were chemically and toxicologically characterized: raw PW, distillate, and post-treated effluent. Raw PW and distillate effluents failed to meet National Pollutant Discharge Elimination System (NPDES) toxicological criteria, causing adverse effects on *P. subcapitata*, *C. dubia* and *V. fischeri* ranging from 84% to 100% at 100% effluent. Characterization identified 64 individual analytes in the feed, including 17 VOCs, 12 SVOCs, 1 PFAS and 7 radionuclides; 65 analytes were identified in the distillate, including 15 VOCs, 14 SVOCs, 5 furans, 4 PFAS and 5 radionuclides. Overall, the post-treatment with GAC and zeolite reduced most of the identified constituents to levels below method detection limits (MDL). This resulted in a consistent toxicity reduction across the WET tests. Specifically, with no observable adverse effects for *P. subcapitata*,*C. dubia* and V. *fischeri* (NOEC>100%). The present study highlights the potential of integrating advanced treatment systems to address the complex matrix of PW and produce an effluent potentially suitable for surface discharge according to NPDES chemical and toxicological criteria.

5.13.T-06 Evaluating the Impact of Oil and Gas Wastewater Dumps in the Permian Basin on Arid Soil Biogeochemistry

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The Permian Basin, underlying New Mexico and Texas, is one of the most productive oil and gas (OG) provinces in the United States. Oil and gas production yields large volumes of wastewater with complex chemistries. The environmental health risks posed by these OG wastewaters are not well understood, particularly in the case of accidental or intentional releases. Starting in November 2017, 39 illegal dumps of OG wastewater were identified in southeastern New Mexico that released approximately 6.4×10^5 liters of fluid onto desert soils. We analyzed changes in soil geochemistry and microbial community composition to evaluate impacts of these releases on soils by comparing soils from within OG wastewater dump-affected zones to corresponding unaffected zones with no known releases. We observed significant ($p<0.01$) changes in soil geochemistry for all dump-affected samples compared to controls, reflecting the residual salts and hydrocarbons left behind by the OG-wastewater release (e.g., enriched in sodium (Na), chloride (Cl), bromide (Br), and percent carbon). We observed significant ($p<0.01$) differences in microbial community structure between dump and control zones including lower alpha diversity in soils from dump areas and differences in phylogenetic composition. Dump-affected soils showed an increase in halophilic and halotolerant taxa, such as members of the Marinobacteraceae, Halomonadaceae, and Halobacteroidaceae, suggesting that the high salinity of the dumped OG wastewater exerted a selective pressure on microbial communities. Taxa related to known hydrocarbon-degrading organisms, e.g., Marinobacter, Salegentibacter, Chromohalobacter, and Alcanivorax, were also detected in the dump-affected soil samples. In contrast, the microbial communities in control soils were dominated by taxa ubiquitous in, and well adapted to, arid and nutrient-deprived soil environments. This study demonstrated that OG-wastewater dumps can lead to shifts in microbial community composition and function towards salt- and hydrocarbon-tolerant taxa that are not typically found in desert soils, thus altering the impacted dryland soil ecosystem. Loss of key microbial taxa, such as those that increase arid soil fertility, or promote plant health, could result in cascading effects to myriad ecosystem services such as loss of flora. Further studies are needed to explore the potential for using halophilic and hydrocarbon-degrading taxa to bioremediate OG-wastewater affected lands.

5.14.A.T Using Mechanistic Effect Modeling to Support Ecological Risk Assessment in the Context of the Endangered Species Act

5.14.A.T-02 Bioenergetic Models to Support ERA for Threatened and Endangered Species

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The utility of bioenergetic models for ecotoxicology has long been recognized. The initial concept of “scope for growth” expanded in the 1990s to incorporate within-organism dynamics, exemplified by Dynamic Energy Budget (DEB) models by S.A.L.M Kooijman. DEB models offer a simplified characterization of physiological rates that can be impacted by contaminants; current applications to ERA use a mechanistic modeling triad of DEB, toxicokinetics(TK), and

toxicodynamics (TD) – recognized for example in recent EFSA publications. A serious impediment with DEB models is their use of abstract dynamic variables, with necessary assumptions relating abstract to observable quantities. Thus, DEB models are typically parameter rich and most applications to ERA focus on model organisms and interpretation of standardized tests. We discuss three modifications to current DEB methodology for new work on threatened and endangered species. 1) *If data to parameterize a model are unavailable, change the model, while still adhering strictly to fundamental bioenergetic principles.* This was demonstrated in a published study of the North Atlantic Right Whale, listed endangered under ESA since 1970. The problem was the availably of different types of data from that used in DEB work. New dynamic variables related closely to interpretable physiology. Key to the dynamics was focus on a new variable, blood, a shared transport medium. We describe ongoing work extending this formalism. 2) *Exploit statistical advances for state space models in ecology*. This derives from experience (1993) modeling Delta Smelt (*Hypomesus transpacificus*), listed threatened under the federal ESA in 1993. Subsequently, there have been advances in state space modeling, including accessible literature and software for ecologists. The payoff is ability to extract reliable insight (including measures of estimability) from limited data, augmented with hypotheses on hidden bioenergetic variables. 3) *Use molecular data to inform bioenergetic models*. This approach utilizes the explanatory power of molecular data to help identify pMoAs. All applications involve model aquatic organisms (algae, zooplankton, fish). A key unknown is the extent to which pMoAs identified for model organisms with molecular tools are conserved in taxonomically similar threatened or endangered species. For example, can SeqAPass give us an idea of protein homology (and thus pMoAs) between model organisms and the focal species?

5.14.A.T-03 Comparison of Stress Responses and Underlying Energetics of Freshwater Mussels Using Life-History Traits and Dynamic Energy Budget Theory

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Freshwater mussels are among the most imperiled taxa globally, threatening their critical role as mediators in the nutrient cycling process. Declines in mussel populations have been attributed to many causes, with contaminants indicated as a significant factor. The physiology of individuals dictates population dynamics, and understanding individual-level responses to the impact of chemicals is therefore imperative to assess the magnitude of risk at the population level. Freshwater mussels exhibit a variety of life-history traits that can be categorized into three groups based on their lifespan, maturation time, and fecundity. To understand whether and how belonging to a specific group dictates sensitivity to certain stressors, we compared individual-level responses to stress. We used a mechanistic effect model relying on Dynamic Energy Budget theory to capture the underlying mechanisms leading to sub-lethal effects, and a traits-based approach to represent multiple species sharing common life-history traits.

We analyzed theoretical stress responses of 48 mussel species, simulating impacts via physiological modes of action related to four key metabolic pathways: energy assimilation, maintenance, growth, and reproduction. We recorded maximum length, age at maturity, and fecundity, and compared these individual-level endpoints among life-history groups.

Results confirm that life-history differences between freshwater mussel groups directly emerge from underlying energetics, primarily due to differences in energy assimilation and maintenance compared to relatively uniform costs of growth. Stress impacts on assimilation had the greatest effect on all endpoints, decreasing maximum length and fecundity on average by 25% and 60%, respectively, and increasing age at maturity by 24%. Impacts on maintenance had a moderate effect, predominantly on fecundity, whereas impacts on growth had negligible biological significance. The short-lived, rapidly maturing mussel group experienced, on average, the lowest increase in age at maturity, and decrease in fecundity, indicating such life-history strategy might be least sensitive to stressor exposure. Findings provide a foundation for population modeling and promote selection of suitable non-listed representative species as surrogates for toxicity testing. Ultimately, this work will contribute to development of effective conservation strategies for freshwater mussel species listed under the Endangered Species Act.

5.14.A.T-04 Refining Risk Assessment of Chemicals by Integrating Laboratory and Exposure Model Outputs into Effect Modeling

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Risk quotients (RQ) form the foundation of Tier I ecological risk assessment and are intentionally conservative by design. They consist in comparing the output of a laboratory experiment (NOEC/NOEL, LD/LC50, or EC25) with the maximum estimated environmental concentration (1-day, 21-day, or 60-day EEC), calculated from environmental fate models like the Pesticide Water Calculator, or residue models used in BeeRex and T-Rex. These RQ are then compared to arbitrary defined level of concerns (LOC) which value depends on whether the RQ calculation is for chronic or acute risk estimation, the taxa of interest, and the whether the risk assessment is for federally listed species or not. However, exceeding a LOC does not necessarily indicate that an individual or a population of a specific species is at risk. The reason is that the calculation of an RQ does not consider the time-dependent nature of chemical exposure or its effects on organisms. Nonetheless, there are recognized methods and approaches that can refine risk assessment by accounting for the variable nature of effects and exposure without compromising the conservatism of the assessment. One such approach is the Toxicokinetic-Toxicokinetic modeling that integrates toxicokinetics (absorption, distribution, metabolism, and excretion of a substance) with toxicodynamics (subsequent biochemical and physiological effects) to predict the relationship between chemical exposure and its toxic effects in living organisms. When combined with the Moving-Time Windows approach, which consists in repetitively estimating effects over an exposure profile while delaying exposure by one day each time, it can identify critical combinations of life stage sensitivity and chemical exposure, resulting in a conservative, yet realistic risk assessment. In this presentation, we will use case studies from the literature to illustrate these concepts and discuss how these approaches and their outputs can enhance ecological risk assessment.

5.14.A.T-05 Toxicity Translation Modeling: Convergent and Divergent Attributes of Five Different Approaches

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Ecological risk assessment is an integral component of environmental protection that relies on a variety of diverse information sources including laboratory studies, field studies, and mathematical and statistical models. Data generated from these sources span levels of biological organization, from the molecular level to up to populations, communities, and ecosystems. To conduct ecological risk assessment at relevant scales for protection goals, ecological risk assessors are therefore challenged with a variety of extrapolation problems. Mathematical and statistical models serve as critical tools for addressing this challenge and conducting transparent extrapolation. In particular, the term Toxicity Translator is used to refer to methods, tools, and approaches that serve to synthesize and extrapolate from apical endpoints measured at, or below, the level of individuals to ecosystem-relevant metrics and are based on population ecology and theory. This presentation will share recent research to understand, categorize, and develop a foundation for toxicity translation modeling. These efforts include comparing convergent and divergent attributes of 5 different toxicity translator models across different taxonomic groups. Through this research, we identify and develop synergistic development paths for toxicity translation modeling and help to formally establish the emerging field of toxicity translation within ecotoxicology.

5.14.A.T-06 An Agent-Based Model of Fathead Minnow for Lower and Higher Tiers of Ecological Risk Assessment

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Mechanistic effect models are valuable and relevant tools that can help overcome some major challenges in ecological risk assessment (ERA), such as extrapolation across scales of biological organization, chemicals, and species challenges. In particular, models can extrapolate data generated from standard test species and consider those characteristics, like life-history traits, that could influence the susceptibility of the exposed populations of listed species. In this study, we built on the existing literature and developed an agent-based model (ABM) for fathead minnow (FHM) to be used as a standard model for diverse ERA purposes. FHM is the most widely used small fish for different regulatory applications in ecotoxicology in North America. Consequenty, data on its life cycle and ecotoxicological laboratory experiments are available. The model is based on Dynamic Energy Budget theory, and lethal and sublethal effects have been implemented through toxicokinetic-toxicodynamic models and by altering different metabolic pathways through physiological modes of action. To ensure transparency, we followed the model-development guidance Pop-GUIDE (Population modeling Guidance, Use, Interpretation, and Development for ecological risk assessment), an approach applicable across regulatory statutes and assessment objectives that greatly facilitates the standardization of modeling development and use in ERA. The model has been developed to integrate different life-history traits, exposure scenarios and routes, as well as environmental variables of interest. Therefore, it can be easily adapted to represent different fish species, and can be applied to estimate risks of different compounds. We first present two case studies showing population-level effects of exposure to chlorothalonil and diazinon. Then, we show how the model could be applied to listed species by comparing the results on FHM with those of a previously published model on listed Cyprinidae. Ultimately, we show that our model could benefit ERA in two ways: (i) by simulating population-level effects for FHM that could possibly be integrated into lower-tier risk assessment; (ii) by extrapolating exposure effects to listed species populations and better guide higher-tier risk assessment.

5.14.B.T Using Mechanistic Effect Modeling to Support Ecological Risk Assessment in the Context of the Endangered Species Act

5.14.B.T-01 Linking Pesticide Exposure Landscapes to Demographic Outcomes: A Case Study for *Bombus affinis* Foundress Queens in Early Foraging Stage

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Agricultural insecticide treatments can have wide ranging impacts to native pollinators and may contribute to population-level impacts that affect species longevity; this is particularly true for endangered species such as *Bombus affinis*, the rusty patched bumblebee. Pesticides are hypothesized to have played a role in the reduction of population numbers, but no formal link has been derived between pesticide use and species decline. In particular, foundress queens may be susceptible to exposures from early-season chemical applications that influence colony survival; loss of these individuals could have significant implications for population persistence. We assessed the potential role that early-season pesticide interactions may have played in declines of *B. affinis* foundress queens by integrating explicit landscape foraging scenarios and crop pesticide exposures from multiple time-specific media into a mechanistic-based queen survival model. To capture variation in pesticide use history as well as variability in the daily and cumulative survival at key life history transition events, we utilized a Monte Carlo simulation method to model scenarios of pesticide use history at the individual field level using a daily difference equation approach, and estimated a daily foraging dose based on habitat desirability and composition. Median cumulative daily queen survival in the period from emergence to nest initiation was high (0.98) but declined between initiation and establishment (0.90) and to the emergence of the first worker brood (0.88). The majority of relevant exposures came from deposition contact with neonicotinoid seed dust and various foliar-applied pesticides during early-season planting. Under the simulated pesticide scenarios, the proportion of queens surviving to full nest establishment was reduced by an additional 7% when compared to natural mortality. This may help to elucidate one of the hypothesized drivers of declines for *Bombus affinis*. Outputs from this work introduce novel methodologies for incorporating spatial dimensions of pesticide exposures into *Bombus* colony cohort dynamics and reinforce the benefit of population metrics for ecological risk assessment.

5.14.B.T-02 Ecological Risk Assessment when Species-Specific Data Are Scarce: How Trait-Based Approaches and Modeling Can Help

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Ecological risk assessments are legally required to ensure that there are no unacceptable risks to living organisms from exposure to chemicals and other anthropogenic stressors. Significant data gaps, however, make it difficult to conduct such assessments for all species that we wish to protect. Consequently, there is growing interest in trait-based approaches because they provide a more functional and context-independent basis for characterizing biodiversity that is useful for biomonitoring, conservation, and management. In this presentation, we discuss how trait-based approaches can support risk assessment and inform decision-making more generally. Through selected examples, we demonstrate how trait-based approaches can be used to identify species that are particularly vulnerable to stressors as a result of their likelihood of exposure, their intrinsic sensitivity, and/or the resilience of their populations. In addition, we show how species can be grouped according to their trait values to help identify species that are broadly representative of other species in their group. This can be helpful for developing mechanistic effect models in which a single model is used to simulate multiple species of the same functional group, i.e., with generally similar life history and ecology. This may be particularly valuable for assessing risks of chemicals and other stressors to species listed under the Endangered Species Act given limitations on the types of data that can be collected for such species. In addition to demonstrating the utility of trait-based approaches, we highlight some of the challenges and areas in which additional knowledge or tools are needed to move the field forward.

5.14.B.T-03 Advantages and Disadvantages of MCnest as a Framework for Ecological Risk Assessment on Listed Birds

Matthew Etterson*, U.S. Environmental Protection Agency*

The US Fish and Wildlife Service Environmental Conservation Online System (ECOS) database lists 100 species of federally listed (threatened or endangered) bird species, with about half of the list endemic to islands. Listed birds are a particular challenge for Ecological Risk Assessment (ERA) because the stressor(s) that led to their listing status are often still operating and are often not the contaminant(s) of interest for the ERA. Further, populations of listed species are likely already declining, making $\lambda < 1$ a meaningless threshold for population level risk assessment. Mechanistic effects models can help us to predict the contribution to declines arising from ongoing non-contaminant stressors and the contaminant of interest. In addition, there is a tension between modeling stressors that operate exogenously on populations, such as habitat loss and introduced predators/parasites, and those that operate endogenously, such as the mechanistic physiological steps that occur along an adverse outcome pathway, following exposure to a contaminant. The recently proposed endogenous lifecycle model (ELM) framework may provide suitable structure for handling this tension and has been incorporated into the Markov Chain Nest Productivity Model (MCnest, USEPA). In conjunction with the incorporation of the ELM analytical capabilities, MCnest has also been expanded to a full annual cycle, which may provide an appropriate framework for investigating the potential influences of environmental contaminants on federally listed birds. I will review the strengths and weaknesses of MCnest in relation to applications to federally listed birds and illustrate with examples. *The views expressed in this abstract do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.*

5.14.B.T-04 A Pragmatic Prioritization of Endangered Species for Effects Modelling Purposes

Ismael M Rodea-Palomares[1] *and Ruoyu Wang*[2]*, (1)Bayer Crop Science, (2)Environmental Safety, Bayer CropScience*

The USEPA and the Services are charged with the responsibility of conducting Ecological Risk Assessments to ensure that the registration of agricultural use pesticides do not result in harm to endangered species. From a practical viewpoint, a no harm conclusion can be linked to the ability to conclude whether an Agency's action will or will not jeopardize (J) the continued existence of populations of endangered species. For many endangered species, it is possible to reach conclusions of No Jeopardy (NJ) based on the standard screening-level ecological risk assessment models and tools. However, by definition, these screening-level tools are very conservative in nature. As a result, for some endangered species, a NJ determination may not be attainable at the screening-level. As jeopardy decisions on the likelihood of an action (e.g., a pesticide use) to affect an endangered species are determined at the population level, population models (or more generally effect models) should be a natural option for better understand and refine the potential effects of pesticides on endangered species. However, population models are scarcely used in ecological risk assessments of pesticides, in part, because with realism comes complexity. Effects models typically require foundational knowledge of the biology, life-history, and habitat preferences of the species. Further, data acquisition might be required for both training and validating the models. This results in significant time and effort being required to build, fit, and validate effects models. Further, there are thousands of endangered species with wildly different taxonomies, biologies, life history traits, and habitat preferences for which developing effects models for each species is an unrealistic goal, and therefore, efforts in prioritization are required. In this talk we will present a pragmatic prioritization of endangered species for the development of effects models in the US CONUS. This prioritization was developed with a clear regulatory focus trying to really identify the species for which the availability of effects models could have the potential to truly inform in meaningful ways the ecological risk assessments of pesticides in substantial acreage in the US

5.14.P-Tu Using Mechanistic Effect Modeling to Support Ecological Risk Assessment in the Context of the Endangered Species Act

5.14.P-Tu-176 Development of a Mechanistic Fish Population Model to Assess Indirect Effects of Environmental Chemical Exposure

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The development and implementation of mechanistic ecological population models are an increasingly important component in efforts to understand and manage risk, especially with endangered species. Mechanistic population models can integrate the most current relevant data at the organismal level to forecast population dynamics under a wide variety of exposure conditions and realistic scenarios. This provides a particularly unique utility when considering indirect effects of environmental exposure at population levels far outside the ‘in vivo’ scope of laboratory experiments. An important example of this involves indirect effects on listed species via trophic links to directly affected prey organisms. The modeling platform developed here can provide forecasts of the population-level consequences on different benthic and pelagic freshwater fish species experiencing exposure-based dietary limitations. The capabilities of the model are demonstrated by presenting a modeling study of the indirect effects of an applied chemical on populations of small freshwater fish species via induced reductions of the insects in their diets. The model is a matrix model, structured for flexible integration of empirical data to inform, among other traits, biologically realistic bounds on species life history, dietary options, weight/fecundity relationships, and exposure scenarios. This assessment shows how differences in these traits manifest into different outlooks for indirect population exposure to a range of concentrations and the types of actionable inference that can follow.

5.14.P-Tu-177 Formulation of a Freshwater Mussel Population Model for Ecological Risk Assessment Using a Standardized Protocol

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Population models provide an *in silico* framework that can supplement, or potentially even substitute time- and resource-consuming field and laboratory studies required for Ecological Risk Assessment (ERA). To be recognized and ultimately used by ERA stakeholders, however, such population models need to be built with a specific ERA objective in mind and offer transparency about their formulation and development process, preferably in a standardized way. Ultimately, the models need to be communicated, both verbally and visually, in the way a non-modeler can understand their purpose, benefits, and limitations. In this study, we use Population modeling Guidance, Use, Interpretation, and Development for Ecological risk assessment (Pop-GUIDE), and combine it with a recently developed, standardized Conceptual Model Diagram (POP-CMD) to formulate a population model for freshwater mussels. Chemical contaminants have been identified as a significant factor contributing to the decline of freshwater mussel populations, however knowledge about the magnitude of these impacts is still limited. The purpose of this population model is therefore to simulate impacts of potential stressors via different physiological modes of action at the individual level and translate these effects to the population level. We discuss the process of model formulation in light of Pop-GUIDE according to 4 phases: model objectives, data compilation, decision steps, and conceptual model. Finally, we present a visualization of the population model in the form of a conceptual diagram considering POP-CMD standardization. The primary goal of this presentation is to foster multi stakeholder discussion around the development of a freshwater mussel population model, utilizing Pop-GUIDE and POP-CMD as tools to effectively communicate and encourage the long overdue standardization in population model formulation for ERA.

5.15.P-Th When 'Off the Shelf' Isn't Good Enough: An Exploration of Higher-Tier Studies in Environmental Risk Assessment

5.15.P-Th-073 What Can Twenty Years of Headwater Stream Monitoring Tell Us About Chemical Presence in the Environment and the Effects on Watershed Conditions

Jennifer Trask[1], ***Zechariah Stone***[2], *Sarah Terrell*[2], *Natalie Walk*[3], *Nathan Snyder*[2], *Sarah E Crawford*[4], *Richard Brain*[5] *and Mark White*[6], *(1)Waterborne Environmental, Leesburg, United States, (2)Waterborne Environmental, (3)Field Programs - Environmental Assessment, Waterborne Environmental, (4)Syngenta, Canada, (5)Syngenta Crop Protection, (6)Syngenta Crop Protection*

The United States (US) Environmental Protection Agency (EPA) required monitoring of a finite number of lotic headwater streams within watersheds highly susceptible to runoff as part of the 2003 reregistration review of atrazine. The purpose of the program was to monitor concentrations during seasonal runoff patterns relative to a conservative aquatic level of concern (LOC) defined by the sensitivity of aquatic plant communities to atrazine exposure. Designing a study to capture concentrations at both low and high stream flow, including peak periods, and combining this with a collection of key environmental data was necessary to enable a thorough and comprehensive understanding of the mechanics and patterns of atrazine transport.

Today, the Atrazine Ecological Monitoring Program (AEMP) continues after two decades of watershed monitoring. The study collects daily (or near daily) atrazine concentrations alongside

high resolution environmental data at stream headwaters within watersheds classified among the upper 20th centile of vulnerability according to the USGS Watershed Regressions For Pesticides Model (WARP). While the program has been conducted in several phases, in total, monitoring has occurred at 77 sites across 13 states for a minimum of two years. Sampling locations were identified on 1st to 3rd order streams at the outlets of watersheds and were equipped with an integrated real-time data delivery system of weather stations, automatic samplers, stream stage measurement stations, and water quality sondes. Early program designs required four-day grab samples coupled with event-based sample collection. As the program matured, the expansion to daily composite sampling was supported through multiple Science Advisory Panels and the desire to support regulatory water model calibration to further refine, characterize, and contextualize potential ecological risks. Many sites have been waived from monitoring over the years for various reasons by EPA and as a result nine sites remain in the program. The presentation will discuss experiences, learnings, and recommendations.

5.15.P-Th-074 A Higher-Tier Field Spray Drift Bioassay Concept to Evaluate Herbicidal Effects to Non-target Plants

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The United States Environmental Protection Agency (USEPA) uses a tiered risk assessment framework to evaluate the potential effects of pesticide active ingredients to non-target organisms. Predictive models such as AgDRIFT® are used at the screening-level to conservatively generate downwind off-field exposure estimates via drift deposition curves. The most sensitive regulatory endpoints, i.e., the effective rate required to affect 25% of individuals (ER25) for non-listed species or the no-observed effect rate (NOER) for listed species, derived from standard guideline vegetative vigor (OCSPP 850.4150) and seedling emergence (OCSPP 850.4100) studies are then benchmarked against these curves. The point at which the regulatory endpoint falls below the corresponding exposure estimate on the drift curve(s) is used to inform the in-field no application zone or 'buffer' to ensure protection of downwind sensitive plants. The fundamental issue with this approach is that it assumes that non-target plants are exposed as would be in-field target weeds, which they are not. In standard guideline studies, test plants are exposed in a chamber via a track sprayer that delivers a direct overhead spray intended to saturate the foliage or soil surface (i.e., full coverage). Conversely, non-target plants do not receive even herbicide coverage on the foliage because spray drift is more likely to contact the upwind (via lateral interception) and top (via deposition) portions of the foliage. In addition, numerous properties of the downwind plant community (e.g., plant density and height, drag characteristics of the foliage, collection efficiency) influence exposure of non-target plants to herbicide drift. What does this mean for farmers in a regulatory context? The results of recent field studies in which non-target plants were placed at different distances downwind of herbicide applications indicate that buffers derived under the current USEPA paradigm are overestimated by 10- to 100-fold. Such unnecessary drift mitigation buffers result in large parts of fields being untreated and, as a result, lower yields. In this presentation, we explore alternative approaches for deriving spray drift buffers for herbicides including a weight of evidence approach that considers all available and relevant information.

5.15.P-Th-075 Effect Factors for the Freshwater Ecotoxicity Impact of Poly Lactic Acid in Life Cycle Assessment

Monica Rodriguez[1] *and Andrea Hicks*[2], *(1)Civil and Environmental Engineering, University of Wisconsin, Madison, (2)University of Wisconsin, Madison*

Bioplastics, such as polylactic acid (PLA), have been proposed as an alternative to fossil-based plastics. Life cycle assessment is one tool which can be employed to assess the environmental impacts of different plastics across their lifecycles. One concern about the environmental impacts of plastics is their potential to become microplastics. In this study an effect factor specific for PLA microplastics is calculated for a freshwater ecotoxicity midpoint and endpoint impact categories. The effect factor developed in this paper is comprised of 9 ecotoxicological data points, that represent 5 species and 3 tropic levels. The data includes 3 freshwater aquatic species and 2 marine aquatic species. The calculated effect factor is 164.24 (11.867-2273.25 CI) PAF m3/kg at the midpoint level, and is 4.106 at the endpoint level, and is comparable to what has been previously estimated in literature for PLA and calculated for other polymer types. The effect factors developed in this study can be used in future comparative life cycle assessment to contrast PLA with other polymer types.

Track 6: Engineering, Remediation and Restoration

6.01.P-Mo Addressing Beneficial Use Impairments at Great Lakes Areas of Concern: Scientific Approaches That Lead to Restoration

6.01.P-Mo-176 Developing the Best Approach to Using Continuous Monitoring Dissolved Oxygen (DO) Data to Assess the Potential Influence of DO on Remediation and Restoration Efforts at Great Lakes AOC Sites

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To assess the effectiveness of remediation and restoration efforts at Great Lakes Areas of Concern sites, the U.S. EPA's Office of Research and Development (ORD), partnering with the Great Lakes National Program Office (GLNPO), have been collecting macroinvertebrate community data as a measure of water/ sediment quality for decades, and started collecting continuous dissolved oxygen (DO) data in 2020. Physical and chemical factors like DO affect the makeup of macroinvertebrate communities, and a comprehensive understanding of the relationship between dissolved oxygen and macroinvertebrate community structure would be very useful in assessing remediation and restoration results. To simultaneously characterize macroinvertebrate community and DO conditions, moorings fitted with Hester-Dendy samplers, continuous DO loggers, and temperature loggers are deployed for six-week intervals across several stations per site, covering a gradient of environmental settings. In this presentation, we report on our progress in developing a framework for distilling 6-weeks' worth of continuous DO data into the most ecologically relevant metrics to discern the effects of DO conditions on these communities during pre- and post-remedy/ restoration samplings (which in some cases can be quite different), and whether those changes could be significant causal factors for differences seen in pre- and post-remedy/ restoration differences in macroinvertebrate communities. Approaches/metrics that we are evaluating include daily mean DO, the amplitude of diel DO cycles (as a measure of DO stability), # days DO dips below threshold values, and the timing of low DO during a deployment. Using non-metric multidimensional scaling (NMDS) plots that show sample grouping (by site, remediation status, etc.), we overlay vectors showing where these macroinvertebrate communities lie on common gradients of indices of water quality (based on community makeup) like the LICI and HBI. Then we plot numerical and categorical DO metrics and use MRPP analyses to determine if there are statistically significant correlations with macroinvertebrate community indices. These analyses will be more powerful in designing a monitoring program as we add community, DO data and the characterization of these localized environments. Ultimately, these factors may be considered during the design phase for these habitat remediation projects.

6.01.P-Mo-177 Approaches to Beneficial Use Impairment Removal in the Muskegon Lake Area of Concern

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The Muskegon Lake Area of Concern (AOC) is a 4,149-acre drowned river mouth lake located in Muskegon County, Michigan, USA. It is located at the terminus of the Muskegon River, the second longest river in Michigan, and flows into Lake Michigan. Muskegon Lake was designated an AOC with nine Beneficial Use Impairments (BUIs) due to several ecological issues, including the loss and degradation of benthic habitat, isolation and fragmentation of coastal wetlands, and degradation of fish and wildlife populations. These issues resulted from historic practices in which sawmill debris, foundry sand, and slag filled emergent wetlands and open water habitats and hardened the shoreline with materials, including pilings and broken concrete. Following the efforts of numerous federal, state, and local partners, the Muskegon Lake AOC is on the cusp of being delisted. A single BUI remains to be removed, Degradation of Benthos. A tiered science-based methodology to address this BUI was established with local restoration targets that are equivalent to those in the Guidance for Delisting Michigan's Great Lakes Areas of Concern. The approach includes the use of Michigan Department of Environment, Great Lakes, and Energy's Surface Water Assessment Section Procedure 51, a qualitative biological and habitat survey protocol for wadeable streams, for multiple tributaries. Additionally, biotic conditions (e.g. survival, density, precent of community composition) for lentic monitoring were established for benthic communities in Muskegon Lake and Bear Lake, which flows into Muskegon Lake, and for riparian wetlands downstream of two restoration sites. Data from the associated monitoring and those which have supported the approaches to remove several other BUIs will be presented, including the Restrictions on Fish and Wildlife Consumption BUI, which was addressed using a comparison study of site-specific fish tissue contaminant levels causing fish consumption advisories in the AOC compared to a control site. Finally, an innovative approach, the Americas Biodiversity Metric, will be presented as an alternative to support future efforts to address the Loss of Fish and Wildlife Habitat BUI at other AOCs. The metric is an adaptation of the regulator-developed Natural England Biodiversity Metric used to deliver net gain policy in the UK and offers a scientifically robust methodology for assessing the biodiversity value of habitats based on size, condition, and conservation priority.

6.01.P-Mo-178 Liver Tumor Frequency in Brown Bullhead in Hamilton Harbour Area of Concern

Mark Ervin McMaster[1], *Erin Ussery*[1], *Jessie Cunningham*[1], *Jim Bennett*[1] *and Bradley Park*[2], *(1)Environment and Climate Change Canada, Canada, (2)University of Manitoba, Canada*

The Beneficial Use Impairment of Fish Tumors and other Deformities was identified in a number of the Canadian Areas of Concern. Hamilton Harbour was one of those AOCs identified that had liver tumor rates that exceeded rates at comparable control locations and were also higher than the overall background rate determined for the lower Great Lakes. During the tenure of this project, we collected brown bullhead from the Hamilton Harbour AOC in 2005, 2007 and 2012 prior to remediation in the harbour. Remediation of Randle Reef began in 2018 with an in-water, double walled engineered containment facility (EFC) over the most contaminated sediment in the harbour. Contaminated sediment surrounding the ECF was then dredged from the harbour and placed into the structure. During this time we again sampled brown bullhead from the Harbour to evaluate the potential for change with dredging activity. In 2023 we again sampled bullhead from the harbour while the final multi-layered environmental topo is being placed on the ECF. The presentation will follow liver tumor frequencies before, during and after the placement of the ECF facility.

6.01.P-Mo-179 Evaluating Approaches for Assessing the Fish Tumors or Other Deformities Beneficial Use Impairment

Amber Bellamy, *Mandy Annis, Amanda Curtis, Lainet Garcia-Rivera, Steph Hummel and Amy Roe, U.S. Fish and Wildlife Service*

The International Joint Commission, a bi-national organization established by the governments of the United States and Canada, identified Beneficial Use Impairments (BUIs), including the Fish Tumors or Other Deformities, to be addressed in Areas of Concern (AOCs) under the Great Lakes Water Quality Agreement (GLWQA). The Fish Tumors or Other Deformities BUI has been designated for 18 of the 43 AOCs in the United States, however it has been removed from 8 of those AOCs. The GLWQA, a commitment between the United States and Canada, provides a framework for state, local, Tribal Nation leadership, and the public to identify causes of and an approach for restoring the BUIs, in addition to establishing restoration criteria for the removal of the BUI. Contaminants in water and sediment, particularly polycyclic aromatic hydrocarbons (PAHs), have been linked to an increased occurrence of internal tumors in fish across Great Lakes AOCs. Management actions including the removal of contaminated sediment should eventually lead to a decrease in tumor prevalence if there are no additional continuous sources of contamination. While there is some consistency across states for determining the status of this BUI, AOC-specific challenges and differing removal criteria have led to some differences in how this BUI is evaluated. We examined the approaches for fish liver tumor evaluation across six states (Ohio, Michigan, Pennsylvania, New York, and Minnesota/Wisconsin), identifying differences in evaluation methods and summarizing temporal trends in tumor prevalence data as it relates to remedial actions (when possible). While fish liver tumor prevalence generally decreases over time across AOCs following remediation (consequently leading to the removal of the BUI), scientific advances have led to the refinement of the causal relationship between fish tumors and exposure to sediment contamination. Our findings are intended to help other AOC personnel planning to evaluate the status of the Fish Tumors or Other Deformities BUI in the future.

6.02.P-Mo-181 Environmental Implications of Oxidative Transformation of 6:2 Fluorotelomer Sulfonate by Common Oxidants

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The application of per- and polyfluoroalkyl substances (PFAS) for commercial and industrial uses dates back decades but their presence and impact to society persist. Some of the most commonly used PFAS in aqueous film forming foams were fluorotelomer-based compounds, one of which is 6:2 fluorotelomer sulfonate (6:2 FTS). Being a polyfluorinated compound, 6:2 FTS contains multiple carbon hydrogen bonds which make the compound particularly vulnerable to oxidation in natural environments. Its degradation produces a plethora of byproducts which are often more persistent and structurally stable, such as perfluorinated compounds. In our current approach to decomposing 6:2 FTS and other PFAS, their oxidative destruction under ambient conditions (1 atm and 20 °C) has been investigated using common chemical oxidants such as persulfate. In this presentation, as a result, we will i) discuss the degradation of 6:2 FTS via conventional techniques; ii) compare the degradation efficacy of 6:2 FTS using different strategies (conventional oxidation vs. advanced oxidation); and iii) demonstrate the degradation pathways of 6:2 FTS using targeted and non-targeted analysis of the degradation byproducts.

6.02.P-Mo-182 Decomposition of PFAS Using Fenton-like Systems under Ambient Conditions: Systemic Combination of an Oxidant and a Transition Metal

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Per- and poly-fluoroalkyl substances (PFAS) are persistent contaminants that accumulate in biological systems, posing a significant risk to human and ecological health. Traditional PFAS removal methods are relatively expensive and energy-consuming. Recent studies have shown that modified Fenton-like systems can degrade certain PFAS. Particularly, persulfate (PS) activated with silver (Ag+) showed a noticeable decomposition of perfluorocarboxylic acids at 20 °C and 1 atm, including perfluorooctanoic acid and perfluorononanoic acid. Nonetheless, there is no research on the reactivity of several other oxidant and transition metal combinations and the role of different oxidation states of transition metals in decomposing PFAS. Previous studies have also shown that combining an oxidant with two transition metals significantly increases the reaction kinetics for decomposing some organic contaminants. In this presentation, we will (i) compare the results of activating chemical oxidants such as hydrogen peroxide and peroxymonosulfate, along with PS under ambient conditions with different transition metals, including Co, Pb, Ni, Cu, Mn, Fe, Zn, Pd, and Ag and (ii) discuss the potential synergistic effect of a co-catalyst (second transition metal) in the metal-oxidant system.

6.02.P-Mo-183 Study on the Decomposition Mechanism of PFAS Via a Silver-Activated Persulfate System Under Ambient Conditions

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Per- and polyfluoroalkyl substances (PFAS) have been widely utilized in the production of food packaging materials, firefighting foams, and fluoropolymers because of their remarkable physicochemical properties. As the United States Environmental Protection Agency recently regulated, PFAS such as perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorooctanesulfonic acid (PFOS) have been widely detected in various drinking water sources. Although the decomposition of PFAS via advanced oxidation processes is promising, they exploit mostly energy-intensive tools such as ultraviolet, ultrasound, microwave, and heat due to the remarkably strong, highly polarized C-F bonds PFAS contain. In recent studies, the potential of the modified Fenton systems to decompose certain PFAS under ambient conditions has been reported. Among various combinations of a common oxidant and a transition metal, in particular, a pair of persulfate and silver showed significant reactivity exclusively toward carboxylic PFAS including PFOA and PFNA. However, the decomposition mechanism of PFAS in the treatment system remains completely unknown and no explanation was made for the reason of negligible PFOS decomposition in the system. In this presentation, as a result, we will i) demonstrate the potential change in the silver speciation during the reaction, ii) elucidate the decomposition pathway of PFAS using targeted and non-targeted analysis of decomposition byproducts, and thus iii) explain the overall decomposition mechanism of some selected PFAS.

6.02.P-Mo-186 Assessment of Microbial Communities in Biologically Activated Carbon Systems Treating Refinery Effluent

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Petroleum refinery wastewater often consists of a diverse array of organic and inorganic pollutants, presenting a formidable challenge for efficient treatment. Therefore, preliminary treatment may be necessary for the receiving wastewater treatment plant (WWTP) to uphold the effectiveness of biological processes within the plant's operation, preventing toxic contaminants from compromising its operational efficiency. This study explores the efficiency of biologically activated carbon (BAC) systems, which employ spent granular activated carbon (GAC) sourced from a drinking water treatment plant packed into columns. These systems, or columns, are inoculated with microorganisms previously exposed to refinery wastewater, aiming to pre-treat refinery effluents before entering the treatment process. The study will test two different inocula: biomass collected from the walls of a manhole in the refinery sewage system and activated sludge from a WWTP. Additionally, two empty-bed contact times (60 and 90 minutes) will be evaluated, providing insights into optimizing operational parameters for maximizing pollutant removal efficiency while minimizing operational costs. Molecular ecology techniques, including 16SrRNA sequencing and PCR (Polymerase Chain Reaction), will be used to characterize microbial communities at different depths within BAC columns to identify the roles of specific microbial groups in the biodegradation process. Additionally, standardized assays for heterotrophic activity and nitrification inhibition will be used to determine whether the BAC treatment effectively mitigates potential inhibitory effects on microorganisms in the aeration basin of the WWTP that receives the refinery effluent. Moreover, standardized assays for heterotrophic activity and nitrification inhibition will assess the effectiveness of BAC treatment in safeguarding microbial communities from inhibitory effects, ensuring the stability and efficiency of biological processes. The anticipated results of this study are expected to advance our understanding of BAC systems, providing insights into microbial interactions and process optimizations necessary for effective treatment. These findings could significantly improve the operational efficiencies of refinery effluent pretreatment, contributing to more sustainable management of industrial effluents.

6.02.P-Mo-187 Harnessing Biocompatible 3D Graphene-Based Nanocomposite for Polycyclic Aromatic Hydrocarbons Degradation: A Sustainable Approach

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Polycyclic aromatic hydrocarbons (PAHs) are a class of emerging contaminant that are of global environmental concern. Due to its hydrophobic properties and limited water solubility, once discharged into the aquatic environment, it inhibits the penetration of oxygen and causes damage to aquatic life. Numerous treatment methods, such as physical and chemical methods, have been explored but bioremediation has emerged as most promising technology for breakdown of PAH. Marine bacterial isolates demonstrated high lipase activity. Lipase accelerates the breakdown of triacylglycerols into glycerol and free fatty acids. Motor oil contains various hydrocarbons, including long-chain fatty acids, which are considered substrates for lipase activity. The presence of lipase accelerates the degradation of motor oil by facilitating the breakdown of complex hydrocarbons into smaller, more readily biodegradable molecules. Indigenous PAH-degrading strains tend to exhibit decrease in microbial activity due to their suspended form; therefore, immobilization using suitable carrier can increase the survival rate. In this study, we used a bio-nano approach to enhance the biodegradation of PAH present in motor oil. Herein, we used macroalgae-associated bacteria, isolated from marine mangrove sediments immobilized on graphene-based nanocomposite foam, which was highly porous, hydrophobic, and super-oleophilic. The low-loading percentage of nanomaterial served as an additional source of carbon, which increased the survival rate and resident time. The FEG-SEM analysis confirmed the trapped bacteria within the pores of the matrix. FTIR analysis revealed the changes in presence of functional groups caused due to the breakdown of PAH. GC-MS analysis was used to identify the synergistically degraded by-products, which were non-toxic in nature. This bio-nano approach is low cost, highly efficient has practical applications in natural environment.

6.02.P-Mo-188 Macro-Algae Biocarbon's Membrane for the Remediation of Organic and Inorganic Pollutants in Soil

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In 2005, the U.S. Environmental Protection Agency included the Vieques, P.R., a site with a legacy of military activities, as a site of ecological risks and human health. Scientific studies have identified high concentrations of metals which are present in explosives, bullets, and other paraphernalia used. Because of these problems, the goal is to establish a means to remediate the soils of Vieques. This study introduces an algae-based biocarbon membrane, developed as a sustainable solution for immobilizing organic and inorganic pollutants. Biocarbon Derived such as biochar, graphite, graphene, and graphene oxide, are recognized for their porosity and adsorption capabilities. Utilizing eco-friendly thermal processes and the algae, as a biocarbon source, we synthesized biocarbon with enhanced surface area and pore structure, optimized for efficient pollutant absorption and distinct properties like increased cation exchange capacity. To augment practical application, we incorporated the biocarbon into sodium alginate membranes, a biodegradable, non-toxic, and cost-effective material, synergizes with biocarbon to improve

mechanical strength and stability while enabling controlled release and adsorption dynamics. The experimental encompasses characterization analyses, including Fourier Transform Infrared Spectroscopy (FTIR), Raman Spectroscopy, and Thermogravimetric Analysis (TGA), to elucidate the functional groups, thermal stability, and structural integrity of the biocarbon. Post-characterization, contaminated soil samples were treated with our algae-based biocarbon membranes. The remediation efficacy was monitored through quantification of heavy metal concentrations using Atomic Absorption Spectrophotometry (AA). Preliminary results demonstrate notable reductions in metal ions, including a 10% decrease in copper. In organic pollutants like perfluorinated compounds, ongoing research is focused on developing targeted remediation strategies. Initial tests on solutions indicate promising potential, with component analysis conducted via Gas Chromatography-Mass Spectrometry (GC-MS). This study presents a pioneering approach to soil remediation, showcasing the feasibility of algae derived biocarbon membranes in mitigating the environmental impact of soil contaminants, thereby contributing to the restoration of ecologically sensitive areas.

6.02.P-Mo-189 Removal of Microplastics from Agricultural Runoff using Biochar: A Column Feasibility Study

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Plastics are extensively used in agriculture, but their weathering and degradation generates microplastics (MPs) that can be carried by runoff into water bodies where they can accumulate and impact wildlife. Due to its physicochemical properties, biochar has shown promise in mitigating contaminants in agricultural runoff. However, few studies have examined its effectiveness at removing MPs. In this study, we assessed MP pollution (>30 µm) in runoff from a farm in the Mississippi Delta and examined the effectiveness of biochar (pinewood and sugarcane) to remove MPs from aqueous solutions. Using micro-Fourier Transform Infrared spectroscopy (µ-FTIR), we observed an average of 237 MPs/L (range 27-609) in the runoff, with most particles identified as polyethylene, polyamide, polyvinyl chloride, polyurethane, acrylonitrile butadiene styrene, and polyarylamide. Biochar columns effectively removed MPs from runoff samples with reductions ranging from 86.6% to 92.6%. MPs of different sizes, shapes, and types were stained with Nile red dye (to facilitate observation by fluorescence) and quantified their downward progress with multiple column volumes of water and wet/dry cycles. Smaller MPs penetrated the columns further, but ≥90% of MPs were retained in the ~20 cm columns regardless of their shape, size, and type. We attribute these results to physical entrapment, hydrophobic behaviors, and electrostatic interactions. Overall, this proof-of-concept work suggests biochar may serve as a cost-effective approach to remove MPs from runoff, and that subsequent field studies are warranted.

6.02.P-Mo-191 Pretreatment of Refinery Wastewater Using Biologically Activated Carbon

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A large volume of wastewater is produced during crude oil processing. Without adequate treatment, pollutants from refinery wastewater may have a significant negative impact on the ecosystem. Refinery wastewater may be combined with domestic wastewater and undergo conventional biological treatment in a wastewater treatment plant. However, depending on the characteristics of the refinery wastewater, additional treatment steps might be needed to avoid negative impacts on biological processes. Granular activated carbon (GAC) has been widely used for the removal of micropollutants in water and wastewater treatment due to its high specific surface area and porosity, GAC also serves as biofilm support media, allowing for the growth of microorganisms able to degrade organic contaminants from wastewater. Thus, the present study investigates the development and use of anaerobic and aerobic biologically activated carbon (BAC) columns reusing spent granulated activated carbon from a water treatment plant as a pretreatment for the primary effluent of a petroleum refinery. The main goal of this project is to determine if BAC columns inoculated with a mixed microbial community previously exposed to the refinery wastewater can reduce the concentrations of toxic contaminants to avoid negative impacts on the activated sludge process in the wastewater treatment plant treating this effluent. Firstly, bench-scale experiments will be performed to evaluate the effect of the empty-bed contact time and respiratory conditions on the removal of nutrients and organic compounds such as benzene, toluene, ethylbenzene, and xylene (BTEX). Empty bed contact times of 60 and 90 minutes will be tested. The concentrations of BTEX, chemical oxygen demand, nitrogen ions, and sulfate will be monitored in the influent and the effluent, as well as the pH and flow rate. Then, a pilot-scale reactor will be designed using the optimal conditions determined in the bench-scale experiments. Molecular ecology analysis will be performed to determine the predominant microbial groups in the columns. At the end of this project, we expect to be able to make recommendations for the design of full-scale BAC columns for refinery wastewater pretreatment. The results obtained in this project may offer an alternative for the disposal of spent GAC in landfills by recycling it for wastewater treatment.

6.02.P-Mo-192 Surface Water Quality for a Proposed Two-stage Ditch Program, Upper Cache River Watershed, Arkansas

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Agricultural runoff transferring sediment particles and nutrients into streams and lakes poses an increasing threat to water quality. These contaminants contribute to eutrophication leading to hypoxic zones in both freshwater and marine aquatic environment. Much of the excess nutrient contamination in the Gulf of Mexico originates from the upper Mississippi River Basin (MRB). The Cache River Watershed in Arkansas is a portion of the MRB had been identified by the Arkansas Department of Environmental Quality (ADEQ) as an area of concern. The ADEQ has identified approximately 515 km of streams in the Upper Cache River Watershed (UCRW) which are almost entirely channelized and are a source of nutrients into the waterways. One method of mitigation of nutrient runoff is construction of two-stage ditches (SDs) in channelized

agricultural streams. The use of SDs is a novel floodplain restoration management technique with the potential to retain contaminants and establish better habitat for enhanced water quality. This design is a practical solution for reducing nutrient pollution in agricultural waterways and a tool for sustainable water management and ecosystem restoration. The Nature Conservancy is installing three SDs in the UCRW to be monitored prior, during, and after construction to determine the efficacy of the SD system. The UCRW's East Slough (EASL) and Big Ditch Creek (BCDI), two sub-watersheds that were previously monitored, have total nitrogen (TN), total phosphorus (TP), and turbidity levels that exceeded state standards. Most dissolved oxygen (DO) violations of state limitations were surpassed by EASL as well as violations for turbidity. Additionally, TP concentrations in BCDI were more than four times higher than the state standard, and TN values were much higher than in any other site sampled for the UCRW. Considering the goal of installation SD systems in the UCRW, we offer here a discussion about the primary construction process and address pre-construction water quality data.

6.02.P-Th Late Breaking Science: Engineering, Remediation and Restoration

6.02.P-Th-192 Oak Ridge Reservation Restoration Synthesis: A DOE Perspective

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Oak Ridge National Laboratory (ORNL) has made significant progress in chemical abatement and restoration of previous perturbations made in efforts related to World War II. Since the development of the Biological Monitoring and Abatement Program (BMAP) in the 1980s, ORNL has generated a unique biomonitoring data set of the surface waters in the ORR that can be utilized in restoration ecology analyses. To date, there has not been a centralized synthesis of restoration efforts on the ORR, though the existence of the efforts has been reported through multiple channels. This synthesis has sequestered the different restoration projects taken by ORNL since its designation as a CERCLA site in the 1980s and the year 2018. Through literature reviews, restoration project entry forms, and interviews with subject experts, this synthesis contains a tabular and spatial database containing in-depth information on 127 restoration projects, including project goals, dates, locations, monitoring efforts, and outcomes. Our goal is to holistically understand the restoration outcomes on the ORR, but also to foster communication between restoration experts and encourage future research in restoration ecology. To this end, we utilize a statistical approach to quantify outcomes of restoration efforts by analyzing species richness within each watershed found in the ORR. Generalized linear mixed models are used to predict species richness by site using a site and year interaction predictor coefficient while accounting for inherent variability between sites and seasons. We extracted the interaction coefficient and analyzed this value using linear regression with a novel weighted restoration scoring system that accounts for number of restoration projects per drainage area. There is a general positive relationship with number of efforts and an increase in species richness, meaning that the efforts thus far have had significant results on the returning of species. We hope to use this model of restoration synthesis and analyses to further encourage research in restoration ecology and how to better quantify restoration efforts.

6.02.P-Th-193 Comparative Detection and Quantification of ESBL *E.coli* Across Shell Creek Watershed, Nebraska: Compartment Bag Test vs Membrane Filtration

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As of 2024, antibiotic resistance (AR) in the United States remains a significant public health concern according to the Centers for Disease Control and Prevention. AR emerges mainly through natural selection (where bacteria mutate and develop resistance), via horizontal gene transfer exacerbated by use, overuse and misuse of antibiotics for veterinary and human health, and from environmental pollution. Surface waters such as rivers, lakes, and streams receive runoff and municipal/industrial discharges loaded with residual antibiotics/transformation products that accelerate the development of antibiotic resistant bacteria and genes, causing potential threat. It is important to develop and implement simple, rapid, accurate and practical solutions available outside the laboratory. To address the urgent need, field deployable methods for detecting ESBL *E.coli* indicator bacteria play a significant role for understanding the AR pattern capturing the onsite scenarios. Traditional methods of culturing bacteria often require controlled environment and sophisticated instruments. The compartment bag test (CBT) has been used in the past as a potable and user-friendly technique for environmental surveillance of indicator bacteria, reducing barriers for field evaluations. The method is designed to be used in place of or in addition to the standard method of membrane filtration (MF). Therefore, the objective of the research was to compare the samples collected in CBT bags on fields and in the laboratory setup with the standard method of MF. Four samples were collected between the upstream to downstream in Shell Creek, including one USGC site for 5 months. A onetime study was conducted to understand the cefotaxime and tetracycline resistance pattern of ESBL *E.coli*. In the rainy season a higher proportion of ESBL *E.coli* were reported, compared to the dry season. Runoff, sediments, watershed characteristics, agricultural and climate were the factors that affected the microbial contamination. The results obtained for CBT and MF were comparable to each other and hence these could be best utilised for surface water monitoring to provide options for surface water surveillance. Both the approaches are reliable and effective for monitoring antibiotic resistance providing essential data for public health and environmental protection efforts.

6.02.P-Th-194 Unraveling Fluorotelomer Biotransformation: Insights From Analytical Chemistry and Microbiology

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For decades, fluorotelomer compounds have been used to produce surfactants and fluorinated polymers in consumer and industrial products. Long-chain fluorotelomers were phased out and replaced with shorter-chained analogues since their biotransformation can result in long-chain perfluoroalkyl carboxylates (PFCAs). Early studies using 14C-labeled fluorotelomer alcohols were conducted to clarify the relationship between fluorotelomer alcohols and PFCAs. Through the use of 14C-labels and high-resolution mass spectrometry, pathways for FTOHs 8:2 and 6:2 were elucidated with high confidence, resulting in the discovery of new transformation intermediates. These studies showed that these compounds undergo a "one-carbon removal process" instead of the classical beta-oxidation. This process involves sequential -CF2- removal

from fluorotelomers, leading to partial defluorination and x:3 acids (such as 5:3, 4:3 and 3:3 acids) formation under aerobic conditions. Despite a relatively good understanding of these pathways, the microorganisms involved are understudied. Our study aims to enrich cultures capable of deep defluorination of fluorotelomer acids for future cost-effective biological treatments. We employed a dual-stage enrichment strategy: initially using diluted media for slow-growing microbes, followed by rich media to accelerate growth and degradation. After a year of enrichment with 5:3 acid, several degrading consortia were obtained. Further testing showed that one consortium degraded 5:3 acid into 4:3, 3:3, and 2:3 acids in 47 days. The consortium's defluorination potential for n:3 acids (n = 2-5) and 6:2 fluorotelomer unsaturated acids was evaluated, with defluorination rates of 24%, 85%, 33%, 55%, and 66%, respectively. Fluoride generation was quantified, which confirmed the extensive biotransformation. Furthermore, based on the dilution plate technique, 23 bacteria were isolated and identified via 16S rRNA gene sequencing. However, none could perform defluorination alone. We also applied metagenomic analysis of 16S rRNA to characterize the bacterial community structure during incubation. This study is the first to identify and enrich microbes responsible for the one-carbon removal pathway in fluorotelomer degradation. The unique culture we developed lays the groundwork for future engineered biological treatments to achieve extensive defluorination of fluorotelomers.

6.02.V Late Breaking Science: Engineering, Remediation and Restoration

6.02.V-01 Mechanistic Insights Into Fast Adsorption of Perfluoroalkyl Substances on Carbonate-Layered Double Hydroxides

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Layered double hydroxide (LDH) with the metal composition of Cu(II)Mg(II)Fe(III) was prepared as an adsorbent for fast adsorption of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). 84% of PFOS and 48% of PFOA in relation to the equilibrium state were adsorbed in the first minutes of contact with 0.1 g/L of suspended μm-sized LDH particles. The adsorption mechanisms of PFOS and PFOA on the CuMgFe-LDH were interpreted. Hydrophobic interactions were primarily responsible for the adsorption of these compounds in accordance with the different adsorption affinities of long-chain and short-chain perfluorinated carboxylic acids. PFOA adsorption on CuMgFe-LDH was strongly suppressed under alkaline conditions while PFOS uptake was only slightly affected in the pH range from 4.3 to 10.7, indicating a significant role of electrostatic interactions for PFOA adsorption. The adsorption of PFOS and PFOA was rather insensitive to competition by monovalent anions. The previously reported ‘memory effect’ of calcined CuMgFe-LDH for sorption of organic anions was not confirmed in the present study. Spent CuMgFe-LDH could be easily regenerated by extraction with 50 vol% methanol in water within 1 h and maintained a high PFOS removal in subsequent usage cycles.

6.03.T Preparing for an Environmental Emergency Response: Disaster Risk Assessment Lessons Learned from the Field

6.03.T-01 Ambient Air Monitoring Following Natural Disasters in Texas

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When a natural disaster like a hurricane occurs, industrial facilities often shut down in advance of the event to mitigate possible damage to the facility and to ensure worker and public safety. When a storm has passed these facilities begin startup activities, and there is the possibility of unauthorized emissions of chemicals into the air from shutdown, upset, and startup activities. The Texas Commission on Environmental Quality (TCEQ) is responsible for monitoring air quality in Texas, and when it is safe to do so, TCEQ deploys monitoring assets after natural disasters to characterize potential impacts of industrial emissions on air quality. To determine the impact of natural disasters on emissions and concentrations of chemicals in air, we evaluated emissions data and air monitoring data from stationary and mobile monitoring instruments collected before, during, and after Hurricane Harvey (2017), Hurricane Laura (2020), Hurricane Delta (2020), and Winter Storm Uri (2021). The TCEQ found that the storms were associated with a range of emissions amounts from industrial facilities, from 480,000 pounds of chemicals for Hurricane Delta to more than 14,000,000 pounds for Hurricane Harvey. Of the chemicals that were released, carbon monoxide, sulfur dioxide, and nitrogen oxide represented the highest quantities of reported emissions for all events. 1.5 million data points of stationary ambient air monitoring data were collected around these events and fewer than 0.001% of the measurements exceeded an appropriate health-protective comparison value. Similarly, more than 2.1 million chemical concentrations were measured using mobile air monitoring instruments and only about 0.01% of the data exceeded a comparison value. Altogether, the TCEQ has collected millions of air monitoring measurements in Texas after natural disasters, and although there are industrial emissions associated with these events, measured chemical concentrations in the air show very few concentrations above health-protective comparison values.

6.03.T-02 Derivation and Application of Comparison Values and Action Levels for Use During Mobile Air Monitoring of Natural Disasters or Industrial Accidents

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The Texas Commission on Environmental Quality (TCEQ) has several ambient air monitoring instruments that can collect instantaneous chemical concentration data during in-motion mobile monitoring surveys. These mobile monitoring devices allow the TCEQ versality in responding to air emissions from natural disasters or industrial accidents. The instruments provide accurate, real-time concentrations, typically in 1-30 second intervals, of select chemicals in ambient air while the monitoring vehicle is in motion. However, instantaneous data present unique challenges for interpretation because there are almost no relevant toxicity studies with brief exposure durations to base health risk determinations. Comparison values don’t exist for evaluating the 1-30 second concentration data either from the perspective of what represents a typical or a high concentration, or for interpreting the toxicity associated with the measured concentrations. Also, there is the need for continuous, real-time review of incoming air monitoring data to provide context to results and to determine appropriate response/actions in the field. TCEQ developed four different types of fit-for-purpose data screening levels for evaluation of mobile air monitoring data. They include an investigation level derived using chemical and instrument-specific baseline detections, as well as three toxicity-based values that trigger increasingly urgent actions. These values are intended to provide guidance to mobile monitoring staff on when to initiate actions such as source investigation, stationary monitoring, and staff exposure mitigation (i.e., leaving the area). The values provide guidance for staff actions but are not intended to overrule the cautionary discretion of the monitoring and field investigation staff, particularly when considering exposure mitigation. The mobile monitoring equipment can be used to rapidly identify abnormally high concentrations of targeted chemicals in ambient air and can help expedite efforts to address them. The mobile instruments provide substantial versatility in where chemical concentrations can be monitored, while also quantitatively measuring a number of different chemicals. The work described here resulted in the generation of fit-for-purpose comparison values that can help the TCEQ and others to prioritize resources for identifying sources, characterizing chemical concentrations, and/or mitigating exposure risk from events that cause chemical releases.

6.03.T-04 Guide for Public Health Response to Cyanobacterial Harmful Blooms in Recreational Fresh Water of Texas

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Cyanobacteria is a type of algae present in all waterbodies that can reproduce rapidly in certain environmental conditions, such as increased temperatures and nutrient loads. In freshwater, cyanobacteria can produce toxins capable of harming humans and animals. During the summer of 2019, several dog deaths suspected from exposures to cyanobacterial harmful algal blooms (HABs) were reported around Lady Bird Lake in Austin, Texas. With technical assistance from Texas Department of State Health Service (DSHS), the city of Austin conducted water and animal biospecimen sampling and testing, as well as provided recommendations to people and pets to avoid contact with water and algae directly at Lady Bird Lake. In Texas, responses to cyanobacterial HABs in freshwater bodies fall on local health departments and waterbody authorities. However, in 2019, there were no coordinated efforts among state and local agencies on HABs and appropriate responses to protect public health. The members of the Toxic Substances Coordinating Committee (TSCC) HAB workgroup decided to develop a statewide guidance document that can be used for responding to HAB events. The TSCC is a legislatively mandated committee hosted by DSHS, where several state agencies meet quarterly to discuss topics related to toxic substances and work together to address certain issues. The TSCC HAB workgroup developed a scientific evidence-based document titled, "*Guide for Public Health Response to Cyanobacterial Harmful Blooms in Recreational Fresh Water of Texas*", first published in March 2022 and revised in February 2024. To date, DSHS has assisted local jurisdictions in Texas for responding to five freshwater cyanobacterial HAB events and related animal exposures. DSHS also facilitates reporting of suspected or confirmed HAB events and exposures to the Center for Disease Control and Prevention's (CDC) One Health Harmful Algal Bloom System (OHHABS).

6.03.T-05 Lessons Learned from a Rapid Response to Invasive Quagga Mussel in the Snake River, Idaho

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Quagga mussels are an invasive freshwater Eastern European mollusk and are a significant ecological and economic problem globally, costing the United States 1 billion dollars per year in damages to water infrastructure and industries. One mussel can produce 30,000–1 million veligers (larva) per year, which can move downstream for up to 30 days without settling. On September 18, 2023, the Idaho State Department of Agriculture (ISDA) discovered a veliger in the Snake River near Twin Falls, Idaho. The ISDA immediately implemented their rapid response plan; Idaho's governor and local, state, and federal agencies made the response a priority. The rapid response team, in coordination with over 19 agencies, debated the plan's treatment options. The state faced a dire question: Is it possible to eliminate a looming quagga mussel outbreak quickly and effectively? The team settled on a molluscicide control of a chelated copper treatment applied for 96 hours at a concentration equal to 1 ppm (1 mg/L). The ISDA and partners-initiated treatment on October 3, 2023, 15 days after the initial veliger discovery. Total loss or dilution of copper within the dissemination area was estimated between 82% and 97%. State and federal agencies collaborated in eliminating quagga mussels, each with their own delegated responsibility monitoring water quality and key aquatic organisms in response to the chelated copper treatment. Water quality returned to below criteria at all sites by October 31, 2023, 28 days after the first treatment, with zero detection of quagga veligers. A fast, aggressive response was necessary to achieve eradication. Other states have attempted eradication, but were unsuccessful, potentially due to a lack of inter-agency collaboration or a slower response time. The ISDA and partner agencies proved for the first time that it is possible to control a potential quagga mussel outbreak with early detection, a rapid eradication response, and inter-agency collaboration—a model for other states across the nation. Ongoing monitoring will be conducted to ensure success.

6.03.T-06 Lessons Learned from Fluorotelomer-Based Aqueous Film-Forming Foam (AFFF) Use During Emergency Response

***Dana McCue**[1] and Reynolds Renshaw[2], (1)EHS Support, (2)EHS Support*

Class B firefighting foams are used to fight flammable liquid fires. Aqueous Film-Forming Foams (AFFF) are reported to be the most widely used and available type of Class B firefighting foam. AFFF was used to control two separate facility fires that are the focus of this presentation. Fire No. 1 was a large incident located near a populated area that fully engulfed a 300,000-square foot facility which stored 3,000,000 gallons of petroleum product. The fire burned for 9 days despite efforts of both professional and local fire fighters. Approximately 1.2 million gallons of fire suppression water and subsequent stormwater were collected in approximately 70 frac tanks. Fire No. 2 was a smaller incident located in a less densely populated area that impacted two structures less than 50,000 square feet which stored 300,000 gallons of petroleum product. The fire burned for less than 1 day and was responded to by the local fire department. Fire suppression water was contained within a 60,000-square foot concrete-lined secondary containment structure. The AFFF used in both fires were C6 fluorotelomer-based that contained per- and polyfluoroalkyl substances (PFAS), in particular 6:2 fluorotelomer sulfonate (6:2 FTS). While effective to control the blaze, PFAS have been shown to cause adverse effects to human

health and the environment. At the time of both incidents, regulations prohibiting or banning PFAS-containing Class B firefighting foam were not in place in either jurisdiction.
The type and size of each fire, how it was handled, and the extent of property damage and environmental impact differed between the events. However, the lessons learned from each incident provide actions that other facilities can perform now to be prepared. Lessons learned are grouped into the following areas for discussion: recordkeeping amongst the chaos (who, what and where); limiting and documenting the extent of AFFF impact (contain, contain, contain) and how that informs future site investigation; treatment and disposal considerations and pitfalls; pre-planning, training and command structure; and, general emergency response (cost efficiency considerations).

6.03.V Preparing for an Environmental Emergency Response: Disaster Risk Assessment Lessons Learned from the Field

6.03.T-03 ArcGIS for Public Health Emergency Response: The Agency for Toxic Substances and Disease Registry's Comprehensive Disaster and Readiness Toolkit (CDART)

Chris Poulet[1], *Elizabeth Irvin*[2], *Teresa Wei Wang*[3,4], *Kelsey Brady*[5], *Timothy Turner*[6] *and* ***Matt Ferguson***[7], *(1)Agency for Toxic Substances and Disease Registry, Office of Community Health and Hazards Assessment, Atlanta, (2)Office of Community Health and Hazards Assessment, Agency for Toxic Substances and Disease Registry, Office of Community Health and Hazards Assessment, (3)Centers for Disease Control and Prevention, Atlanta, United States, (4)Office of Community Health and Hazard Assessment, Agency for Toxic Substances and Disease Registry, Office of Community Health and Hazard Assessment, Atlanta, (5)Agency for Toxic Substances and Disease Registry, Office of Innovation and Analytics, (6)Office of Community Health and Hazard Assessment, Agency for Toxic Substances and Disease Registry, Office of Community Health and Hazard Assessment, (7)ATSDR - Region 7*

The Agency for Toxic Substances and Disease Registry's (ATSDR) Comprehensive Disaster Assessment and Readiness Toolkit (CDART) represents a digital suite of adaptable tools that can enhance disaster preparedness, response, and recovery. CDART facilitates real-time situational awareness and field activity visibility by incorporating operational GIS capabilities, app-based data collection, and critical information dashboards. The CDART Team at ATSDR originally developed and piloted the use of app-based environmental assessments for healthcare facilities during Hurricane Maria Response and Recovery Operations in 2017 and 2018 in partnership with the Puerto Rico Department of Health and Puerto Rico Planning Board. The CDART Team also modified the toolkit for Puerto Rico's COVID-19 vaccine distribution efforts. Most recently, the CDART Team provided virtual assistance to the Hawaii Department of Health during the September 2023 Maui Wildfire Response by building a modified CDART form and dashboard as a proof-of-concept to track health services capacity provided for displaced community members at a subset of hotels on the island of Maui. Currently, the CDART Team maintains a roster of ATSDR staff to provide virtual or field assistance to partners during hurricane season. This presentation will provide attendees with an overview of the toolkit, as well as how the CDART Team can provide technical assistance and training to partners across federal, state, territorial, and local health departments for utilization and deployment of the CDART toolkit to meet their emergency response needs.

6.04.P-We Risk Assessment, Remediation, and Restoration: Applying Interdisciplinary Approaches to Creating Successful Remediation and Restoration Projects

6.04.P-We-188 Ecological Risk Assessment, Risk Management, and Restoration: The New Jersey Approach

***Brian S Yates**, Yates Environmental Sciences*

The practicability of restoring ecosystems is an often overlooked component of risk-based remediation. Retrospective research on wetland mitigation success in New Jersey, suggests that restoring mature forested wetlands has a <10% success rate compared to >90% for emergent wetlands and scrub/shrub habitats. If not conducted carefully, remediating mature forested wetlands could cause a natural resource injury. We present the case of a trap & skeet shooting range adjacent to a forested wetland complex that has been highly contaminated by lead. Conventional ecological risk assessment lines of evidence (e.g., tissue sampling, food chain modeling) were used to develop a preliminary remediation goal (PRG) of 150 mg/kg based on a NOAEL for the American woodcock. However, remediating to the PRG would destroy 20 acres of mature, forested wetlands. A risk management decision was developed which allowed for preservation of forest habitats (with lead levels up to 20X the PRG) while the PRG was applied to emergent habitats. This allowed for maximal risk reduction while minimizing adverse effects to sensitive forested wetlands.

6.04.P-We-190 Assessing the Robustness of Statistical Models in Evaluating PCB Exposure Trends in Fish Amid Variable Lipid Content

***Ying Wang**[1], John Kern[2], Solomon Gbondo-Tugbawa[3] and Juliana Atmadja[3], (1)WSP USA, Burnaby, (2)Kern Statistical Services, (3)WSP USA*

Remediation of contaminated sediments often targets reducing contaminant levels in fish tissues for the protection of wildlife and human health. Fish tissue concentrations represent a time- and space- integrated concentrations in water and sediments, making them valuable for long-term monitoring of remedy effectiveness. Concentrations of lipophilic chemicals, such as polychlorinated biphenyls (PCBs), in fish tissues are influenced not only by environmental exposure but also by fluctuations in lipid content. This makes it difficult to isolate changes in fish tissue PCB concentrations due to exposure from those caused by lipid variations. We compared three statistical models using simulations to assess how well they estimate PCB trends when lipid varies with time and measured with error. The models included: (1) a multiple linear regression model with lipid content as a covariate; (2) a linear regression with lipid-normalized PCB concentration; and (3) a non-linear multiple regression with both lipid content and non-lipid organic matter (NLOM) as covariates. All models performed well when lipid content remained constant with time. However, when lipid content decreased over time, multiple regression models (1 and 3) tended to overestimate PCB trends, whereas the lipid-normalized model (2) consistently underestimated the PCB trends, except when its assumptions of a linear PCB-lipid relationship and no PCB partitioning into NLOM were met. Our findings suggest that multiple regression models (1 and 3) and lipid-normalized model (2) likely provide upper and lower bounds for the true PCB trend, with the discrepancy reflecting the degree of collinearity between lipid content and time. Conclusions: This study highlights the importance of evaluating lipid-time collinearity for accurate assessment of PCB trend in fish. The extent of this collinearity depends on the magnitude of trend in lipid content and its within-year variation, and can be quantified by regressing the logarithm of lipid content against time (e.g., R-squared value). To identify true changes in fish tissue PCB concentrations due to changes in exposure, we recommend selecting fish species or tissue types that exhibit minimal lipid-time collinearity. The results from this study can be generalized to assess trends of other lipophilic contaminants besides PCBs.

6.04.T Risk Assessment, Remediation, and Restoration: Applying Interdisciplinary Approaches to Creating Successful Remediation and Restoration Projects

6.04.T-01 The East Palestine OH Train Disaster - A Continuing Saga of People, Ecosystems, and Places

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The train derailment disaster in East Palestine, OH occurred more than 15 months ago. Despite the efforts to remediate the aftereffects, many impairments to ecological and human health remain. The myriad of contaminants, singly and in mixtures, and the timing of multiple releases to a variety of media continues to challenge the scientific understanding of the ongoing and potential consequences. Adding to the complexity are the differing views of involved groups in determining sampling, analytical, and interpretation approaches. The efforts of one independent group of investigators will be presented, integrating targeted data collection across a variety of environmental media essentially covering the time period from the initial event to present and focused on more persistent and combustion-created pollutants than those, such as vinyl chloride, benzene, and butyl acrylate, directly released from the damaged and burned railcars.

The targeted environmental data for water, sediment, soils, garden crop, and residences will be augmented by human and ecological data as well as experiential and photographic documentation. These show a pattern of consequences of both the initial incidents and subsequent remediation efforts exacerbated by extreme natural events occurring multiple times over the ensuing timeline. Mixtures of chemicals dispersed by this disaster and cleanup have created human exposure unprecedented in the US. Given the lack of quantitative standards for exposures to mixtures, medical monitoring and testing of those that may have been exposed is imperative. This presentation will also address the trends in potential human health effects from exposures to date.

6.04.T-02 Biomimetic Extraction with Polydimethylsiloxane as an Indicator of Toxicity of Petroleum Mixtures Before and After Sediment Remediation

Magdalena Rakowska[1]*, Thomas Parkerton*[2] *and Danny D. Reible*[1]*, (1)Texas Tech University, (2)EnviSci Consulting*

Assessment of total petroleum hydrocarbons from contaminated sites demands routine and reliable measurements at low levels. This includes post-remedy, which are often challenged by limited detection due to high efficiency of amendments used. Further, typical analyses often focus on single constituents. Biomimetic extraction (BE) with Polydimethylsiloxane (PDMS) has been demonstrated as a fast and sensitive method for determining toxicity of unresolved hydrocarbon mixtures in aquatic systems. The method measures a pooled response of the bioavailable fraction of petroleum hydrocarbons (total area), which is translated to molar concentration on the PDMS sampler. The molar concentration is then related to toxic thresholds in target lipid of aquatic organisms. The primary objective of this study was to evaluate the effect of activated carbon (AC) on reducing bioavailability of unresolved hydrocarbons. Three sediments with a variety of petroleum hydrocarbon compositions were evaluated with a non-depletive BE approach using standard 30 µm PDMS fibers. The assessment was conducted for sediments with and without AC addition. Sediments were also evaluated with a depletive BE approach for determination of total extractable BE using PDMS coated sheets. Solvent extracts were analyzed using large volume injection GC-FID. The effectiveness of AC was then assessed by calculating percent reductions and was further used for calculating apparent sorption coefficients for AC (K_{AC}). The highest total BE was determined for IHC sediment (225 µmol/g), whereas the lowest for LDW (7.8 µmol/g) with 6 µm PDMS sheets. The non-depletive and depletive BE data were used to estimate BE partition coefficients K_{BE}, L/kg. The AC yielded BE reductions ranging from 25 to 92% depending on the sediment and AC dosage used corresponding to K_{AC} of 48800 to 396000 L/kg. The effectiveness of AC was inversely correlated with the amount of organic matter and the presence of NAPL in the sediments. The collected data was used to build an estimate of the long-term toxicity of sediments under natural recovery, in-situ capping with AC amendment and in-situ treatment with AC. The CapSIM model was used to predict BE in the surficial sediments over time in each scenario. Results and parameter values provide a basis for predicting BE as a toxicity indicator over time for in-situ treatment and capping in addition to routine screening of sediments at contaminated sites to support design of treatment remedies.

6.04.T-03 Ecological Restoration of Degraded Peatlands in Sungai Tohor, Sumatra, Indonesia

Charles Lee[1] *and Yee Ching Chong*[2]*, (1)University of Newcastle (Australia), Singapore, (2)PM Haze, Singapore*

Peatlands are the largest natural terrestrial carbon store, which sequesters 0.37 gigatonnes of carbon dioxide CO_2 a year. Damaged peatlands are a major source of greenhouse gas emissions, annually releasing almost 6% of global anthropogenic CO_2 emissions. Peatland restoration is therefore crucial in emissions reductions, thereby mitigating climate change impacts. In the Sungai Tohor area in Riau, Indonesia, its rich peatland is drained to support monoculture plantations (pulpwood) leading to massive fires, land subsidence, and flooding. The village communities would not be able to survive in the mid to long term without restoring their degraded lands to a healthy peatland ecosystem. Since 2019, the University of Newcastle (Australia) has collaborated with 2 NGOs - People's Movement to Stop Haze (PM Haze, Singapore) and Ekonomi Kreatif Andalan (EKA, Indonesia), in a peatland restoration project. The main objective is to restore the damaged Sungai Tohor peatland consisting of 3 hectares - 30% burnt and 70% degraded land. The tasks involved: (a) peatland vegetation replanting; and (b) rewetting of peatland groundwater. Re-vegetation comprises of planting more than 10 peatland vegetation species sourced from natural peatland, as well as sago palm - the main cash crop of the villages. Re-wetting tasks include raising the groundwater by canal blocking to prevent further drainage of peatlands. The restoration efforts have expanded to about 25 hectares of damaged peatlands. Sustained restoration and continuous monitoring over 5 years have produced crucial data to demonstrate an excellent case study of a successful restoration program. This research study will present key findings and challenges in re-vegetation and re-wetting in the restored peatlands.

6.04.T-04 TCEQ Natural Resource Trustee Program and Ecological Service Analysis Under the Texas Risk Reduction Program

***Michael Cave**, Texas Commission on Environmental Quality, Austin, United States*

The Texas Commission on Environmental Quality's Natural Resource Trustee Program (NRTP) fulfills the agency's role as a designated state Natural Resource Trustee to act on behalf of the public in Natural Resource Damage Assessment (NRDA) matters, including assessing natural resource injuries caused by oil spills or hazardous substance releases; determining the nature and extent of restoration required to compensate the public; and ensuring responsible parties implement or fund the restoration. The NRTP works in close coordination with the other Texas Natural Resource Trustee agencies, including the Texas Parks and Wildlife Department, Texas General Land Office, United States Fish and Wildlife Service on behalf of the U.S. Department of the Interior, and the National Oceanic and Atmospheric Administration (collectively, the Texas Trustees). The NRTP also coordinates the referral and review of certain ecological risk documents to the other Trustees in accordance with Texas Risk Reduction Program (TRRP) rules, including Ecological Service Analysis (ESA). In accordance with TRRP Remedy Standard B (30 Tex. Admin. Code §350.33), when either the initial concentrations of chemicals of concern (COCs) within environmental media exceed only the ecological Protective Concentration Levels (PCLs), or when there will be residual concentrations of COCs above the ecological PCLs following completion of a human health response action, a responsible party can consult with the Texas Trustees to potentially conduct an ESA of the affected property. Under an ESA, a responsible party can evaluate the effects of reasonable and feasible remediation alternatives with respect to present and predicted losses of ecological services and provide compensatory ecological restoration either alone or in combination with some type of active response action (e.g., hot spot removal) or passive response action (e.g., natural attenuation) for the affected property. This 12-minute presentation will provide an overview of the work of the NRTP and the ESA process.

6.04.T-05 In Situ Toxicity Identification Evaluation: Prototype Improvements and Field Verifications of a Technology for Detecting Stressor-Causality Linkages

***Austin Crane**[1], D Bart Chadwick[2], Haixiang Mao[3], Seth Strauss[1], Aiden Nicholson[4], Grace Thompson[3], Gunther H Rosen[5], Molly Colvin[5] and G Allen Burton Jr.[4], (1)University of Michigan, Ann Arbor, United States, (2)Coastal Monitoring Associates, (3)School for Environment and Sustainability, University of Michigan, (4)University of Michigan, (5)Naval Information Warfare Center Pacific*

In aquatic ecosystems with numerous chemical stressors, it is crucial for practitioners to be able to identify the contaminant classes present and rank them based on toxic impacts. Previously developed methods for doing this, such as the US EPA Toxicity Identification Evaluation (TIE) method, can be confounded by sample manipulation artifacts and temporal limits of sample collection. To address these shortcomings, the in-situ Toxicity Identification Evaluation (iTIE) system was designed as a broadly applicable technology capable of identifying and ranking contaminant classes of concern at a variety of aquatic sites. In the current prototype, site water is sampled either from the water column or from sediment pore spaces, gently oxygenated, differentially fractionated by an array of sorptive resin matrices, and exposed in-situ to test organisms. Different resin treatments, including HLB for general organics, C18 for nonpolar organics, WAX for PFAS, and zeolite for ammonia, can be selected depending on site. Additionally, different test organisms, including embryo-larval fish, amphipods, and daphnids, can be selected depending on site and purpose of study. The resulting fractionated water can be preserved for future analysis. The iTIE system has undergone a series of technological assessments and updates, including: 1) the addition of a booster pump to improve water movement into the through the system; 2) verification that the pore water sampler, a Trident-style probe, successfully isolates pore water without inadvertently sampling overlying water; 3) modification of the oxygenation system to ensure sufficient performance at sites with high dissolved sulfide content. Following technological optimizations, the current prototype will be field-tested at a variety of sites throughout 2024. The system will be tested using embryo-larval fathead minnows (*Pimephales promelas*) at several sites in southeast Michigan, some of which may be contaminated with ammonia, polycyclic aromatic hydrocarbons, pesticides, and PFAS. The system will also be tested using embryo-larval topsmelt (*Atherinops affinis*) at a marine site in San Diego, California that contains high dissolved sulfide concentrations, among other contaminants. Results to date affirm that the iTIE system is a highly sensitive, cost-effective technology with the ability to strengthen stressor-causality linkages in ecological risk assessments based on weight of evidence.

6.04.T-06 Quantifying Specific Discharge and Dissolved-Phased PAH Mass Flux Using Sediment Bed Passive Flux Meters to Optimize Sediment Cap Design for Intertidal Sediments

***Dan Lavoie**[1], David Finney[1] and Bernadette Wright[2], (1)Jacobs Engineering Group, (2)U.S. Environmental Protection Agency Remedial Project Manager*

Migration of non-aqueous phase liquid (NAPL) is impacting intertidal areas known as East Beach and North Shoal at the Wyckoff/Eagle Harbor Superfund Site in Bainbridge Island, WA. The remedy selected to address these discharges includes dredging and capping. To determine if dissolved-phase PAH discharge also needs to be treated in the sediment cap, dissolved-phased PAH concentrations and rates of discharge within transition zone water at the base of the proposed cap were quantified. Sediment bed passive flux meters (SBPFMs) were deployed during a sediment cap predesign investigation to measure time-averaged upward/downward discharge rates and associated dissolved-phase PAH mass discharges within the transition zone for 2 weeks. SBPFMs were installed at 11 East Beach and 15 North Shoal locations within the 2-4 foot interval below sediment surface for a period of two weeks. Using a PushPoint, collocated transition zone grab samples were also collected at 4 East Beach and 6 North Shoal SBPFM locations to measure dissolved-phased PAHs during a single ebb-tide event. The SBPFM data allowed for estimates of net upward specific (upward minus downward) discharges and associated mass discharges across the study areas. The average net upward discharges for East Beach and North Shoal were 0.55 and 0.15 cm/d. Consistent with past ebb tide event observations, the highest measured specific discharges were observed between the 0 and +2.5 foot beach elevation contours. Observations of NAPL staining on some SBPFMs correlated to elevated PAH mass discharges which when converted to average concentrations indicted that individual constituents were in excess of effective solubilities. When excluding the NAPL-affected results, total dissolved PAH concentrations from SBPFMs ranged from not detected to 2,294 µg/L at the East Beach and 1.2 to 3,128 µg/L at the North Shoal. The transition zone grab sample results generally agreed with SBPFM concentrations, providing additional confidence in the SBPFM data set when the differences between the methods were considered. The SBPFM results confirmed the conceptual site model by verifying the primary areas of discharge within the cap target treatment zones. Excluding the NAPL-affected results, SBPFM results provided a range of time-weighted average transition zone concentrations that will be used to inform dissolved contaminant transport modeling for the purposes of cap design in the East Beach and North Shoal areas.

6.05.T The Trinity River Past, Present, and Future: Management of an Urban Watershed in a Growing City

6.05.T-01 State of the River, State of the City

***Mattie Parker**, City of Fort Worth*

6.05.T-02 Streams and Valleys' Confluence: The Trinity River Strategic Master Plan

***Stacey Pierce**, Streams and Valleys*

6.05.T-03 Recognizing the Resource: Promoting Biodiversity Through Watershed Stewardship in TRWD's Urbanizing Watersheds

***Aaron Hoff**, Tarrant Regional Water District*

6.05.T-04 The History of Aquatic Research and Risk Assessment on the Trinity River, Texas

***Scott D Dyer**, Biology & Kinesiology, LeTourneau University*

The Trinity River is the longest river whose headwaters and confluence with the Gulf of Mexico exist entirely within the borders of Texas. Throughout its length, its role providing drinking water and irrigation to agriculture is unrivalled in the state. The area surrounding the upper Trinity is highly urbanized. Discharges of municipal wastewater provide the largest source of flow during 7Q10 low flow. Due to its urbanized nature, the Trinity has been a source of several scientific inquiries to determine its biological quality as it has the potential for assessing a “worst-case” scenario for chemical risk assessments, particularly those that emanate from domestic wastewater treatment plants. This presentation will illustrate how work on the Trinity has provided prognostics that have been and can be used for other urbanized rivers and streams in the U.S. Focus will be made on studies in the 1980’s through the 2010’s. These studies include diagnostics from stressed wastewater plants in the 1980s to sophisticated studies conducted on surfactants conducted between 2000 and 2020. I was present in the 1980s when the University of North Texas became the focal point of biological and chemical research on the Trinity and helped develop the surfactant monitoring and modeling studies. This presentation is dedicated to the late Dr. Kenneth L. Dickson, a science leader, researcher, mentor, and friend from the University of North Texas that popularized ecological education to include aspiring environmental stewards from primary and secondary schools through post-graduate school.

6.06.P-Mo Tools, Methods, and Approaches for Natural Resource Damage Assessment

6.06.P-Mo-193 Enhancing the Federal Natural Resource Damage Assessment and Restoration Process Through Bayesian Networks: A Case-Study on the Little Mississinewa River, Indiana

***April D Reed**[1] and Wayne G Landis[2], (1)Western Washington University, Bellingham, United States, (2)Western Washington University*

The Federal Natural Resource Damage Assessment and Restoration (NRDAR) program gives

Tribes and certain government agencies the authority to assess injury to natural resources and to pursue and implement compensatory action for any resources lost or injured due to unlawful releases of chemicals into the environment. This study was centered around the development of a Bayesian network (BN) decision support tool tailored to the needs of Natural Resource Damage Assessment and Restoration (NRDAR) practitioners. The goal was to design a probabilistic BN tool that could lend quantitative insight into natural resource injury. A case study was used to develop and demonstrate the tool's functionality and propriety for NRDAR purposes. Our case study focused on the fish resources of an inactive polychlorinated biphenyl (PCB)-contaminated Superfund Site in mideastern Indiana, the Little Mississinewa River (LMR), and the larger Mississinewa River, into which the LMR drains. A BN model was created to describe the causal relationships between PCBs released into the LMR environment and the resulting injury to fish resources across the study site. The BN model includes three common adverse effect pathways for PCB exposure in fish - mortality, growth, and reproductive effects. The BN also includes a combined mortality + growth (M+G) effects pathway and a combined largest effects model (CLEM) pathway. Each pathway's endpoint is an injury determination node which gives a probabilistic estimation of an injured or uninjured decision based on site-specific fish tissue concentration and toxicity data for the specified pathway. The probability distributions from the Bayesian network's CLEM percent effects results have been linked to spreadsheets that automate injury calculations with measurement in units of discount service acre years (DSAYs). Using the BN tool, probabilistic injury determination and quantification were performed for individual spatial subregions as well as the entire LMR study site. This study demonstrates that BNs can be used to characterize relative injury across a spatial gradient as well as over time. The preliminary sensitivity analyses indicate that, in all subregions, fish species is an important factor related to injury determination and that some fish species are more prone to injury than others.

6.06.P-Mo-194 From Leslie to Lefkovitch and Beyond: Standardizing the Construction of Matrix Population Models for Quantifying Loss of Ecological Resources

Timothy Alan Walker[1]*, Carolyn Meyer*[2] *and Holly McChesney*[3]*, (1)Arcadis NA, (2)ARCADIS, (3)Arcadis U.S.*

Matrix population models (MPMs) are an increasingly popular tool for quantifying population-level endpoints in both natural resource damage assessments (NRDA) and ecological risk assessments (ERA). MPMs can provide a framework that links reductions in life stage-specific vital rates caused by environmental contaminants to population-level endpoints including abundance, population growth rates, and extinction rates. Recently, a review of matrices published in the COMADRE database revealed that more than half of the published papers reviewed contained key matrix construction errors. Here, we propose a standardized methodology for the construction of MPMs that is intended to reduce errors in matrix construction. This method synthesizes existing methods from the MPM literature to provide a generalized framework using three basic matrices: survival, reproduction, and transition. These three basic matrices can be combined in a desired life history sequence to reproduce any discrete time matrix model and can fit any desired census timing (e.g. pre-breeding or post-breeding). The primary benefits of this method are that it is easy to learn; census time selection is intuitive; it scales well with added complexity; and it provides a framework for identifying errors in existing matrices. To complement this method, we also recommend a switch in the standard graphical representation of MPMs. Using this alternative graphical representation, the construction of matrices for complex, multi-step life histories can be accomplished without the need for advanced knowledge of matrix operations. The approach and its benefits are illustrated with examples demonstrating how model results are affected by both incorrect formulations and simplifying assumptions. A reduction in errors in matrix construction could significantly improve the accuracy of results obtained via MPM analyses and increase the acceptability of their use within the regulatory framework of NRDAs and ERAs where model results are often controversial due to the high consequence of their results for both the responsible and affected parties.

6.06.P-Mo-195 Mine the Data Gap: Methods to Bridge Spatio-Temporal or Tissue Data Gaps for NRDA

Amber Stojak[1]*, Carolyn Meyer*[2]*, William Stiteler*[1] *and Timothy Alan Walker*[3]*, (1)Arcadis, Raleigh, (2)ARCADIS, (3)Arcadis U.S.*

The understanding of chemical exposures in key media over spatial and temporal scales is crucial for determining injury to biological resources. Data gaps in that understanding often exist when conducting cooperative Natural Resource Damage Assessments (NRDAs) in historically contaminated sites. Such NRDA evaluations and estimations of injury typically begin at the time of release of a hazardous substance or in 1981, whichever is more recent, and injury estimates continue through time and into the future. If any spatio-temporal data gaps in chemical concentrations for media of interest exist (and they always are uncertain for the future), methods are needed to first fill in data gaps of chemical concentrations in abiotic media over time or area, including projections into the future, and then to link concentrations to effects on receptors or their habitat. For NRDAs covering large areas, we recommend identifying and understanding data gaps by 1) compiling and mapping available data from all involved parties 2) conducting an extensive literature and public database search in the media of interest in the region (including unimpacted locations) and 3) identifying co-located data to develop bioaccumulation (BAF) and trophic transfer functions (TTF) that assess concentration changes between media. This foundation of data can then be analyzed for trends to identify data gaps in space or time. We have applied the following methods in various NRDAs to fill existing gaps: 1) an interpolation modelling approach (e.g., inverse distance weighting) that can cover spatial or temporal gaps or both, 2) a decision flow chart for selecting the best interpolation method for data gaps 3) a Monte probabilistic method to simulate values for missing years, 4) an assessment of the correct spatial and temporal scale for statistical estimates of chemical exposure and 5) future predictions using past trends and considering climatic or management changes expected. Once data gaps are filled at the abiotic level (e.g. sediment, soil, or water), BAFs can be used to fill the biotic tissue data gaps (e.g. fish tissue or bird eggs). We discuss how to avoid pitfalls often observed in application of these methods (e.g., use of non-site specific BAFs, lab TTFs, use of incorrect scale or method of data averaging). Employing and exploring these methods and results can lead to a better understanding of potential injury.

6.06.T Tools, Methods, and Approaches for Natural Resource Damage Assessment

6.06.T-01 The Bird Mercury Tool: Derivation of New Toxicity Reference Values and Application to Injury Assessments

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Birds are often used as bioindicators of environmental mercury contamination and modeling tools are needed to help practitioners quantify injury to birds during Natural Resource Damage

Assessments (NRDA). We completed a comprehensive review, summarized data from 168 studies, performed a series of Bayesian hierarchical meta-analyses, developed new toxicity reference values for multiple bird tissue residues and diet, and quantified the effects of methylmercury on birds using a benchmark dose analysis framework. Lethal and sublethal effects of methylmercury on birds were quantified in nine biologically relevant endpoint categories and three age classes. Effective mercury concentrations where there was a 10% reduction (EC10%) in the production of juvenile offspring, histology endpoints, and biochemical markers were substantially lower than those for survival and behavior of juveniles and adults. Within the egg age class, survival was the most sensitive endpoint. Body morphology was not particularly sensitive to mercury. We developed toxicity reference values using a category that combined survival and reproduction endpoints for juveniles, because juveniles were more sensitive to mercury toxicity than eggs or adults. Adult blood-equivalent mercury concentrations (µg/g ww) and egg-equivalent mercury concentrations (µg/g fww) caused low injury to birds (EC1%) at 0.09 and 0.04, moderate injury (EC5%) at 0.6 and 0.3, high injury (EC10%) at 1.3 and 0.7, and severe injury (EC20%) at 3.2 and 1.8, respectively. Mercury concentrations in the female's diet (µg/g dw) caused low injury to juveniles when maternal diet was 0.16, moderate injury at 0.6, high injury at 1.1, and severe injury at 2.4. We used these derived dose-response relationships to develop *The Bird Mercury Tool* which provides practitioners a simplified way to quantify injury to birds caused by mercury pollution. The practitioner can enter mercury concentrations in most bird tissues or diet and the associated metadata, and *The Bird Mercury Tool* will return the estimated injury to birds based on the derived statistical models. We will provide an example of how *The Bird Mercury Tool* can be used to both quantify injury to birds and estimate the percentage of the sampled bird population that is considered injured using data from an anonymized active NRDA case.

6.06.T-02 Using Tree Swallows to Assess Injury and Restoration Efficacy for Riparian NRDAR in Montana

*Brian Balmer[1], Megan Fylling[2], **Bridger M Creel[2]**, Ben Colman[2], Johanna Kraus[3], Creagh Breuner[2], Joseph Skorupa[4], Jacob Martin[4] and Travis S. Schmidt[5], (1)U.S. Fish and Wildlife Service, Washington, (2)University of Montana, (3)U.S. Geological Survey, (4)U.S. Fish and Wildlife Service, (5)U.S. Geological Survey*

The primary goal of the U.S. Department of the Interior's (DOI) Natural Resource Damage Assessment and Restoration (NRDAR) Program is to restore DOI Trust Resources (i.e., federally threatened and endangered species, migratory birds, and National Wildlife Refuges) that are impacted as a result of anthropogenic contaminants released into the environment. Throughout the state of Montana there are 21 Superfund Sites at various stages of remediation and restoration, as well as a variety of other point and non-point anthropogenic sources of contaminants including agriculture production, commercial/residential growth, livestock grazing, mining development, and natural gas/oil exploration. In Montana, much of the historical focus has been on threats to aquatic environments. However, the transport of contaminants from aquatic to terrestrial food webs and associated effects throughout freshwater-riparian ecosystems are a critical component to evaluate linkages associated with contaminant exposure. Tree swallows (*Tachycineta bicolor*) have been used extensively in North America as sentinels to assess the spatiotemporal extent of anthropogenic contamination in freshwater-riparian ecosystems. This species is a mid-level consumer and aerial insectivore that is ubiquitous across a wide range of habitats including lentic and lotic ecosystems. Tree swallows are secondary cavity nesters that readily use nest boxes, which allows for installation of boxes at sites of particular interest, permits reliable collection of biological samples, and allows for routine monitoring to determine reproductive success. Since 2021, nest box colonies have begun to be established in four Montana riverine basins (Bitterroot, Clark Fork, Missouri, and Kootenai) with varying levels and types of anthropogenic activity. The development of this comprehensive nest box network throughout Montana's watersheds provides a standardized sampling methodology to evaluate the scope of aquatic-terrestrial contamination at impacted sites, success of restoration projects, and long-term monitoring to assess changes in contaminant concentrations/profiles over spatial and temporal scales.

6.06.T-03 Trophic Magnification Model: An Innovative Approach to Predict Tissue Concentrations Based on Sediment Contamination and Trophic Transfer

Fabrizio Bonatesta*, Andrew McFadden, Matt Rissing, Claire Lay, Jamie Holmes and Jeffrey M. Morris, Abt Global*

Trophic magnification (e.g., biomagnification) is a process where contaminants accumulate in organisms at higher trophic levels within a food web. This phenomenon, observed with methylmercury, perfluorooctanesulfonic acid (PFOS), and many other organic contaminants, can lead to adverse effects in higher trophic level organisms even when nominal concentrations in environmental media are relatively low. One approach for assessing exposure and adverse effects of these chemicals focuses on the development and application of a Trophic Magnification Model (TMM). At many natural resource damage assessment (NRDA) sites, practitioners are tasked with estimating adverse effects of contaminants on higher trophic level organisms based on sediment data. Our model aims to quantify the relative uptake of contaminants, trophic magnification within the food web, and the link between sediment contaminant concentrations and the magnitude of the trophic magnification. We have used the TMM to evaluate transport of contaminants from sediment into organisms at various NRDA sites based on assigned trophic levels, preferably calculated using site-specific stable nitrogen isotope data. We calculate trophic magnification slopes (TMS) and intercepts for geographic sub-sections of the sites based on spatially interpolated sediment contaminant concentrations. We use these slopes and intercepts to model tissue contaminant concentrations at multiple trophic levels based on sediment concentrations. By integrating sediment contaminant concentrations, TMS, and tissue contaminant thresholds, the TMM serves as an innovative tool for assessing adverse effects to high trophic level organisms based on uptake from sediments and trophic magnification in the food web. This model can also be used to derive sediment thresholds or assess sediment cleanup goals.

6.06.T-04 Spills in Streams: Using Benthic Macroinvertebrate Data for Injury Quantification and Restoration Scaling

Serena Ciparis*, U.S. Fish and Wildlife Service*

When a hazardous substance spills into a stream, adverse effects often occur rapidly. A visible fish kill can result in a quick response and direct counts of affected fish may be used for quantification of injury. However, if a fish kill is not visible, or if fish cannot be sampled, alternative methods for injury quantification are necessary. Benthic macroinvertebrates are relatively sedentary, easy to sample, sensitive to many contaminants, and are food for many species that are Federal or State trust resources. Many states conduct or require benthic macroinvertebrate sampling as part of water quality monitoring programs, and the data may be used to determine baseline conditions. Benthic macroinvertebrate data were used successfully for injury quantification, restoration scaling, and damages determination in a Natural Resource Damage Assessment and Restoration (NRDAR) case in the eastern United States. The trust resource was a Federally listed threatened fish species and one dead individual was collected. Injury was determined as the mean percent loss of the proportion of three sensitive taxa, Ephemeroptera, Plecoptera, and Trichoptera, over space and time. A Resource Equivalency Analysis (REA) was used to quantify total injury and scale restoration, as in-stream and riparian habitat improvement projects. Detailed methodology, comparison with the Habitat Equivalency Analysis (HEA) approach, as well as the benefits and potential drawbacks of using benthic macroinvertebrate data for assessment and damages determination in NRDAR cases, will be discussed.

6.06.T-05 Recommended Methods for Developing Tribal Cultural Loss Claims

Sherry Kircher[1] *and Kristin Skrabis*[2]*, (1)U.S. Department of the Interior, United States, (2)Department of the Interior, Office of Policy Analysis*

The mission of the U.S. Department of the Interior's (DOI) Natural Resource Damage Assessment and Restoration (NRDAR) Program is to "restore natural resources injured as a result of oil spills or hazardous substance releases into the environment." NRDAR is a partnership between States, Tribes, and Federal agencies and consists of two parts - damage assessment and restoration implementation. The methods used to calculate ecological damages, such as Habitat Equivalency Analysis (HEA) and Resource Equivalency Analysis (REA), are well known and documented. However, ways to determine lost Tribal cultural use of natural resources may not be as familiar to most case teams. In this discussion, we will present a variety of tools and methods used to estimate Tribal cultural lost use of natural resources, including mapping, interviews and surveys, ethnographic studies, cultural activity videos, and Tribal standards, many of which have been identified by Tribal co-Trustees. The goal of this presentation is to discuss current methods given the legal constraints of NRDAR and does not suggest that these calculations can capture or replace the deep losses incurred by the Tribes.

6.06.T-06 Best Practices for Streamlined Approaches to NRDAs

Nadia Martin*, IEc*

Conducting a natural resource damage assessment (NRDA) can be a long and challenging process, particularly for complex sites with multiple contaminants, long histories of operations and releases, multiple injured resources, and/or numerous potentially responsible parties (PRPs). However, even at simpler sites, the process can be long, particularly if there are uncertainties in the extent and magnitude of contamination or if the parties involved are focused on areas of disagreement as opposed to areas of agreement. As such, Trustees and PRPs may consider streamlined approaches to quantifying injuries and damages, where appropriate, to help resolve NRDA claims more efficiently and effectively – saving time and resources and achieving natural resource restoration sooner. Type A NRDAs under the Department of the Interior’s (DOI) regulations are simplified assessments requiring minimal field observations, as opposed to Type B NRDAs, which are site-specific procedures for detailed assessments. Although current Type A procedures have not been updated in recent decades and include outdated formulas for modeling in specific environments, this concept of a simplified or streamlined assessment is included in the regulations as an option for trustees where appropriate. Further, the DOI recently released a Notice of Proposed Rulemaking for public comment, regarding updating Type A assessments.

Streamlined approaches could include models for all or portions of the injury assessment, restoration scaling, and damages determination steps of the NRDA process. Models could utilize established NRDA methodologies (e.g., equivalency analyses) and standard or default input parameters. Successful streamlined approaches will be flexible, incorporate best practices, include processes for utilizing site-specific data, contain clearly documented data sources for default inputs, have a clear nexus between the scope of injuries and restorative damages, and a pathway to transfer knowledge or inputs from settled and confidential cases. In this talk, I will draw on past case examples to provide recommendations for when streamlined approaches may (or may not) be appropriate; discuss best practices, standard inputs, and possible data sources; discuss examples of streamlined assessments that led to successful resolutions of NRDA claims; and suggestions for how to transfer knowledge between cases.

Track 7: Policy, Management and Communication

7.01.T Combating Misinformation-Disinformation in Environmental Science: Potential Opportunities and Responsibilities for Scientists

7.01.T-01 An Introduction to Combating Mis- and Disinformation in Science

***Patrick D. Guiney**, University of Wisconsin, Madison*

Dr. Guiney will kick this Special Session off with an introductory presentation by presenting case studies of mis- and disinformation in science gathered by all three co-chairs. The emphasis of this talk will be on defining key terms, presenting pertinent examples, and offering our perspectives on best practices for combating mis- and disinformation in science. Misinformation has become an influential and growing problem within science communication. These can be simple online posts, overstatement of sound bites in press releases and journal article abstracts, and maybe the most troubling, the known or unintentional agendas driven by pundits. Misinformation can also be deliberate and purposefully misleading or perhaps even more common, unintentional, or taken out of context and repeated for public consumption. For example, several years ago SETAC North America began an initiative to distribute SETAC Multi-briefs to help inform our scientific community of instances where information that was regularly cycling through various online outlets regarding how scientific findings were being communicated. It was the contention that informed scientists are better equipped to continually address situations when science misinformation is encountered. As scientists, we also encounter more entrenched and often deliberate misinformation through the wide-spread outbreak of predator journals (i.e., rapid publication, lack of editorial policies, broad and often undefined scopes), that appear to have editorial boards or peer-review processes, but in actuality they do not exist. Many of these outlets publish articles that have clear advocacy agendas, using the opportunity to hide under the cover of peer-reviewed literature. As scientists in the SETAC community, we can utilize our peer-reviewed journals, annual meetings, focus topic meetings and curated workshops to continually look for ways to provide forums and opportunities, by citing, sharing, and building on relevant and accurate science information that has been evaluated and continues to be cross-checked through the exchange and assessment of our scientific peers. However, the best way for us to address misinformation in science and its communication to decision makers is to identify examples when we encounter them and provide opportunities for discussion as to how to guard against the use of misinformation when it is observed. We propose potentially two categories of actions that we might take, one being preemptive pre-bunking of scientific misinformation and the other de-bunking of scientific misinformation when it is encountered. This presentation's goal is to provide potential resolutions when it is encountered. These examples can serve as "best practices" that can be effectively implemented to address misinformation in science communication.

7.01.T-02 The Role of Professional Research Librarians in the Curating of Scientific Research Outputs, the Peer Review Process, and Science Communication in the Age of Artificial Intelligence Applications

***Matthew Von Hendy**, Green Heron Information Services*

Up to 25% of the citations, data sets and research claims they return are fake--i.e., 'source hallucination'. Source hallucination has major profound implications for all aspects of the scientific peer review process and science communication. Many environmental scientists incorporate ChatGPT and other artificial intelligence (AI) applications into their research activities including their literature searching and reviews. While these new technologies have tremendous potential, in terms of research they also have a major problem: with up to 25% of the citations, data sets and research claims they return are fake. Source hallucination has major profound implications for all aspects of the scientific peer review process and science communication. AI experts agree that there is no effective way of fixing this issue with AI applications, so source hallucination is not going away anytime soon. It is important to consider this issue in the context of what is happening with literature searching today with regard to peer review publication. Most scientists would agree that they have a professional and moral obligation to ensure that their work submitted for peer review is accurate. In theory, authors and peer reviewers play important roles in ensuring the quality of the research. What happens in practice tends to be somewhat different. My very unscientific surveys indicate that up to 75% of scientists rely on Google or Google Scholar for their searching and verification of citations seldom happens. Theoretically, peer reviewers are supposed to verify citations, but this does not happen much in practice. Peer reviewers are not paid and are overworked. With the on-going transition that scientists are making to using ChatGPT/other AI applications instead of Google for their literature searching, the issue of source hallucination is a major problem that is happening now. It will only accelerate in the coming months. So, if the traditional safeguards in the scientific peer review system are already not paying a lot of attention to fact checking and citation verification, how are they going to deal with the tidal wave of fake citations coming their way courtesy of ChatGPT and other AI applications? The current system operates on the assumption that every citation is correct. In 3-5 years, we may have to operate on the assumption that every citation is WRONG until proven real. This talk concludes with some steps that authors, peer reviewers, editors, publishers, and other interested parties can take now to help this situation. We will also give some consideration to what peer-review might look like--it probably will not be dead, but it will be critically ill.

7.01.T-03 Case Studies From the Endocrine Disrupter Area of Science

***Ellen M. Mihaich**, ER2*

In science, we are taught about the requirement for replicability of responses, the corroboration of results between studies, in order to determine the reliability of our data. We are also taught that we should test hypotheses using all relevant and reliable data, not just generate them from the data we find that fits the hypothesis we prefer. But all too often, when it comes to communication, we are left wondering, not knowing if what we are hearing is truly corroboration or consensus instead of just a message repeated over and over again that becomes "truth." Nowhere has the science of hypothesis generating and use of alarming headlines been more prevalent than in the area of endocrine disruption. There are many reasons for this, some innocent given the developing nature of the tools used to identify and understand the "problem," some a function of putting policies and regulations in place and forcing the science to comply, and some because it is easy to become entrenched in what we believe must be true. We are all guilty of taking shortcuts in judging how plausible something is because we all have limited resources. It takes effort to understand all sides of an argument, and to test all possible hypotheses. And society, social media, and the press do not help because we all love a good soundbite. But facts matter and the truth starts with us, ethical scientists. In this talk we will explore ways to recognize the illusion of truth, how to make hypothesis testing rather than hypothesis generating the norm, and how to hold others accountable for introducing or repeating an unproven theory or false narrative. Among other examples, a recent post on sperm counts, found on a leading internet medical information site, will be used as a springboard to illustrate the dangers of believing in what sounds like a plausible "truth."

7.01.T-04 The Antidote for Mis and Dis Information

***Christopher J. Borgert**, Applied Pharmacology & Toxicology*

Science relies on honesty, which implies transparency and an attempt at objectivity. Misinformation and disinformation are forms of lying that can arise from factual ("honest") mistakes or intentional deception, but ambiguities of motive render these difficult to distinguish. Similarly, mischaracterization and misinterpretation could be honest or intentional mistakes, or pure deception. By extension, one might ask whether is there an element of intent when scientists render conclusions without characterizing uncertainties and without disclosing legitimate alternative interpretations, and whether "selective" data reporting is also a form of lying? These issues are at the heart of the misinformation / disinformation debate. In contrast, post-modern, relativist philosophy – which is largely accepted by scientists and societies that rely on science – contends that truth is relative, and that science cannot be held to standards of honesty since no person or corporate body is thoroughly transparent, objective, or knowledgeable. Thus, the misinformation / disinformation controversy presents an unresolvable dilemma to the post-modern world, for if objectivity and transparency do not exist, then neither does misinformation and disinformation. Ironically, the voices that most ardently decry the need to control misinformation and disinformation are the same voices that insist, de-facto, that truth, facts, biology, and even physics are relative constructs, the parameters of which they alone are entitled to define. Scientists must consider this dilemma with great sincerity, for if truth is relative, how can information regarding the physical world be defined and understood corporately? Can science be advanced through application of relativist theories, and if so, how can misinformation and disinformation be defined in relative terms? I contend that it cannot, that the current misinformation / disinformation controversy is a veiled acknowledgement that it cannot, and that those who would wage war against misinformation and disinformation do so disingenuously in the pursuit of power over science, people, and society. If science is to survive this attack, scientists must be prepared to argue the case for objective truth, transparency, and honesty, and to defend the legitimacy of information gained through established methods of scientific inquiry.

7.01.T-05 How Miscommunications Can Persist for Decades in Our Scientific Literature

***Serena Ciparis**, U.S. Fish and Wildlife Service*

A firsthand experience, where the reporting of a study was misrepresented in the media, supporting sensational headlines and propagating misconception of the results. Scientists in academia, government, and industry are tasked with producing high-quality data to answer questions relevant to their profession. Ethical principles and codes of conduct generally ensure unbiased study design, data collection, statistical analyses, and reporting of results. However, pressure to achieve outcomes deemed successful by funding agencies, supervisors, or colleagues with senior standing can ultimately affect how results are utilized and published. In addition, after publication, the information can be misrepresented in the media to the benefit or detriment of the scientist or research subject. Effects of these scientific miscommunications can persist for decades. Using a combination of personal experiences and popularized research, this presentation will illustrate situations leading to scientific communication going awry. What happens when a senior colleague publishes collaborative research in a predatory journal despite protests from the other co-authors? Or, what if the media misrepresents statements in a publication to support sensational headlines that continue to propagate a misconception from a 20-year-old study? Using these examples, the following questions will be addressed: Do institutional pressures create susceptibility to scientific miscommunication? What should scientists do when communication of results does not go as planned? What are some appropriate responses, and when is a response warranted (or not)? Finally, are resources available to scientists faced with communication dilemmas? The goal of this presentation to foster a sense of community among scientists faced with cringe-worthy communication of their work and to generate discussion to provide examples of practical approaches for scientists to avoid or remedy future communication mishaps.

7.02.P-Th Community-Based Participatory Research Approaches in Environmental Toxicology and Monitoring

7.02.P-Th-076 Applying Community-Based Participatory Research to Ecotoxicology

***Helen C Poynton**[1], Valeria Hernandez Talavera[2], Lorena M Estrada-Martinez[3], Kiani Meléndez[1] and Rosalyn Negrón[1], (1)University of Massachusetts Boston, (2)University of Massachusetts Boston, Boston, United States, (3)School for the Environment, University of Massachusetts, Boston*

Community-Based Participatory Research (CBPR) is an approach to health research that traces its roots to popular education movements and participatory action research. It is centered on social justice and integrates socioeconomic and environmental factors. Community engagement is critical to understanding the complex stressors that impact community health and necessary for advocating for policies that safeguard public health. A fundamental aspect of CBPR principles is a cooperative process where community and academic partners contribute equally to the research process and engage in co-learning, capacity building, and systems development. While CBPR has long been established in public health research, its application to environmental toxicology and ecotoxicology is a recent development. Moreover, the advantages of employing CBPR to study ecological health may not be immediately apparent. Here, we describe the collaborative benefits of CBPR in ecotoxicology and the process of establishing a community-academic partnership through a case study in Vieques, PR. The multi-year CBPR partnership, known as Vieques, Ambiente, Salud y Acción Comunitaria ([VASAC] Vieques, Environment, Health, and

Community Action), was formed in 2021 to address environmental and ecological health injustices stemming from sixty years of U.S. Navy military exercises and weapons testing in Vieques, PR. The primary objective of the ecological health team is to explore the various pathways and interactions through which the U.S. Navy's presence in Vieques and associated contaminants have influenced Vieques' ecology. Decades of previous studies using conventional methods failed to identify any significant ecological risk, fostering a sense of distrust between the community and scientists. This project relies on close, transparent collaborations between community members and the scientific team to uncover toxicity and environmental degradation sources. By recognizing the residents of Vieques as experts in their island's environment, we have engaged in co-learning that has opened up new avenues of investigation and revealed toxicity in areas previously deemed low-risk. CPBR is a potent approach to make ecotoxicology more meaningful to community members and translate the outcomes of our work into actions that safeguard the environment.

7.02.P-Th-077 Assessment of Metal Accumulation in Blue Land Crabs (*Cardisoma guanhumi*) as Bioindicators of Environmental Pollution on Vieques Island, Puerto Rico

Vanessa Cecilia Alvarez Carrillo[1], *Liz M Diaz Vazquez*[2], *Valeria Hernandez Talavera*[3], *Helen C Poynton*[4], *Sebastian Guerrero Hidalgo*[1], *Stephanie Soto Ramos*[1], *Alanis Sanchez*[5], *Kalianne Burgos*[6] *and Giam Bosques*[1], *(1)Chemistry, University of Puerto Rico-Río Piedras Campus, Puerto Rico, (2)Department of Chemistry, University of Puerto Rico, Rio Piedras, Puerto Rico, (3)University of Massachusetts Boston, Boston, United States, (4)University of Massachusetts Boston, (5)Biology, University of Puerto Rico-Río Piedras Campus, Puerto Rico, (6)Interdisciplinary Studies, University of Puerto Rico-Río Piedras Campus, Puerto Rico*

For over sixty years, the United States Navy used the island of Vieques, Puerto Rico, for military training and munitions testing, including ship-to-shore bombing practice and live-fire drills. The closure of these Navy facilities in 2003 ended these activities; however, the environmental legacy of contamination associated, especially with unexploded ordnance, continues to be a cause for concern. This pollution risks leaching toxic metals into the ecosystem, affecting both soil and marine life, including the Blue Land Crab (*Cardisoma guanhumi*), an essential species for the ecology, economy, and food sources from the island of Vieques. This study quantifies the concentrations of nine metals (Cu, Co, Cd, Ni, Zn, Mg, Mn, Pb, Li) associated with military debris on the island or essential metabolic pathways in crabs, using Flame Atomic Absorption Spectrometry (FAAS) to assess health risks to local consumers. Tissue samples from male and female Blue Land Crabs were analyzed and collected in military-restricted zones, accessible areas, and residential locations during the summer of 2023. These areas are notable for being actively used by local fishermen and the community. The measured concentration levels of the metals followed the order Cu > Mg > Zn > Mn > Ni > Co > Cd > Pb > Li in the areas under study that are located on the North Coast (NC) and the South Coast (SC); Northwest (NW) and Southwest (SW); Southeast (SE) and Restricted Areas (RA). This research aims to elucidate the extent of metal contamination in crabs and lays the foundation for identifying organic compounds in *C. guanhumi* tissues related to military activities. By examining the impact of decades-long military operations on the environment of Vieques and its marine organisms, this study lays the foundation for understanding and mitigating the ecological effects of military practices in the area.

7.02.P-Th-078 Evaluation of Youth Participatory Science Program on Groundwater Quality Management

Sara Brock-Contreras[1] *and Daniel D Snow*[2]*, (1)University of Nebraska Lincoln, United States, (2)University of Nebraska*

Domestic wells are unregulated in the United States and private drinking water quality is virtually unknown. Potentially contentious interactions challenge investigating the quality of water for the millions of people using these wells for daily household use. Participatory science (PS) conducted within high schools offers scientists an opportunity to collect data about well water quality, offer hands-on learning opportunities for students, increases diversity of participating scientists, and operate within tight-knit communities. While PS offers many benefits to individual participants and research teams, little is known about the usability and effectiveness of PS data by decision-makers. “Know Your Well”, a high school-level well sampling program, trains student-scientists to sample, test, and communicate results about domestic well water quality to local decision-makers and community stakeholders. This CBPR study examines stakeholder perceptions of data salience and legitimacy and willingness to pay for treatment and on-going monitoring. Cooperation with governing boards of Nebraska’s Natural Resources Districts provides an opportunity to evaluate regional-scale groundwater management decision-making and resulting human health effects. Understanding student connections provides an opportunity to conduct perception surveys after students’ presentation of findings with follow-up interviews to contextualize responses to salience, legitimacy, and willingness-to-pay questions. We hypothesize that an increase in perceptions of salience and legitimacy in the PS data, due to the level of trust the community members have for members of their own community, will affect decisions made by policy makers. We expect that decision-makers may support groundwater monitoring done by the student-scientists, when they are less likely to support other monitoring programs. Proper instruction and training ensure higher PS data quality, increasing trust decision makers place in the monitoring results. This study highlights how a domestic well PS project can enhance our understanding of management of well water quality and introduce evidence-based research to the next generation of scientists and decision-makers. The program’s results have helped well owners make the decision to start treating their water and have yielded interesting water quality maps illustrating where contaminants exceed safe levels including nitrate, arsenic, and uranium.

7.02.P-Th-080 Assessing Environmental Health Literacy and Air Quality Concerns in the Mississippi Delta

Amelia Craze Smith[1] *and Courtney Roper*[2]*, (1)University of Mississippi, (2)BioMolecular Sciences, University of Mississippi*

The Mississippi Delta is a culturally diverse, yet historically underserved region made up of 18 counties in western Mississippi. Numerous health disparities exist in the region due to factors such as poverty, food deserts, and inadequate access to health care. Environmental stressors, such as air quality, can exacerbate existing health conditions. While air quality monitoring has been severely limited within the region, satellite data has identified many areas in the Delta as communities with environmental justice concerns related to the air pollutant, fine particulate matter ($PM_{2.5}$). Due to the lack of $PM_{2.5}$ compositional data in the Delta, as well as other potential environmental concerns, our goal was to assess the Environmental Health Literacy (EHL) of the Delta community and determine knowledge regarding air quality. EHL is defined as understanding that environmental exposures can be linked to adverse health outcomes and higher

EHL leads to improved overall health. Assessing the EHL of a community that has documented environmental justice concerns, such as $PM_{2.5}$ exposure, is vital to future educational and advocacy programs in the region. Our goal is to increase available $PM_{2.5}$ data for the Mississippi Delta, promote environmental health literacy, build rapport to ultimately perform indoor air sampling, address resident health concerns, and communicate our findings using methods selected by the community to support education and remediation efforts. The project objective is to perform community surveying and $PM_{2.5}$ sampling in an area that has limited previous data collection. Community outreach and attendance at regional meetings through collaboration with organizations invested in Delta communities, including both academic and community groups, is ongoing. EHL survey (IRB protocol 24x-135) responses have begun to be collected after advertisement throughout community spaces in the Delta (i.e. community centers, libraries, and local businesses). Anonymous responses will be used to determine sources and pollutants of concern for characterization as well as preferred report-back methods of data. $PM_{2.5}$ sampling (weekly and real-time) is being conducted on four farms within the Delta with anticipated differences based on season and sampler location. Ultimately the goal of this work is to increase the EHL of community members in the Delta through targeted educational and environmental sampling efforts.

7.02.T Community-Based Participatory Research Approaches in Environmental Toxicology and Monitoring

7.02.T-01 A Community-Science Approach to Characterizing Seasonal and Spatial Variation in Exposure to Drinking Water Disinfection Byproducts

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Drinking water disinfection byproducts (DBPs) are one of the most common health-based regulatory violations for drinking water in the United States. DBPs have been consistently associated with increases in bladder cancer and more tenuous links exist with other health effects. The problem is particularly acute in rural drinking water systems. For example, the Martin County Water District in eastern Kentucky experienced more than a decade of quarterly MCL violations for trihalomethanes (THMs) and haloacetic acids (HAAs). Rural drinking water systems in Appalachia face challenges both due to the complexity of the distribution networks required in the mountainous remote landscape and declining resources available for maintaining and operating infrastructure. There is also widespread distrust in institutions leading many to avoid drinking tap water. Epidemiological and exposure studies are hampered by poor exposure classification for DBPs which exhibit strong seasonal and spatial variability. Further, regulatory monitoring schemes usually only involve quarterly sampling at a small number of monitoring locations. This project used community-based scientists to gather high spatial and temporal resolution data on concentrations of THMs and haloacetonitriles (HANs) in two counties in Eastern Kentucky. Exposure concentrations were related to seasonal variation in source water characteristics and distribution network characteristics. These data will help to inform small drinking water systems on methods for reducing exposure to DBPs.

7.02.T-02 BRAIDED Food Security: Indigenous, Community-Led Mercury Monitoring in Traditional Foods

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Accessing healthy food resources is a significant challenge for rural coastal Alaskan communities, which are predominantly comprised of Indigenous Alaskans who rely on subsistence foods that are nutritionally and culturally important. Addressing these concerns is urgent, as food safety and security are increasingly threatened by environmental toxicants and climate change, and requires approaches that enable communities to make proactive decisions and respond to rapid changes in essential resources. Many food-safety monitoring programs in geographically isolated communities currently rely on contributory citizen science models, where citizen-collected samples are sent off-site to academic or agency research labs for analyses. This model has been shown to enhance participant knowledge and engagement, but it is prone to delays in communicating results, and may magnify inequities, technological gaps, and distrust. The BRAIDED project is a community-led research partnership that addresses these challenges by centering all steps from identifying the problem, to data collection, analysis, and processing within the community, with support from research and technology partnerships. As part of a pilot project, the Aleut Community of St. Paul Island (ACSPI), Alaska identified concerns surrounding food security and safety, specifically related to mercury contamination of marine resources. In 2024, we (1) developed on-site research capacity for analyzing voluntarily donated traditional foods for mercury, (2) expanded community engagement in data collection, analysis, and interpretation by leveraging “two-eyed seeing” approaches to scientific training and educational programming, and (3) developed a digital dashboard for data archival, accessibility, and sharing capacity to facilitate information utilization and resilience, within and between communities. The proposed pathway will be applicable for a range of geographically isolated, rural communities working to incorporate local and Traditional Knowledge and scientific data on food safety into natural resource management decisions.

7.02.T-03 Community-Based Participatory Research Approach to Rebuild Trust in Ecological Risk Assessment in Vieques, PR

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Between 1941 and 2003, Vieques, an island municipality of Puerto Rico (P.R.), was utilized by the U.S. Navy and NATO allies for conducting military exercises involving live ammunition, ordnances, storage, processing supplies, and waste disposal. The historical military activities on Vieques have resulted in the presence of various chemical stressors, leading to a lack of trust among residents. As a result, many residents are hesitant to participate in research studies or

collaborate with scientists. To address this, our study employed a community-based participatory research (CBPR) approach to gain insights into current environmental health concerns from the community's perspective. This approach allowed the community of Vieques to take ownership of the research process and provided them with early access to the data.

Despite over a decade of chemical analyses in sediments, soils, water, and biota, no toxicity assessment had been conducted on Vieques, which represented a significant gap in understanding the impacts of contamination. Working with the Community-Academic Steering Committee (CASC), we identified ten locations of concern for the residents of Vieques, including lagoons and coastal beaches. With our community partners, we hypothesized that lagoonal sediments may be polluted with organic and inorganic chemicals due to weathering of ordnances, bullets, and other military waste. Using a Genomic-based Environmental Health Assessment (GEHA), we assess the toxicity of lagoon sediments by exposing the amphipod *Parhyale hawaiensis* to sediments and identifying molecular stress responses using RNAseq. Fifteen out of twenty-nine sediment samples from eight locations caused greater than 50% mortality to *P. hawaiensis* after 96-hour exposure, and sediments from restricted areas caused complete mortality after a 1:4 dilution with clean sand. Measured contaminants, including metals and PFAS, do not account for this high mortality. Gene expression analysis on survivors from these sediment exposures may help us identify the class of compounds responsible for toxicity and guide further chemical analysis. This data will allow the community to better understand past military exercises' adverse effects on environmental health in Vieques, P.R., demand further remediation of contaminated sites, and make informed decisions for the future of their coastal natural resources.

7.02.T-04 Community Based Participatory Research in Large Scale Environmental Monitoring Programs: Think Globally, Act Locally

__Alan S. Kolok__[1] and Tate Libunao[2], (1)University of Idaho, United States, (2)University of Idaho, United States

The field of environmental monitoring is being shaped by two significant, though somewhat contradictory, paradigms. The first is community-based participatory research (CBPR), an approach where researchers, organizations, and community members collaborate equitably on all aspects of a research project. In such projects, community members are often motivated to participate because the research has a direct, or perceived, effect on their health and well-being due to the project being conducted in the participant's immediate locale. The second is GeoHealth, a research approach that seeks to understand the relationships between people, location, time, and environmentally driven impacts upon health. With respect to the fate, transport, and biological impacts of chemicals, GeoHealth can encompass studies conducted on scales from local to global. Reconciling these two approaches is problematic, particularly with respect to large scale monitoring programs. Indeed, the interplay between researchers and community members would appear to be particularly one-sided, as researchers benefit from crowdsourced monitoring campaigns, while providing little back to community members. The lack of return on investment, from the perspective of the community, can lead to disinvestment from the campaign. For the last four years, we have been administering the Crayfish Mercury Project, a monitoring project that aims to cover the entire Columbia River Basin. We have found it possible to involve multiple, geographically distinct, communities in monitoring programs by tailoring the outcome of each campaign to the needs and desires of each community group independently. In the Pacific Northwest, this involves recognizing that indigenous communities, for example, have different goals and expectations with respect to environmental monitoring campaigns than do non-native community groups. The same is true for K-12 groups relative to adult community organizations, such as the Spokane Riverkeepers. By allowing each group in our monitoring program to self-select their goals and responsibilities, we have been able to monitor environmental concentrations of mercury in crayfish throughout the entire Columbia River Basin.

7.02.T-05 Development of a Community-Centered Harmful Algal Bloom Monitoring Strategy for the Sacramento-San Joaquin Delta

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Cyanobacterial harmful algal blooms (HABs) are a noxious impediment to water quality, recreation, and public health for communities of the Sacramento-San Joaquin Delta (Delta). HABs are a consistent occurrence throughout the Delta and there are concerns that blooms will increase in frequency and severity due to climate change. Despite HABs posing a recurrent and persistent threat, especially in the last 20 years, a consistent and coordinated approach to monitoring HABs does not exist and there is no program funded to do so. Given the broad attention on this issue, regulators, concerned citizens, and resource managers identified a need for increased coordination amongst the many groups that work on HABs in the Delta to align efforts. In response to this call, the Delta Stewardship Council hosted a workshop in Fall 2022 to best identify nexuses where HABs monitoring and data collection can be optimized, and to develop a monitoring strategy to support decision making with the use of best available science. The workshop brought together 154 participants over two days to hear community priorities for HABs monitoring in the Delta. The information and collaboration at the workshop set the foundation for the development of a monitoring strategy. The monitoring strategy was developed throughout 2023 with additional targeted engagement with Tribes, environmental justice experts, and community members. The monitoring strategy was finalized in summer 2024 and implementation is ongoing. By centering community needs through the development of the strategy as well as continuing to engage broad community participation through implementation of the strategy, we hope to conduct work that is reflective of community priorities. In this talk, we will share the process associated with developing a monitoring strategy with the input of diverse voices, what we learned from collaborators, including regulatory partners and community-based organizers.

7.03.P-Th Ensuring Scientific Integrity: Strategies for Assessing Study Reliability and Bias in Ecotoxicology

7.03.P-Th-081 Trends in Quality and Risk Assessment Applicability of Microplastic Ecotoxicity Studies

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Francisco Estuary Institute, (8)Gradient Corporation, (9)Bangladesh Agricultural University, Bangladesh, (10)University of Birmingham, United Kingdom, (11)Southern California Coastal Water Research Project

The Toxicity of Microplastics Explorer (ToMEx) aquatic organism database contains over 280 microplastic ecotoxicity studies that have been scored for quality and applicability for risk assessment based on the quality criteria outlined by de Ruijter et al (2020). We used this data to assess the overall reporting quality of microplastic effects studies and evaluate the relationship between quality scores and various factors including time (year of publication), taxonomic group, whether adverse effects were observed, and if journal impact factor was linked to conformity with reporting criteria. Data uploaded into ToMEx were ranked for the conformity to quality assurance and quality control criteria and split into two categories based on whether the study reported the appropriate technical criteria and if information pertinent to risk assessment was provided. Studies reliably reported most technical criteria with the exception of background contamination, chemical purity, suspension homogeneity, exposure verification, and laboratory preparation. However, much of the data from studies within ToMEx does not support risk assessment criteria. Biological endpoints, food availability, and exposure time were the only criteria where most of the data met reporting criteria. Overall study quality and applicability to risk assessment has not changed over time (by year of publication), although a very weak (but significant) increase in technical quality over time was observed. We also found a very weak (but significant) positive trend between study quality score and journal impact factors, as well as a weak (but significant) negative correlation between quality and whether a study found a significant effect at any exposure concentration tested. Quality score does appear to vary significantly depending on study organism, with fish studies generally having lower risk quality criteria scores and algae, annelids, crustaceans, and mixed community studies generally having higher scores. This analysis highlights uncertainties underlying the current state of knowledge of microplastics ecotoxicity, data gaps in the microplastics ecotoxicity literature, and provides a framework for assessing aggregated ecotoxicity data quality and it’s applicability to risk assessment.

7.03.T Ensuring Scientific Integrity: Strategies for Assessing Study Reliability and Bias in Ecotoxicology

7.03.T-01 Applications of the Semi-Automated Study Quality Assessment and Reporting Evaluation (SQuARE) Tool for Evaluating Dataset Quality and its Use Across Diverse Scientific Domains

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The evaluation of individual study quality is one of the most time-intensive aspects of the systematic literature review process, as it involves the evaluation of available data against variable criteria by human experts. Current study quality assessments rely on manual judgment calls that are highly specific and generate quality metrics that can only be used for the specific problem being addressed. Ensuring that study characteristics and reporting requirements are met is essential in determining whether data are suitable for risk assessment, as well as other applications. Therefore, there is a need to increase the efficiency by which study quality criteria are assessed by automating processes. To this end, we developed the Study Quality Assessment and Reporting Evaluation (SQuARE) tool, a semi-automated framework to integrate extracted data and flexibly evaluate it against any quality criteria needed for a given project. Using Python and Excel to integrate and evaluate extracted data against user-specified reporting criteria, this platform allows for evaluation of individual study quality with minimal input from domain experts after the initial criteria specification. By generating summary quality scores for each study and implementing quality thresholds, the SQuARE tool quickly identifies studies considered sufficient quality for inclusion. To demonstrate the customizability of the SQuARE tool across diverse scientific domains, we show here its use in various case studies using data across species and chemicals from both guideline studies (i.e., EPA-recommended methods) and in vitro and in vivo non-guideline studies. To better accommodate the specific requirements of a guideline study, the tool was adapted to 1) allow for different criteria assessment based on the purpose of the review; 2) allow for increased specificity when evaluating numeric criteria; 3) allow for the evaluation of different data levels and subsets; and 4) restrict criteria evaluations to specific data types within an evaluation group. Overall, the SQuARE tool provides a framework and methodology for assessing the reporting quality of individual studies and extracted datasets, having the potential to significantly reduce time and labor in study quality assessment efforts. This work demonstrates the broad applicability of the SQuARE tool and highlights the ability to customize for use across diverse scientific domains. *This abstract does not necessarily reflect U.S. Environmental Protection Agency policy.*

7.03.T-02 A Systematic Literature Review of Fish Short Term Reproduction Assay (FSTRA) Studies

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The Fish Short Term Reproduction Assay (FSTRA) is part of the U.S. Environmental Protection Agency's Endocrine Disruptor Screening Program Tier 1 screening test for chemicals intended to identify compounds that may have potential to interfere with the estrogen, androgen, or thyroid pathways. The FSTRA is a 21-day aquatic exposure assay designed to measure fish reproductive success and other potentially mechanistic, endpoints. The peer-reviewed literature contains reports of studies that assess the effect of substances on a variety of endpoints in fish which were performed using methods that were similar to the FSTRA guideline (Guideline 890.1350). A systematic literature review was performed to identify chemicals assessed in high-quality *in vivo* studies that can potentially be used as "reference chemicals" as part of a performance-based validation of high-throughput assays and computational models for potential alternatives to the current FSTRA Tier 1 screening test. A two-stage search of the literature was performed - first using an expert-generated search string, and then using an ontology-driven expansion of the original search string. Expert reviewers screened 2,286 references through a process of reviewing titles and abstracts followed by the full text of articles before performing a study inventory of experiments contained in each article and extracting data from relevant study experiments. Quality control checks were conducted at each phase of the process. Screening focused on fecundity, among other requirements, such as chemical exposure and study type, to qualify for passing each review phase. Of the 2,248 references reviewed at the title/abstract phase, 342 passed to full text review, while full text review yielded 124 studies to be inventoried. Study inventory and data extraction, which will yield an estimated dataset of 93 articles and 118 unique chemicals, is close to completion. In addition, data is being extracted from 68 Data Evaluation Records (DERs) that summarize studies performed according to the FSTRA guideline. Finally, study quality will be assessed using an internally developed tool that automatically compares extracted data to expert-specified criteria. Data extracted from the literature contain toxicological endpoints and chemical identifiers to potentially support validation of high-throughput assays and computational models as alternatives to *in vivo* animal testing. *This abstract does not necessarily reflect U.S. Environmental Protection Agency policy.*

7.03.T-03 Identification and Curation of Ecological Toxicity Data for Per- and Polyfluoroalkyl Substances (PFAS) Using the ECOTOXicology Knowledgebase Protocols

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Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic chemicals, some of which are known to be persistent in the environment, bioaccumulative in organisms, and toxic at relatively low levels. Evaluating potential ecological impacts and risks associated with PFAS is challenging due to the large number of PFAS and the long list of ecological species possibly exposed to PFAS in the environment. Thus, chemical assessments must initially rely on existing empirical data, computational models, and predictive tools, followed with targeted new toxicity testing. Our objective was to identify and make readily available ecological effects data for PFAS using the well-established protocols of the ECOTOXicology Knowledgebase (ECOTOX, www.epa.gov/ecotox). PFAS literature searches were conducted annually from 2018-2023, with over 14,000 PFAS searched in 2022 and 2023. The ECOTOX systematic protocols were applied to: 1) screen literature with pre-defined inclusion criteria (e.g., ecologically-relevant species, verifiable CASRN, endpoint and control reported); 2) annotate publications at full-text review for test compound(s), test species, exposure type(s), and effect type(s); and 3) curate data for inclusion in ECOTOX with study details, test conditions, and toxicity results. Full-text review and data extraction are on-going, with prioritization of PFAS that are included in Agency research or active assessments / criteria development. To-date, ecological toxicity data have been identified and curated for 360 PFAS from >1,600 publications, with over 800 unique test organisms. Over 40% of the records are for PFOS and PFOA, while many other PFAS are data poor. PFAS toxicity data are available for aquatic and terrestrial vertebrates, invertebrates, and plants; however, distribution across taxa is uneven with notable under-representation for groups such as amphibians, reptiles, and birds. 64% of publications measured traditional growth, reproduction, and mortality toxicity endpoints, and 41% include measurements for biochemical, cellular, or genetic effects. This multi-year effort provides: 1) a literature inventory of PFAS ecological toxicity to inform future research by quantifying existing data and identifying data gaps for species, toxins, and endpoints; and 2) detailed extracted toxicity data to support PFAS ecological risk assessments and development of PFAS criteria / benchmarks. *This abstract does not necessarily reflect U.S. Environmental Protection Agency policy.*

7.03.T-04 Meta-Analysis of the Ecotoxicological Data for Per- and Polyfluoroalkyl Substances (PFASs) in Freshwater Environments

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Despite the growing concern about the ecological risks of per- and polyfluoroalkyl substances (PFASs), there is a lack of comprehensive understanding of the actual ecotoxicity of these substances. Using peer-reviewed data and a probabilistic method, we provide scientific base of toxicity data quality for ecological risk assessment of PFASs. The comprehensive review of 82 studies revealed that only 22% (score>7 based on a semi-quantitative evaluation) offered high-quality ecotoxicity data on freshwater environments. Probabilistic species sensitivity distributions (PSSDs) were constructed based on datasets with different data qualities, from which predicted no-effect concentrations (PNECs) were derived. We found that using datasets comprising low-quality data resulted in an underestimation of the PNECs of PFASs in freshwater settings. The impacts of functional groups and carbon chain lengths were statistically examined by comparing PNECs and the available data for Daphnia magna and Danio rerio. The results suggest that given the significant disparities in toxicity among PFASs, the proposition of regulating PFASs as a family necessitates further critical and scientifically informed discussion. This study emphasizes the importance of measuring PFAS concentrations in both organisms and environmental media. Furthermore, it highlights the pressing need for improving long-term biological exposure toxicity experiments and expanding the breadth of PFAS research to facilitate ecological risk assessments and the development of regulatory standards.

7.03.T-05 The Toxicity of Microplastics Explorer 2.0 - Are We Moving the Needle Forward on Microplastics Toxicity Research?

Leah Thornton Hampton[1]*, Stephanie B Kennedy[2], Andrew Barrick[3], Dana Briggs Wyler[1], Bethanie Carney Almroth[4], Scott Coffin[5], Win Cowger[6], Darragh Doyle[4], Eden Hataley[7], Sara Hutton[8], Anna Tuulikki Kukkola[9], Magdalena M. Mair[10], Ezra L Miller[11], Laura Monclus[12,13], Emma Sharpe[14], Samreen Siddiqui[15,16] and Alvine C Mehinto[1], (1)Southern California Coastal Water Research Project (SCCWRP), (2)ToxStrategies, United States, (3)Auburn University, United States, (4)University of Gothenburg, Sweden, (5)California Office of Environmental Health Hazard Assessment, (6)Moore Institute for Plastic Pollution Research, (7)Department of Physical and Environmental Sciences, University of Toronto Scarborough, Canada, (8)GSI Environmental, (9)University of Birmingham, United Kingdom, (10)Statistical Ecotoxicology, University of Bayreuth, Germany, (11)San Francisco Estuary Institute, (12)Norwegian University of Science & Technology, Norway, (13)Norwegian Geological Institute, Trondheim, Norway, (14)Environment Science, Western Washington University, (15)Oregon State University, (16)King County Water and Land Resources Division.*

The Toxicity of Microplastics Explorer (ToMEx) was released as an open source, open access database and web application for microplastics toxicity three years ago for the purpose of data exploration, visualization, and analysis, providing an invaluable tool to the research community. Yet, the peer-reviewed literature has continued to grow exponentially, soon rendering ToMEx out of date. To ensure the continued utility of ToMEx, a crowd-sourcing approach was utilized to create a global workgroup to update ToMEx by extracting data from additional studies published since the original release. Through this process, both the aquatic and human health ToMEx databases roughly doubled in size. While a greater diversity of species and test particle characteristics are now represented in the aquatic organisms database, diversity within the human health database remains more limited. Select analyses previously conducted using the original database were repeated using the new, ToMEx 2.0 database to determine if new or additional trends or insights could be identified. Specifically, a previously developed framework for developing health-based microplastic thresholds for the state of California was applied using the newly updated database to determine if thresholds would be improved. However, data fit for the purpose of threshold development were still limited as they were for ToMEx 1.0, and confidence intervals surrounding each threshold remained wide. This was mostly likely due to the fact that the quality of studies published since the first iteration of ToMEx has not substantially improved. Given the effort required to update the ToMEx database, it is suggested that future updates only incorporate studies and data that meet strict screening criteria to ensure data utility. Despite this, ToMEx remains a useful tool for the research community, and this exercise demonstrates that large, coordinated data-mining efforts are feasible.

7.04.T Contributions of Three SETAC Lone Star Legends: Professors Kenneth L. Dickson, W. Thomas Waller and C. Herb Ward

7.04.T-02 Jim Lazorchak

***James M Lazorchak**, Office of Research and Development, U.S. Environmental Protection Agency*

7.04.T-03 Teresa Norberg-King

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7.04.T-04 Richard Wenning

***Richard J Wenning**, Ecosystem Services Economics, Montrose Environmental Solutions, Yarmouth, ME*

7.04.T-06 Ruthanne Thompson

***Ruthanne Thompson**, University of North Texas*

7.05.P-Th General: Policy, Management and Communication

7.05.P-Th-082 Adapting Systematic Review Data to the Adverse Outcome Pathway Framework: Annotating Non-Mammalian In Vitro and In Vivo Androgen Receptor Data

***Patricia Ceger**[1], Jaleh Abedini[2], Bethany Cook[2], Shannon M Bell[2], Kristan Markey[3], Sarah Vliet[4] and Scott G Lynn[5], (1)RTI International, (2)Environmental Informatics, RTI International, (3)U.S. Environmental Protection Agency, (4)Office of Research and Development, U.S. Environmental Protection Agency, (5)U.S. Environmental Protection Agency*

Systematic literature reviews (SR) aim to collect and integrate large bodies of data in such a way as to make data consistent, computable, and comparable. The adverse outcome pathway (AOP) framework was developed to facilitate linking information describing molecular initiating events through one or more key events (KEs) to adverse biological outcomes. Both require large amounts of human effort and expertise that conceptually could flow from SRs to AOPs. To explore what would be needed to accomplish this, we conducted a case study attempting to map data from a cross-species androgen receptor (AR) systematic review to existing KEs in AR AOPs. The SR provided *in vitro* data on AR binding and transactivation (TA) for 14 different species, and *in vivo* data for 41 species, representing several hundred endpoints. The endpoints

were collected as a non-standardized free text (e.g., survival vs mortality), limiting comparability within and across species. We standardized and manually mapped the data to Level of Biological Organization (LOBO), Object, Process, and Action using the KE in the AOP Wiki as a source for endpoint terms. The in vitro data had 131 of 264 records that mapped to AR TA. The *in vivo* data had 226 of 1891 records that were directly mappable to 31 different KEs, *(e.g.* increased vitellogenin mRNA), and covered 6 LOBOs, 8 Actions, 124 Objects, 36 Processes, and 327 Phenotypes. Where possible, we proposed standardized terms where they did not already exist, (*e.g.* increased gonadosomatic index). Some mapping and standardization was complex and required additional interpretation. (*e.g.* defining LOBO for whole animal homogenate when an effect is localized to an organ). This complexity hinders reproducible interpretation and comparison of results, impeding regulatory decision-making and risk assessment processes.
By standardizing and adopting clear guidance for data collection and annotation, we enhance the comparability of data generated across test methods and species, thereby advancing the potential use of SR data in the AOP framework. This study illustrates differences in experimental methodologies and endpoints and highlights the difficulties in aligning non-specific endpoints observed in vivo with mechanistic assays conducted in vitro. The lessons learned here are broadly applicable to similar efforts seeking to align information coming from multiple species.
This abstract does not necessarily reflect U.S. Environmental Protection Agency policy.

7.05.P-Th-083 Probabilistic Risk Assessment: An Underutilized Tool for Human Health and Environmental Regulatory Criteria Development

***Camille Flinders*[1]*, Brad Barnhart*[1]*, Emily Morrison*[2]*, Paul Anderson*[2] *and Wayne G Landis*[3]*,*
(1)NCASI, (2)Arcadis U.S., (3)Western Washington University

Probabilistic Risk Assessment (PRA) is a collection of risk assessment methods that incorporates uncertainty and variability to estimate the likelihood of exposure, risk, or hazard. In contrast to other risk assessment approaches that often rely on single point estimates (typically, upper bound values) for the various inputs into a risk equation which are applied across all members of an exposed population, PRA facilitates transparency regarding uncertainty and risk estimates among different members of an exposed population (including sensitive groups or life stages). PRA allows policy-makers to input exposure parameters as distributions to develop target-specific, science-based margins of safety and exposure criteria that can be transparently linked to the actual population exposure characteristics and the stipulated risk targets selected for the population, which overcomes the phenomenon known as ‘compounded conservatism’ stemming from multiple worst-case assumptions. Despite the recognition of the benefits of probabilistic methods and their use in certain programs (e.g., Superfund), PRA is underutilized to evaluate risk and develop regulatory criteria for the protection of human health, aquatic life, and air quality. This may be due to the absence of guidance from the Environmental Protection Agency supporting benchmark and criteria derivation using a PRA approach, perceived difficulty in developing PRA-derived criteria among agency personnel, and lack of freely accessible calculation tools. This presentation describes various probabilistic methods suitable for environmental risk assessment and the benefits of their use, typical distributions of environmental data, and challenges to implementation. It also provides an overview of a special publication series that advances the understanding of probabilistic methodologies and their versatility for robust, transparent, data-based environmental risk assessment and standards derivation across a range of media that align with regulatory objectives to protect aquatic and terrestrial biota, human health, and vulnerable populations.

7.05.P-Th-084 Emerging Trends in Grouping Chemicals for Regulatory and Toxicological Purposes

Emma Williams, EIT[1], Jessica G Lambert[1], ***Colin Hogan***[2] *and Patricia Underwood[3], (1)Noblis, (2)Noblis, United States, (3)DoD*

Historically, chemicals have been evaluated and regulated on a chemical-by-chemical basis. As pressure to accelerate the identification, assessment, and mitigation of risks from exposure to hazardous substances mounts, more regulatory entities are taking a broader class-based approach to chemical risk evaluation and management. This poster highlights the rationale for class-based chemical regulation; describes various existing frameworks for grouping chemicals for toxicological purposes and/or regulation; provides examples of past, proposed, and future chemical groupings; and discusses impacts and recommendations for the Department of Defense (DoD) Chemical and Material Risk Management Program (CMRMP). This assessment will compare different chemical grouping framework approaches using flame retardants as a case study. This includes a discussion of the Environmental Protection Agency's (EPA's) approach to grouping and evaluating flame retardants in clusters (e.g. Chlorinated Phosphate Esters, Cyclic Aliphatic Bromides), and the European Chemicals Agency (ECHA) Regulatory Strategy for Flame Retardants. We will discuss differences and similarities in these approaches, trends, and challenges and opportunities associated with grouping chemicals for regulatory or toxicological purposes. The ability of DoD and the defense industrial base (DIB) to reliably source chemicals is dependent on global regulations that often increase the risk of chemical obsolescence and supply chain impacts. Determining how and when groups of chemicals will be regulated thus becomes a vital step towards chemical risk management planning and supply chain security. A better understanding of how chemicals are grouped leads to more proactive risk management. As the regulatory process adapts to a shifting landscape, the DoD's CMRMP is adapting its chemical risk management process to better approach class-based regulations.

7.05.P-Th-085 Benefits and Drawbacks Associated with Implementation of Longevity Plans in Colorado to Support Site-Specific Water Quality Standards

Ashley Romero*, Jeniffer Lynch and Sarah Skigen-Caird, GEI Consultants*

The state of Colorado, like many other states, allow for the adoption of site-specific standards for surface waters where an indicator species procedure (water effects ratio), recalculation procedure, use attainability analysis, or other site-specific analysis has been completed. Once an appropriate level of data collection and analysis has been completed and deemed acceptable by the Colorado Department of Public Health and Environment (CDPHE), Water Quality Control Commission (WQCC), and the U.S. Environmental Protection Agency (EPA), the WQCC may adopt site-specific standards as supported by the site-specific study results. For segments assigned aquatic life classifications, where factors other than water quality substantially limit the diversity and abundance of species present, the WQCC and EPA may adopt site-specific acute or chronic standards as determined to be appropriate based upon available information regarding the waters and habitat. Once adopted, these site-specific standards remain in effect until superseded by revised standards promulgated pursuant to Regulation No. 31: "The Basic Standards and Methodologies for Surface Water (5 CCR 1002-31)", as administered by the Colorado WQCC. Beginning in 2019, the site-specific standards adopted by the WQCC were required to include a Longevity Plan to ensure that site-specific standards can be reviewed during subsequent triennial reviews, as required by federal and state rule (Federal Clean Water Act Section 303(c)(1) and Colorado Water Quality Control Act Section 25-8-202(f)). Implementation of a Longevity Plan supports the evaluation of the continued relevance of a site-specific standard to ensure that any changes in site conditions are adequately captured and remain protective of aquatic life. However, one requirement of the site-specific plan consists of extensive collection and analysis of data and other relevant information on an annual basis that may deter the proposal of updated, more relevant water quality criteria altogether. A case study will be presented from a site in Colorado which employed the use of hardness-based equations for cadmium and zinc based on the EPA's recalculation procedure. The implications of data-heavy Longevity Plans will be explored, both the benefits and drawbacks.

7.05.P-Th-086 The Awareness and Inclusion of Environmental Education in High Schools Curriculum: A Current Piecemeal Approach

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Environmental education (EE) is required to create awareness on the importance of protecting and managing the environment for a sustainable use. The introduction of EE at early stages in life was suggested by researchers as a method that will not only provide knowledge but influence attitude and practice later in life. The current study reviewed the current policy, curriculum, delivery, and challenges associated with the implementation of the policies around Environmental Education teachings at High Schools from different countries. A systematic review of journals in the field between 2007 – 2024 was used. This involved literature search on policy, curriculum, and implementations strategies from different countries. Reports from the study showed that EE is included in the curricula of most of the high schools from different countries but with low implementation strategies. EE is currently not a stand-alone subject but housed in different subjects and extensively taught in science subjects in high schools. Teachers' attitudes and knowledge of environmental issues remains a major setback in the teachings of the subject. Lack of infrastructure, time and teachers' availability are factors affecting EE at high school. Clear direction is needed for the implementation of the EE in High Schools. A subject that teaches EE as a subject may be considered so that it is not viewed as a supporting subject by the students.

7.05.P-Th-087 EPA GIS Tool Application for Displaying Water Chemistry and Freshwater Aquatic Life Criteria Values for Metals (MetALiCC - MAP) - An Update

Luis Cruz*, Michael Elias and Kathryn Gallagher, U.S. Environmental Protection Agency*

The United States Environmental Protection Agency (EPA) has developed 304(a) aquatic life criteria (ALC) for metals using bioavailability models to enable users to derive criteria that reflect site-specific water chemistry conditions. Water chemistry data inputs necessary to run metal bioavailability models may not be available or may be incomplete for some ambient waters. The EPA applied the United States Geological Survey's NWIS (National Waters Information System) database in a Geographical Information System (GIS) map application to provide access to an interactive, nationwide database to support states, tribes, and stakeholders with the derivation of bioavailability-based freshwater ALC values when site-specific water chemistry data are not available. The GIS application presents data using two primary interfaces: 1) map layers with water chemistry and calculated ALC displayed at selected levels of organization (MetALiCC - MAP), and 2) heat maps presenting the distribution of pH, DOC, and hardness in surface waters (Interpolation Maps) of the conterminous U.S. Currently there are ALC layers for aluminum, cadmium, and copper. A recent update includes layers for statewide ALC (aluminum, cadmium, and copper) added to the map application using NWIS data. The EPA is now developing map layers for 1) territories and states outside the conterminous U.S. (Alaska, Hawaii, and Puerto Rico), and 2) map layers using states' provided data (non NWIS data) to improve geographical coverage.

7.05.P-Th Late Breaking Science: Policy, Management and Communication

7.05.P-Th-195 Advancing Science-Based Innovations for the Safe and Sustainable by Design (SSbD) Concept

Annegaaike Leopold[1]*, Michelle Bloor*[2]*, Ksenia J Groh*[3]*, Leo Posthuma*[4]*, Hans Sanderson*[5]*, Hanna Schreiber*[6]*, Christoph Schuer*[7] *and Paul Thomas*[8]*, (1)Calidris Environment BV, Netherlands, (2)United Kingdom, (3)Eawag - Swiss Federal Institute of Aquatic Science and Technology, Switzerland, (4)National Institute for Public Health and the Environment (RIVM), Netherlands, (5)Aarhus University, Denmark, (6)German Environment Agency (UBA), Vienna, Austria, (7)Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland, (8)KREATiS SAS, France*

SETAC Europe has a Sounding Board (SB) in support of its membership of the European Commission's (EC) High Level Roundtable for the Implementation of the Chemicals Strategy for Sustainability (CSS). It was established in 2021 by the SETAC Europe Council. The SB aims to support the implementation of the CSS. In 2023 – 2024, the SB undertook a series of Safe and Sustainable by Design (SSbD) stakeholder consultations, in collaboration with the policy experts from the EC. The SSbD vision is an integral component of the European Union's Green Deal and its CSS. SSbD embodies a proactive framework for the assessment and management of chemicals and products through the integration of innovative safe and sustainable assessment streams. While most components of the current SSbD framework are not original, the concept of including all elements in a single hierarchical evaluation to demonstrate the safety and sustainability of chemical substances, preferably already at the design stage, in a common way across the chemical sector, is certainly novel. SSbD assessment requires availability of relevant data and science-based tools and models that allow characterisation of the afore-mentioned safety and sustainability aspects. It needs to accommodate both qualitative and quantitative data as per the innovation stage, which presents a wide array of scientific and practical challenges. The stakeholder consultations focused on a series of questions, including which conceptual methods, practical tools and datasets could be developed and made available for use in SSbD assessment; or to improve the scientific basis for, and utility of, the SSbD concept, and what aspects need to be incorporated into the SSbD framework that have not yet been considered. The three-stage consultation process consisted of two online events, followed by an in-person workshop held during the 24th SETAC Europe Annual Meeting in Seville, Spain, on the 6th of May, 2024. A road-mapping approach was applied, which encouraged all participants to first formulate an aspirational goal that will help advance SSbD, identify the problem associated with this goal, and finally outline the stepping stones that could help deliver the goal. This poster will give examples of developed roadmaps and will share the key outcomes of this consultation series, as presented to the European Commission.

7.05.P-Th-196 Study on a Co-Benefit Policy Frameworks for Driving Toward Large Emission Reduction of Air Pollutants and Greenhouse Gases in Asia Region Using Policy Simulation With Cost-Benefit Analysis Models

***Eiji Komatsu**, Daiju Narita[2], Tazuko Morikawa[3], Daichi Yamada[4] and Masamitsu Hayasaki[3], (1)The University of Tokyo, Japan, (2)Japan Automobile Research Institute, Japan, (3)Hiroshima University, Japan*

East Asia’s high economic growth in recent decades has accompanied corresponding high growth in air pollution levels and carbon dioxide emissions from the region. As a consequence, a significant part of global carbon emissions now originates from East Asian economies.
In this study, a cost-effectiveness analysis model (CEAM) for air pollutants reduction measures, which evaluates abatement measures based on their marginal cost, was developed in order to analyze policies to attain clean air in urban areas of Japan and East Asia. We found a 10-fold gap in reduction efficiency with and without considering cost-effectiveness in the case of the study on Japan. Policy simulation results showed that it was infeasible to meet the pollution standard levels set by the WHO guidelines (2021) in Japan by implementing all the reasonably available control technologies as estimated by the CEAM and the Community Multi-scale Air Quality (CMAQ) model. This indicates additional large-scale reduction introducing the maximum technically feasible reduction measures (MTFRs) and decrease in transboundary pollution transport from neighboring countries in East Asia are necessary. The MTFRs for air pollutants could bring about large greenhouse gas (GHG) reductions, but they are very costly. In this study, an analytical methodology that takes into account the relationship between policies to attain GHG long-term targets and clean air was developed. By finding optimal countermeasures addressing the two goals, co-benefit strategies of GHG and air pollution reduction were studied. Finally, by conducting policy simulations for Japan as well as East Asian countries using the developed methodology, we address the co-benefit policy frameworks in East Asia region in this study.

7.05.P-Th-197 Acute Health-Based Screening Level Derivation for Cyanotoxins (Microcystin, Cylindrospermopsin and Anatoxins)

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Certain environmental conditions can cause algae to reproduce rapidly in bodies of fresh and marine water, leading to algal blooms. These blooms, often known as harmful algal blooms or HABs, have the potential to cause harm to human health, aquatic ecosystems, pets, and livestock. Periodically, the Texas Commission on Environmental Quality (TCEQ) is asked to address these HABs in drinking and surface water. While there are no federal maximum contaminant levels (MCLs) for cyanotoxins, the United States Environmental Protection Agency (US EPA) and other agencies have developed screening levels for various types of cyanotoxins. The present work shows the development of acute (short-term) oral toxicity factors and associated health-based screening levels for both drinking water (humans) and for consumption of algal mats/crust

by dogs and livestock. A literature search was conducted for peer-reviewed literature and regulatory documents that could be used as a basis for developing acute (short-term) oral toxicity values (i.e., reference doses (RfDs)) for humans, dogs, cattle, and horses. Existing screening levels are presented, as well as candidate reference dose (RfD) values for humans and animals. The RfD values were derived from studies of 30 days or less using standard approaches from US EPA and TCEQ. Short-term screening values were then derived for microcystin-LR, cylindrospermopsin (CYN), anatoxin-a, and dihydroanatoxin-a. These values are helpful when emergency situations arise and TCEQ needs to provide support to other state agencies to protect public health.

7.05.V General: Policy, Management and Communication

7.05.V-01 Breaking Boundaries: Harnessing New Approach Methodologies for Effective Chemical Safety Evaluation

***Gustavo Bastos Machado**, University of Guelph, Guelph, Canada*

In recent years, the field of New Approach Methodologies (NAMs) has witnessed remarkable progress, fundamentally transforming the landscape of chemical hazard and risk assessment. This presentation is dedicated to exploring the latest advancements in various NAMs techniques, their diverse applications across industries, and the evolving dynamics of regulatory acceptance and reliability. From cutting-edge in vitro assays to sophisticated computational models like QSAR and read-across, complemented by Omics technologies, NAMs offer promising alternatives to conventional animal testing methods. However, while regulatory agencies acknowledge the potential of NAMs, their acceptance hinges on factors such as reliability, accuracy, and relevance to specific regulatory requirements. Robust scientific evidence demonstrating the validity and predictivity of NAMs for assessing chemical safety is typically demanded by regulators. Through insightful discussions and illustrative case studies, our presentation will delve into the successes, limitations, and future directions of NAMs, providing attendees with invaluable insights into their implementation and impact on chemical safety assessment. One compelling case study we will examine involves a cosmetic material seeking regulatory approval. Through a meticulous compilation of NAMs data, including read across, QSAR predictions, and in vitro studies, a weight of evidence report was formulated to support the substance notification. This comprehensive approach effectively demonstrated the safety profile of the cosmetic material, showcasing the practical application and effectiveness of NAMs in regulatory submissions. We will analyze the key strategies employed, challenges encountered, and the ultimate success achieved in gaining regulatory approval, offering attendees actionable insights into navigating similar regulatory pathways

7.06.A.T Metals: Current Affairs and Recent Developments

7.06.A.T-01 A Best Practices User's Guide for Sediment Porewater Passive Sampling for Inorganic Constituents of Concern

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Passive sampling using dialysis samplers ("peepers") is a common approach for measuring the availability of metals and other inorganics in aquatic matrices. Peepers function by allowing sediment porewater or surface water to equilibrate with water contained in an isolated compartment via passive diffusion through a semi-permeable membrane. After an equilibration period, the peeper is retrieved, and the peeper solution is analyzed for metals and reported as a concentration in water. This presentation will highlight a recently produced Best Practices User's Guide (BPUG) that captures lessons learned from a 3-year research effort to address the lack of standard guidance for using peepers to evaluate metal availability in sediment and water. The objective of the research effort is to enhance the standardization of, and confidence in, peeper usage for passive sampling of inorganic constituents – especially for the target metals that were the focus of our evaluation. The BPUG highlights three components of our work: 1) A detailed literature review that examined past and present best practices for peeper preparation, deployment, retrieval, and data analysis; 2) A series of laboratory experiments to validate best practices for peeper usage, specifically addressing methods for end-users to be able to prepare, ship, store, process, and preserve peeper samplers; and 3) An example field demonstration in which peepers were deployed in surface sediment and surface water at Naval Base San Diego, San Diego, California. The BPUG incorporates these elements of the project, and is organized as best-practice answers to frequently asked questions (FAQs) for investigators wanting to measure metal availability in sediment and water. In addition, the BPUG provides practical resources for peeper end users, including: 1) A peeper preparation Standard Operating Procedure (SOP); 2) a peeper deployment, retrieval, and processing (SOP); and 3) An Excel file for interpreting results from peepers deployed in short (pre-equilibrium) durations. This presentation will highlight key findings of our review, laboratory experiments, and field demonstration, as well as provide an overview of the BPUG resources. Overall, we believe the BPUG will be a useful resource that enables end-users to successfully prepare, deploy, and retrieve peepers, as well as interpret and use peeper data in a decision-making context at sediment sites under regulatory oversight.

7.06.A.T-02 Assessment of Heavy Metal Contamination in Sediment and Mussels from Ennore Estuary, India

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Industrialization near the coastal habitats has increased heavy metals' presence and levels in marine ecosystems in various ways. Ennore estuary is one of such vital coastal habitats located on the Southeast coast of India, supporting diverse flora and fauna, and is severely affected by discharges of industries activities. The present study focuses on assessing the concentration of heavy metals (mercury and cadmium) in sediment and Charru mussel (Mytella strigata) surrounding coal thermal power plants in the Ennore estuary. Mercury (Hg) and cadmium (Cd) concentrations in sediment ranged from 0.003- 0.041mg/kg and 0.14- 0.75 mg/kg, respectively. The analysis of the mussel samples showed that the concentration ranged between 0.029- 0.055 mg/kg for Hg and 1.85- 18.5 mg/kg for Cd. The calculated biota-sediment accumulation factor (BSAF) for mussels indicated effective accumulation of both metals. The increasing industrial activities indicate the estuary's enrichment and bioaccumulation of Hg and Cd, which pose an increased toxicity risk to the local organisms in this coastal environment. The results of this study will provide information to assist in developing strategies to protect the Ennore estuary and the ecosystems it supports.

7.06.A.T-03 Production of Biological Sulfides Leads to Increased Arsenic Dissolution in Sediments from a Potable Aquifer

***Kayleigh Millerick**[1], Amrika Deonarine[2], Soroush Ghasemi[2], Montana Montez[2] and Negar Shaghaghi[3], (1)Civil, Environmental, and Construction Engineering, Texas Tech University, (2)Texas Tech University, (3)Chemical Engineering, Texas Tech University*

Water scarcity in the American Southwest has led to increased demands on groundwater resources in a region where subsurface aquifer waters often contain dissolved arsenic. One attractive attenuation strategy for utilities is *in situ* arsenic precipitation; arsenic can be precipitated under sulfidic reducing conditions via complexation with sulfides, commonly producing orpiment-, realgar-, and arsenopyrite-like phases that remain in the subsurface during groundwater withdrawal. In this work, we explored the potential for arsenic immobilization in groundwater intended as a municipal drinking water source by promoting indigenous bacterial sulfate-reducing activity. Bench-scale microcosms were prepared containing aquifer sediments and water from four different aquifer depths and were amended with acetate, propionate, or a sulfate-reducing enrichment to promote biological sulfide production. Results showed increased dissolution of arsenic in sulfate-reducing microcosms amended with carbon substrates over the 4-month study period. Total dissolved arsenic concentrations were ~50 ppb in microcosms with active sulfate-reducing activity, approximately double what was observed in autoclaved and unamended controls. As(III) was the dominant dissolved species detected, with As(V) also observed, but bioactive microcosms also produced a third inorganic arsenic species that may be a thiolated form of arsenic. Overall, our findings suggest that precipitation of arsenic may not always occur under anaerobic, sulfidic conditions and underscore the need for a detailed biogeochemical analysis before pursuing immobilization strategies.

7.06.A.T-04 Exposure and Effects to the Benthic Community of a Large Western US River Treated with Copper to Eradicate Quagga Mussels

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Copper-based chemical treatments are commonly used to eradicate invasive mussels in closed water bodies such as ponds and small lakes, but their use in large rivers has been limited. In October 2023, in response to an early detection of invasive quagga mussels (*Dreissena rostriformis*), a six-mile reach of the Snake River in southern Idaho was treated with 19,300 kg of chelated copper over 11 days. In the days leading up to the treatment, we initiated a monitoring strategy to help evaluate the exposure and effects of the treatment on the non-target benthic community downstream. Immediately prior to and at one month following the treatment, we surveyed the benthic community composition (macroinvertebrates and periphyton) and measured bed sediment copper concentrations at one site upstream and six sites downstream of the treated reach (0.5 to 65 miles downstream). We also collected high-frequency water samples at upstream and downstream locations to assess copper exposure throughout the treatment and to compare observed biological effects to predicted toxicity based on the biotic ligand model. Lastly, we calculated water column copper loads at 10 miles downstream of the treatment to determine the proportion of the initial copper mass removed from the water column via sorption to macrophytes or sediment and biological uptake. Together, these samples offer an unprecedented opportunity to evaluate the downstream effects of a large-scale copper treatment on the non-target benthic community in a major river. Findings from this study can help inform watershed managers tasked with controlling invasive dreissenid mussels while also protecting culturally, economically, and ecologically important non-target species.

7.06.A.T-05 Spatial and Temporal Concentration of Heavy Metals in an Ecologically Important Australian Freshwater Environment, Lake Colac

***Urmi Nishat Nini*[1]**, *Aaron Schultz*[2], *Beata Ujvari*[3], *Benjamin Long*[4], *Samantha Harriage*[4], *Damien Callahan*[3], *Will McCance*[5] *and Michael Thomas*[5], *(1)Deakin University, Burwood, Australia, (2)Deakin University, (3)Deakin University, Australia, (4)Federation University, Australia, (5)Barwon Water, Australia*

Australian regional freshwater resources are increasingly exposed to a growing number of existing (heavy metals) and new chemicals, known as contaminants of emerging concern (CECs) including, industrial and household chemicals, pesticides, manufactured nanomaterials, and nano/microplastics. Lake Colac, an important freshwater aquatic ecosystem in regional Victoria, receives water inputs from the town's wastewater treatment facility, stormwater, and local agricultural runoff into two creeks that flow into the lake. These water inputs combined with recreational activities (hunting, fishing, and boating) are potentially increasing contaminant levels in the lake and the residing flora and fauna, along with EPBC-listed threatened species native to the lake, are at risk. Therefore, this research project aimed to identify the risks from heavy metal contamination and determine if the current ecosystem health of Lake Colac is supportive of the survival of the residing biodiversity. Freshwater glass shrimp (*Paratya australensis*) were selected as a bioindicator species to assess the health of the lake and were sampled from five different locations seasonally (every three months) over a twelve month period. Triplicate surface water samples were also collected at each sample time from eight different locations at Lake Colac. Heavy metal concentrations were then measured in the surface water and acid digested shrimp samples using NexION 350 inductively coupled plasma mass spectrometer (ICP-MS; PerkinElmer). Elevated concentrations of copper and zinc were recorded at each sample location and time in the surface water and shrimp samples and were above safe water guideline limits. Low concentrations of lead, silver, cadmium, chromium, and nickel were also recorded. The detection of elevated concentrations of copper and zinc at Lake Colac highlights the risks that contaminants pose to ecologically important freshwater environments in Australia.

7.06.A.T-06 An Icy Issue: Investigating the Repercussions of Chronic Nickel Exposure to a Key Arctic Fish Species, the Arctic Char (*Salvelinus alpinus*)

Connor B. Stewart[1]*, Emily Rogevich Garman*[2]*, Elizabeth Middleton*[2]*, Christian Schlekat*[2]*, Daniel Alessi*[3]*, Anne Cremazy*[4] *and Tamzin Blewett*[5]*, (1)Biological Sciences, University of Alberta, Edmonton, Canada, (2)NiPERA, (3)University of Alberta, Canada, (4)Institut National de la Recherche Scientifique, Canada, (5)Biological Sciences, University of Alberta, Canada*

Nickel (Ni) is an essential trace metal of significant economic importance, particularly in the context of transitioning to green energy. Over the past decade, global demand for Ni extraction has surged, but despite this there is limited understanding of the environmental impacts associated with this increased demand. Arctic environments are at risk of such contamination, with significant Ni mining occurring in Northern zones. Nickel toxicity is dictated by its bioavailability to organisms in the receiving environment, with many Arctic freshwaters predicted to have high Ni bioavailability due to their abiotic composition (hardness, pH, DOC). Additionally, the unique physiology of Arctic organisms may alter their responses to Ni exposure, making extrapolation of results from temperate model organisms less feasible. Arctic char (*Salvelinus alpinus*) are an important salmonid species in Arctic ecosystems, but the physiological responses of these fish to chronic Ni exposure remains unstudied. To address this, we exposed juvenile Arctic char to a range of environmentally relevant Ni concentrations for sixty days to investigate the consequences of chronic Ni exposure and the physiologic mechanisms of toxic action. This work evaluated impacts on growth and survival, respiratory toxicity via gill histology and metabolic oxygen consumption, and ionoregulatory perturbance via tissue specific ion analysis and Ni accumulation. These experiments found significant growth rate reductions with a 60-day EC_{20} (Ni concentration causing a 20% reduction in growth rate) of 436 µg/L and significant mortality at higher concentrations, with a 28-day LC_{10} (Ni concentration killing 10% of exposed organisms) of only 1,111 µg/L, a concentration well within the range of Ni observed near Arctic mines. Additionally, relevant Ni concentrations caused significant alterations to the gill structure of exposed fish. These results taken together will provide insight into the sensitivity of Arctic char, furthering our understanding of the risks posed by Ni to unique Arctic biota.

7.06.B.T Metals: Current Affairs and Recent Developments

7.06.B.T-01 Development of a Biotic Ligand Model Package as a Web Application in R

Kelly E Croteau *and Robert Santore, Windward Environmental*

The biotic ligand model (BLM) is a widely used computer program that can be used for predicting the effects of water chemistry on the bioavailability and toxicity of metals. The BLM has been used for setting water quality criteria in the United States, Canada, and the European Union, and has historically been freely available as a program that runs on the Microsoft Windows operating system. Since the BLM was first released in 2001, there have been numerous versions of Microsoft Windows (e.g. Xp, Vista, 7, 8, 10, 11) and the BLM has been continually updated in response. However, the availability of the model as a program that requires installation can be challenging for users in corporate or government settings which may require help from an administrator to install programs. Furthermore, the availability of the model as Microsoft Windows software can be a barrier to users with other computer platforms. To address these problems, a version of the BLM has been developed in R, with a web interface developed as a Shiny application. This version of the BLM allows users to access the model by simply

visiting a web site, which will allow users access without installation and for any operating system. This version of the BLM is also distributed as a package in R, which will allow users to incorporate model calculations in their own R workflow. In this presentation we will discuss the development and release of this version of the BLM and how users can access it as either a Shiny app or an R package. We will discuss how the R and desktop versions compare in computation performance, the compatibility of the models to provide similar results, and additional benefits seen from the change in hosting. A road map for future development will also be discussed.

7.06.B.T-02 Multilevel Concentration-Response Models Can Improve Ecological Risk Assessment: A Case Study on Effects of Copper to Fish

***Ryan Hill**, Brian Pyper and Sean Engelking, Azimuth Consulting Group, Canada*

Risk assessors can use concentration-response models to estimate the expected magnitude of effect corresponding to a particular concentration or dose of a chemical, or to derive a site-specific concentration or benchmark dose that corresponds to a specific magnitude of effect that is of interest to decision-makers. Fitting concentration-response relationships to data for individual experiments is routine work for toxicologists. However, risk assessors are typically not interested in specific experiments alone, but rather in the full range of possible concentration-response relationships that reflect variation among experiments. Mixed-effects or hierarchical models, collectively referred to herein as multilevel models, are suitable for such data sets because they simultaneously fit a global mean relationship while accounting for variability among subsets of the data. Here we demonstrate a case study of multilevel concentration-response modelling of the effects of copper on salmonids. Using studies compiled to support development of aquatic life guidelines and criteria in Canada and the US, we extracted raw concentration-response data from either the publications or through contact with study authors. Our final data set focused on survival as the endpoint, and included 21 experiments from 7 studies. We fit several generalized linear mixed-effect models to the data that allowed for differing intercepts or slopes among studies and experiments. Once a preferred random-effects structure was identified, we then incorporated known toxicity modifying factors of copper (dissolved organic carbon, hardness, pH) as covariates and identified a preferred final model. Last, to more fully account for uncertainties, we re-fit the preferred model in a Bayesian framework. The Bayesian approach allows us to estimate a full probability distribution for the magnitude of effect at a particular copper concentration and particular values for toxicity modifiers, while accounting for variability in concentration-response relationships evident among studies and experiments.

7.06.B.T-03 Update on the Development of U.S. Environmental Protection Agency's Aquatic Life Ambient Water Quality Criteria (AWQC) for Metals

***Christine Bergeron**, Kathryn Gallagher, Joseph R. Beaman, Luis Cruz, Kristen Prossner and Brian Schnitker, U.S. Environmental Protection Agency*

The U.S. Environmental Protection Agency (EPA) develops Aquatic Life Ambient Water Quality Criteria (AWQC) for toxic chemicals as national recommendations of ambient water concentrations that will protect against adverse ecological effects to aquatic life resulting from exposure to a pollutant found in water. States, territories, and tribes may use the recommended criteria in developing water quality standards, or they may develop their own criteria. EPA is systematically updating AWQC for metals over time to reflect new toxicity studies and approaches for considering how water chemistry parameters (e.g., pH, dissolved organic carbon

[DOC], and hardness) may affect metal bioavailability and subsequent toxicity to aquatic species. After conducting comparative analyses of bioavailability models in collaboration with eight metals associations under a Cooperative Research and Development Agreement (CRADA), EPA intends to use multiple linear regression (MLR) models to update AWQC for metals due to their relative simplicity (lower number of input parameters and data collection requirements), transparency, and ease of use compared to the Agency's current approach for copper which relies on a biotic ligand model (BLM). EPA developed MLR bioavailability models for individual metals starting with lead, nickel, and zinc. EPA reviewed the underlying toxicity studies, evaluated water chemistry ranges for pH, hardness, and DOC necessary to cover the range of US conditions, and investigated pooling taxa in the models. The EPA MLR bioavailability models were externally peer reviewed: the external peer review comments were generally favorable. EPA is currently developing draft updated Aquatic Life AWQC for lead, nickel, and zinc using the peer reviewed bioavailability models. This presentation will discuss the current EPA bioavailability models and the status of the criteria updates for metals.

7.06.B.T-04 Why Isn't Dietary Exposure Considered in Water Quality Criteria Development for Metals?

***William J. Adams**, Environmental, Red Cap Consulting*

Diet as an important exposure route for metals has been assessed in several detailed publications with the conclusion that while it plays a role in bioaccumulation its role in toxicity is minor. This has been challenged several times in the past and most recently when multiple linear regression (MLR) models USEPA plans to use for metals water quality criteria (WQC) underwent peer review. This criticism has prompted a further assessment with a view to identifying where dietary exposure has been used in developing WQC and where it might be used. The existing US WQC explicitly considers diet as a key route of exposure for mercury (food chain accumulation and potential for wildlife exposure) and for selenium with dietary exposure resulting in fish embryo deformities. Laboratory studies have previously evaluated the potential for dietary effects of arsenic, cadmium, cobalt, copper, silver, and zinc in addition to mercury and selenium. For the most part a lack of effects has been observed. An exception to this is for arsenic where dietary effects have been shown for rainbow trout and fathead minnows. Additionally, a large body of bioaccumulation factors for arsenic have shown little relationship with water exposure concentrations suggesting diet may be important. This presentation will evaluate previous efforts to develop dietary WQC and compare mercury and selenium WQC approaches with what might be done for arsenic. A model approach for arsenic will be presented and suggestions for how to assess which metals should be assessed for the importance of dietary exposure.

7.06.B.T-06 Integration of Environment, Health, and Sustainability Metrics: Zinc Case Studies

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Increasing requests from stakeholders across mineral value chains demand information on various aspects of material composition, risk to health and environment, and emissions footprint. As a result, industry strategies to support measurable progress towards zero pollution and decarbonization are becoming increasingly sophisticated. While individual companies take specific measures to address customer and jurisdictional requirements, industry sectors are integrating decades of research and development to identify metrics and pathways towards achieving decarbonization and reducing environmental emissions. The development of credible systems for assessing life cycle impacts (ISO guidance, GHG Protocol) or chemical management (GHS CLP, risk assessment) considerations for products have been individually challenging. Using decades of research from the zinc industry on material flow analysis and life cycle assessment (LCA), decarbonization scenarios were developed. In an additional step, environmental emission scenarios from zinc production and use (e.g., EU REACH dossier) were used to link source apportionment with risk assessment and abatement scenarios. A synergistic result of the analysis illuminated zinc's "value in use" that can equate to massive intractable carbon emission reductions from steel and concrete sectors. Results demonstrated that an integrated framework for combining LCA and risk assessment enables innovation. This presentation will offer a framework assessing the spectrum of benefits and impacts for materials along a value chain.

7.06.P-Tu Metals: Current Affairs and Recent Developments

7.06.P-Tu-178 Distribution of Trace Metals and Rare Earth Elements in Surface Sediments in the Canadian Arctic: Establishment of Geochemical Baseline

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An exhaustive spatial portrait of trace metals and rare earth elements (REE) distribution across the Canadian Arctic was conducted based on 141 marine surface sediments samples collected during the ArcticNet 2016-2019 and 2022 missions aboard the Canadian Coast Guard Ship Amundsen. This study allows a comprehensive assessment of the current state of metal pollution in the region using a geochemical survey coupled with pollution indices and regional backgrounds measured with pre-industrial sediments. Major and trace element concentrations in sediments were measured by partial acid digestion and mass spectrometry and represent the extractable fraction, i.e. the most mobile fraction available to organisms. Results revealed significant spatial variability in trace metals and REE concentrations across the region, with a clear West-East component. Highest metals contents are observed in Beaufort Sea and Amundsen Gulf region (western Canadian Arctic), highlighting the Mackenzie River as an important source of trace metals. Conversely, REE are predominantly clustered near the Canadian Shield coasts, in the south central and eastern Canadian Arctic. The distribution pattern of REE normalized to Post-Archean Australian Shale show a small enrichment in light REE compared to heavy REE. Enrichment factor and Geo-accumulation index indicate generally low metal enrichment or contamination levels, although a few samples exhibit moderate enrichment / contamination in As in Beaufort Sea and Jones Sound. Sediment quality was also assessed using the Canadian guidelines and most of the samples presented metals concentrations below the Probable Effects Level. Concentration surpassing this threshold were however measured in some samples in As, Cu and Ni in Amundsen and Coronation gulfs and in Jones and Lancaster Sounds. Overall, trace metals and REE distribution in the Canadian Arctic exhibit natural concentrations that reflect surrounding geology and regional sedimentary processes.

7.06.P-Tu-179 Implementing Metal Bioavailability in Australia and New Zealand: The Road to Finalisation

Jenny Stauber[1]*, Jennifer Gadd*[2]*, Rick van Dam*[3]*, Aleicia Holland*[1]*, **Erin Smith***[4]*, Adam C Ryan*[5]*,* *Elizabeth Middleton[6], Graham Merrington[7], Adam Peters[7] and Iain Wilson[7], (1)La Trobe University, Australia, (2)Hydrotoxy, New Zealand, (3)WQadvice, Australia, (4)Copper Development Association, (5)International Zinc Association, (6)NiPERA, (7)wca environment, United Kingdom*

Draft default Water Quality Guideline (WQG) values for copper, nickel, and zinc have been developed for application to freshwaters in Australia and New Zealand. These WQG values are more complex than previous hardness-based WQG values, as they also depend on pH and DOC. Experiences from other regulatory jurisdictions that have successfully implemented bioavailability-based approaches for metals (e.g., Europe and North America) indicate that to ensure effective regulatory uptake, additional efforts beyond those normally considered for non-metal WQG values are required.This project, which started in early 2024, aims to deliver the key implementation steps for regulatory application of WQG values. This will facilitate routine and consistent regulatory uptake, and therefore improved assessment of potential Cu, Ni and Zn risks in Australian and New Zealand fresh waters. This poster will outline the strategy we are taking, and the progress we have made in this 18 month programme. The main tasks are: 1) Collection and collation of surface water chemistry data, and development of a database, for the key factors that affect bioavailability – to enable the derivation of a reference WQG representing 'high' bioavailability conditions; 2)Derivation of the reference WQG value for each substance of interest, to be used in a tiered risk-based approach with consideration of potential differences in the freshwaters found in New Zealand and Australia; 3) Development of a simplified tool for predicting local bioavailability at sites; 4) Development of guidance for the use of the simplified bioavailability tool; 5) Development of training materials for user groups and stakeholders that covers the tiered risk-based approach and use of the reference WQG values. As the focus of the project is for regulatory uptake of the developed tool, the last 3 tasks are being undertaken with the use of a regulatory 'user' focus group to ensure that the final product is fit-for-purpose and meets the needs of the target audience.

7.06.P-Tu-180 Implementing Bioavailability for Metal Water Quality Guidelines in Australia and New Zealand: Collecting and Collating Water Chemistry Data for Guideline Derivation

*Jennifer Gadd[1], Aleicia Holland[2], Jenny Stauber[2], Rick van Dam[3], **Erin Smith**[4], Adam C Ryan[5], Elizabeth Middleton[6], Graham Merrington[7], Adam Peters[7] and Iain Wilson[7], (1)Hydrotoxy, New Zealand, (2)La Trobe University, Australia, (3)WQadvice, Australia, (4)Copper Development Association, (5)International Zinc Association, (6)NiPERA, (7)wca environment, United Kingdom*

Water Quality Guidelines (WQG) have recently been developed for copper, nickel, and zinc for Australia and New Zealand's freshwaters. As these are bioavailability-based and rely on measurement of pH, hardness or calcium and dissolved organic carbon (DOC), a critical first step on the journey to their implementation is the collection and collation of national surface water chemistry data. These data are imperative as they enable the derivation of reference WQG, representative of high bioavailability conditions. This then allows the use of a tiered risk-based approach to bioavailability implementation. The data collected demonstrate inherent differences in the water chemistries of Australia and New Zealand, and so guideline values have been derived for each country individually. The approach used for the derivation is similar to the approach currently applied in Europe for Environmental Quality Standard derivation.

This poster will outline the data that have been collated (primarily existing monitoring data from state/regional authorities), the spatial extent of that data for each country, why different reference values are required for each country, any uncertainties and gaps in the collected data and the strategies being undertaken to fill these that can be readily applied by others starting on similar journeys.

7.06.P-Tu-181 Understanding the Role of Water in the Mining Industry: Metrics, Risks, and Sustainability

***Erin Smith**[1], Raymond Philippe[2] and Mark Fellows[2], (1)Copper Development Association, (2)Skarn Associates, United Kingdom*

Water plays a crucial yet often overlooked role in the mining industry, impacting various aspects of operations and sustainability. Unlike greenhouse gases, water's significance lies in its multifaceted influence on operations, regulations, reputation, and climate change adaptation. Stakeholders must assess relevant metrics to gauge water-related risks effectively. Asset-level water analysis emerges as a critical tool, offering insights into operational efficiency, resilience, and environmental impact. Operational water management metrics, including consumption and efficiency, are pivotal for mitigating risks and ensuring environmental and social responsibility. Financial risks, such as production disruptions, underscore the need for water metrics at both asset and corporate levels. Despite limited asset-level disclosure, efforts are underway to quantify and benchmark water impacts, guiding informed decisions and enhancing the sector's sustainability.

7.08.T Overcoming Risk Communication Challenges: Strategies to Create Real Behavior Change for Better Communication

7.08.T-01 Confirmation Bias and the Proliferation of Erroneous Research and Publication Bias

***Shane de Solla**[1,2] and Karen A Kidd[3], (1)Environment and Climate Change Canada, (2)McMaster University, Hamilton, Canada, (3)McMaster University, Canada*

Attempts to replicate previously published studies has revealed a “hidden” crisis in science. The successful replication rate (%occurrences where the replicating study has the same results as the original study), is roughly 50%; roughly the same likelihood as obtaining “heads” by randomly flipping a coin. Further, studies that fail to be replicated successfully appear to be biased towards false positives and have more citations, than studies that are successfully replicated. “Natural selection” may favour poor research designs and data analyses, as studies that have surprising results or large effects sizes are rewarded by a greater number of citations and therefore influence. Further, journals are often biased towards publishing significant results as opposed to “null results”, again leading to overestimation of effect sizes. Confirmation bias may also contribute to the proliferation of false positives, as researchers may have cognitive biases towards hypotheses that support their world view, without due consideration of alternative hypotheses. Although “ancient history”, Bradford Hill’s seminal work on causality published in 1965 (e.g., strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy), may aid researchers in avoiding making strong inferences in the face of relatively weak evidence. Our inability to successfully replicate previous research, and tendency towards publishing false positives, corrodes the credibility of scientific method and practitioners, and weakens scientific practices. We must do better.

7.08.T-03 Enhancing Climate Resilience - Creating Strategy from the Community Perspective

Diane Henshel[1]*, Brittany Sanders*[2] *and Amanda Smith*[2]*, (1)Indiana University, (2)O'Neill School of Public and Environmental Affairs, Indiana University - Bloomington*

Climate change is creating increased extreme weather events, including increased risk of flooding from severe, precipitous storms. These flooding events have cost Great Lakes states $7.19 trillion over the past decade, an increase of 64% over the prior 6 years (in 2024 dollars). Despite the best current efforts of governments to prepare for extreme events, individuals and families can expect little help from municipalities, states, the federal government or even NGOs in the hours to days following an extreme weather-precipitated event. The closest source of help would be one's immediate family, co-residents, and neighbors. A preliminary Great Lakes storm risk history and perception survey (2019 – 2020) of residents in United States Great Lakes watershed counties indicated that: erosion is of greater concern than heat, flooding or drought; 80% of respondents have no training to deal with disasters; 50% have no emergency kit prepared for a disaster; 20% live in communities with no disaster programs in place; and 10% live in communities where storms have closed services at least once within the last 5 years. We assumed that individual vulnerability would be decreased by a more extensive network of family, neighbors, friends, and other connections who could help in such an extreme weather-precipitated emergency. The Great Lakes survey also indicated that a connection network (measured as a Community Network Index) is enhanced by involvement in almost any type of community or business group, including social media. To enable greater community and individual level resilience, we developed a draft Community Resilience and Adaptation Guidance and associated decision support tools (in Excel and Miro) that uses reframed Community-Based Critical Infrastructure categories (Energy-related home utilities, Communication, Transportation, Food, Water, Personal Care) to take an individual through their home's flood and storm vulnerabilities, to identify and prioritize possible adaptations by Critical Infrastructure category. This Guidance will be tested in focus groups before it is revised and posted online for general use. This work has used results from the storm and flood risk history and perception survey to identify key areas of vulnerability in the US Great Lakes watershed to drive the development of a community-based group-help approach encouraging community-based adaptations to increased community resilience to the increasingly severe flooding in the region.

7.08.T-04 Comprehensive Resources for Microplastics Messaging: What Utilities Need to Know

Alma Beciragic[1] *and* ***Linda Mortensen***[2]*, (1)Arcadis, Raleigh, United States, (2)Arcadis*

Microplastics (MPs) have been detected across the world in almost every place investigated; thus, it is no surprise that MPs have likewise been detected in water supplies, including both surface and groundwater sources. With many occurrence studies garnering media attention, consumer concern over MPs in drinking water has continued to grow. This trend is likely to accelerate with the passage of Senate Bill 1422, which requires the California State Water Board to formally define MPs in drinking water, mandates the adoption of standard analytical methods, and requires four years of MPs monitoring in select water systems with inclusion of results in Consumer Confidence Reports (CCRs). With utilities rightly focused on more acute priorities (such as lead, PFAS, and infrastructure repair), most have limited institutional knowledge of MPs. However, such knowledge may become critical in the future; New Jersey recently passed related legislation for monitoring MPs, and a similar bill is currently proposed by the New York City Council. Water Research Foundation (WRF) Project 5155 – Developing Strategic Consumer Messaging for Microplastics in Drinking Water Supplies – was initiated to equip utilities with the resources to appropriately respond to consumer inquiries. In addition to a statistically significant survey of consumer messaging receptiveness, WRF 5155 also compiled important contextual information about MPs issues (occurrence, sampling, water treatment removal efficacy, etc.) to further gird utilities for the inevitable wave of consumer concern. This presentation will discuss the results and main conclusions of the recently published WRF 5155 study. In tandem with WRF 5155, a diverse group of volunteer stakeholders was convened to develop additional language for utilities to be able to use in their CCRs. This "Consumer Messaging Workgroup" (CMW) – operating under the purview of the California Water Quality Monitoring Council – identified six topical categories for CCR language modules; each was designed to be clear, concise, and reader-friendly while also ensuring the language can be used by any utility across the country, at its discretion. The main objectives of this presentation are to: 1) Bolster industry knowledge on MPs in drinking water (occurrence, treatment, analysis challenges, remaining research gaps) 2) Communicate lessons learned regarding effective MP messaging, and 3) Raise awareness of the resources generated by WRF 5155 and the CMW.

7.09.P-Tu Two-Eyed Seeing: Bridging, Braiding and Weaving Indigenous Ecological Knowledge with Western Science to Inform Science

7.09.P-Tu-182 Environmental Protection for Indigenous Use - Athabasca Chipewyan First Nation Research and Water Policy "tu bet'a ts'ena (With Water We Live)"

Timothy Bebeteidoh[1] *and Mandy L Olsgard*[2], *(1)Athabasca Chipewyan First Nation - Denel Lands and Resource Management, Canada, (2)Integrated Toxicology Solutions, Canada*

A multi-year collaborative project describing how Indigenous community knowledge can inform scientific studies and be relied on to develop Policy for Nation to Nation Treaty discussions and engagement and consultation with industry and government stakeholders will be presented. In 2019, the Athabasca Chipewyan First Nation (ACFN), Fort McKay First Nation (FMFN) and Mikisew Cree First Nation (MCFN) started a research project that would eventually re-define surface water and sediment quality criteria in the Lower Athabasca River and downstream surface waters and support the development of Nation specific (ACFN) Water Policy. The presentation will discuss the process and methods relied on to integrate principals of western environmental science (i.e. toxicology, limnology, hydrology, risk assessment) and Indigenous ways of knowing (i.e. cultural practices, traditional food and medicine use, community member risk tolerance) and the tiered approach to establish criteria based on non-degradation and health risk criteria for Indigenous water use categories (consumption of traditional foods, medicine and surface water, trapping furbearing mammals, the health of wildlife (birds and mammals), and aquatic ecosystem health). Results will be presented in the context of the ACFN Water Policy "*tu bet'a ts'ena (With Water We Live)*". Application of the methodology to other ecosystems (i.e., terrestrial) will also be presented to provide the audience with an understanding of how the approach reflects ecosystem health, Indigenous ways of life, and s.35 Treaty Rights rather than any single environmental media (i.e. soils, water, air) or receptor group (i.e. aquatic life, mammals, birds, plants).

7.09.T Two-Eyed Seeing: Bridging, Braiding and Weaving Indigenous Ecological Knowledge with Western Science to Inform Science

7.09.T-01 Archaeology, Heritage, and Transdisciplinary Research in the Aleutian Islands, Alaska: Faunal Refuse from Ancestral Unangam Middens Provide Insight to Natural Mercury Dynamics

Julie Avery[1]*, Nicole Misarti*[2]*, Caroline Funk*[3]*, Scott Shirar*[4]*, Joshua Reuther*[4]*, Todd O'Hara*[5] *and Lorrie Rea*[2]*, (1)University of Alaska Fairbanks, United States, (2)University of Alaska Fairbanks, (3)University at Buffalo, (4)Museum of the North, University of Alaska Fairbanks, (5)Texas A&M University*

Mercury is a toxicant of global health concern with natural sources from volcanic, fire, glacial and other processes. Understanding mercury dynamics with minimal anthropogenic contribution will inform how climate change has and will impact future environmental mercury concentrations. In the Aleutian Islands of the North Pacific, Indigenous (Unangax) People have inhabited Unalaska Island for more than 9000 years, and their middens (refuse heaps) have been preserved for at least 5000 years. Excavations of middens in 7 ancestral villages [spanning 5000 to 200 years before present (yBP)] provided skeletal remains of otariids [Steller sea lion (*Eumetopias jubatus*), Northern fur seal (*Callorhinus ursinus*)] and Pacific cod (*Gadus macrocephalus*), that were examined for total mercury concentrations ([THg]). All three species remain of cultural and subsistence importance today. Midden sites were located within 8 to 20km of the current city of Unalaska. Materials excavated from ancestral villages were utilized following Memoranda of Agreement between Unangam representatives and University of Alaska. Mean (n=444) otariid compact bone [THg] ranged from 6.7 to 516.1µg/kg dw (1µg/kg detection limit) and varied by site. Two sites inhabited 5500-3200yBP (UNL-048) and 2500-1800yBP (UNL-092) had the greatest mean [THg] (99.7±75.6 and 88.1±92.1µg/kg, respectively) compared with the other 4 sites (range 44.7±39.3 to 69.4±71.5 µg/kg; $p<0.001$). In all sites, [THg] within age class were highly variable; however, pups (75.3±74.0µg/kg) had greater [THg] than adults 56.9±59.1µg/kg ($p=0.014$). At UNL-050 (3692 to 2756 yBP) where both cod (n=299) and otariid (n=52) specimen were available, mean [THg] was greater in cod (90.3±88.9µg/kg) compared with otariid (30.2±11.7µg/kg; $p<0.001$) bone specimens. Otariids and cod harvested by Unangam, Qawalangin and Qigiiĝun Tribe members, reveal differences in [THg] over time, between age classes, and among species. The differences (and high within group variability) were likely influenced by environmental conditions, and feeding ecology of these species, as well as ancestral Unangam harvesting strategies.

7.09.T-02 Traditional Marine Resource Consumption Risk-Based Monitoring Program for a Working Harbour

Ariel Blanc[1]*, Amy Corp*[2]*, Mark Larsen*[2]*, Eric Crawford*[3]*, Fiona Wong*[4] *and Michelle Havey*[2]*, (1)Anchor QEA, Seattle, (2)Anchor QEA, (3)Transport Canada, Canada, (4)Public Services and Procurement Canada, Canada*

Victoria Harbour, British Columbia, is centrally located within the traditional territory of Esquimalt Nation and Songhees Nation (First Nations) and has been a busy commercial and industrial harbour since the mid-1800s. Contaminants in sediment are elevated from the harbour's historical past, and there are ongoing sources of contaminants to the harbour, including stormwater. Targeted early action and source control have addressed some contamination, and decades of monitoring data suggest recovery is occurring in both sediments and sea life.

Dungeness crab hepatopancreas dioxin/furan and PCB toxic equivalent concentrations decreased by >75% between 1995 and 2019. The recent human health risk assessment identified potential risk to First Nations consumers of sea life at traditional quantities and rates. While there is not current regular harvesting of sea life by First Nations community members or the public, these findings indicate sediment and tissue contaminant reductions to date have not been sufficient to reduce risks below thresholds for frequent consumption of traditional marine resources. First Nations have expressed a strong desire to resume harvesting sea life from the harbour. A source control and sediment management plan (SCSMP) outlining the remedial strategy for the harbour that includes coordination with First Nations is being developed. The SCSMP will include elements of active remediation, source control, and monitored natural recovery. A monitoring program for the next 80 years has been developed to track harbour recovery through implementation of the SCSMP. This monitoring program includes four cycles of sampling occurring over progressively longer periods as harbour conditions improve. A complete updated harbour sediment and sea life tissue dataset will be collected during each cycle. Sea life samples will be processed consistent with traditional processing methods as shared by First Nations. Caged bivalves will also be tested to evaluate impacts of surface water quality on bivalves, which are marine resources historically harvested from the harbour. Updated risk estimates for First Nations consumption of individual sea life types and the mixed diet will be quantified with the sea life tissue data collected from each sampling event. This information will be provided to First Nations and may be used to inform modification to the harbour sea life consumption advisory. A decision-making framework has been developed to identify potential monitoring data outcomes and responses throughout the duration of the program.

7.09.T-03 Interweaving Traditional Knowledge and Western Science in the San Francisco Bay Delta

*Aaron Nicole Angel[1], Eva Bush[2], Morgan Chow[2], Jill Harris[3], **Tricia Lee**[3], Dylan Stern[3] and Xoco Shinbrot[3], (1)The Freshwater Trust, (2)Delta Stewardship Council, Delta Stewardship Council, (3)Delta Stewardship Council*

The Sacramento-San Joaquin Bay Delta (Delta) in California is a complex environmental landscape, challenged by climate change and other anthropogenic pressures. Recognizing these pressures, Western, or mainstream science and management have begun to embrace more holistic views of natural resource management and conservation in the Delta. Place-based knowledge and land stewardship practices of Indigenous peoples typify what is known as Traditional Knowledge (TK). TK is an adaptive, participatory, and experiential process built through multigenerational observation. Building inroads for TK in agency processes and products fulfills California State directives, aligns with federal initiatives, and advances the missions of the Delta Stewardship Council (Council). With this motivation in mind, staff at the Council are conducting research to evaluate current and future opportunities to interweave TK and mainstream science within Delta management practices. Council staff conducted a team-based literature review of peer-reviewed and grey literature using a snowball sampling approach to evaluate how TK is applied and interwoven with mainstream science in estuarine socio-ecological systems. Interviews with Tribes provide further perspectives on how best to co-produce knowledge in the Delta. The team-based literature review evaluated a total of 89 articles, with 40% coming from Indigenous scholars. We will share key themes including the similarities and differences between TK and mainstream science, participatory approaches and barriers and solutions to interweaving knowledges, as well as exemplary applications of TK with science. In addition, Council staff are developing a “Best Practices for Interweaving Traditional Knowledge and Delta Science and Management” which highlights six strategies for the Council and other audiences to consider. As the Council explores how to best co-produce knowledge, we see how recognizing TK as a separate knowledge system that complements mainstream science is an important step in the right direction.

7.09.T-04 Cote First Nation Baseline Health Assessment Focused on the Introduction of Bison to the Community

Kelly Jones[1] *and Theresa K Lopez*[2]*, (1)Tetra Tech Canada, Canada, (2)Tetra Tech*

In the winter of 2021 Cote First Nation (CFN) introduced a herd of bison to the community and have perceived an assessment of the health of the bison is needed and wish to engage the Youth & Elders in the community to educate them in the teachings of CFN history, language, identity and culture. This project will help foster a community re-connection to traditional ways by teaching herd management and ecological conservation. This research aims to note the baseline health of the CFN community, incorporate elder’s knowledge of the role of bison in Cote life, and engage youth in the ways of their culture to sustain traditional practices. CFN will engage Elders to document the role of bison in the Cote First Nation culture, including practical and spiritual connections to the bison. This assessment incorporates field surveys, interviews of elders by the youth, and engaging the community to create a path forward for the bison project; to create employment opportunities within the community related to the bison, such as herd management and butchering, abattoir services, selling of arts and crafts from the bison, and eventually training opportunities for other First Nations. As well, there is a push to completely rid all CFN lands of pesticide/herbicide use. The lands may have been contaminated by aerial spraying on non-Cote Band lands. To build a successful program of bison introduction and integration to the CFN community, this research will gather traditional knowledge and introduce youth to traditional foods, food harvesting, and cultural practices. The cultural and environmental benefits of bison reintroduction to Indigenous communities have been anecdotally noted while the benefits of traditional foods diet have been scientifically noted. The short-term objectives are to: 1) Document the current conditions of the lands to which the bison have been reintroduced in Cote First Nation; soil sampling and a biologic inventory will be conducted. 2) Engage Elders to document the role of bison in the Cote First Nation culture, including practical and spiritual connections to the bison; 3) Engage youth in order to create a path forward for the bison project; and 4) Document the changes brought to the environment by the re-introduction of bison. The long-term objectives are to create economic and employment opportunities within the community related to the bison, and incorporate the bison into the Yearly Youth Cultural Camp as traditional food, crafts, and cultural items.

7.09.T-05 Increasing Access to the Environmental Sector Through Overnight, Experiential Learning Field Courses

Rachel Kusuma Giles*, Ecology and Evolutionary Biology, University of Toronto, Canada; Field Research in Ecology and Evolution Diversified, Canada*

There is a clear underrepresentation of Indigenous, Black and/or Racialized (BIPOC) individuals in the environmental sector that only intensifies higher up the career ladder. Field Research in Ecology and Evolution Diversified (FREED) is an organization whose mandate is to address barriers to accessing field work alongside creating a curriculum that is led by majority BIPOC early-career professionals for BIPOC undergraduate students. In our presentation, we will share how our FREED curriculum integrates accessibility, multiple knowledge systems, including Indigenous knowledge, and diverse learning opportunities into our events that make FREED a unique and meaningful experiential learning experience for students. Additionally, we will share some data from surveys where we assessed the impact of these accessible, experiential learning experiences for BIPOC undergraduate students in field work and the environmental sector. Specifically, we surveyed how student perceptions of their own skills and confidence in field work, their sense of community in conservation and ecology sectors, and their likelihood of pursuing a environmental-based career changed after participating in FREED.

Track 8: Systems Approaches

8.01.P-We Biodiversity Metrics for Improved Chemical Management

8.01.P-We-191 Assessing Risks to Biodiversity from Exposure to Chemicals - Findings of an ECETOC Task Force on Current and Future Research Directions

Anja Gladbach[1], David A Bohan[2], Marta Baccaro[3], Sian Ellis[4], Franziska Enzmann[5], Jutta Hellstern[6], Emily Rogevich Garman[7], Sarah A. Hughes[8], Philippe Lemaire[9], ***Jan-Dieter Ludwigs**[10]*, *Lorraine Maltby[11], Christoph Mayer[12], Anna-Maija Nyman[13], Hans Sanderson[14], Simran Sandhu[15], Aaron Stoler[16], Marco Thines[17], Johannes Tolls[18] and Nika Galic[19], (1)Bayer AG - Crop Science Division, Germany, (2)INRAe, France, (3)European Commission - Joint Research Centre, Brussels, Belgium, (4)Corteva Agriscience, United Kingdom, (5)Henkel, Denmark, (6)F. Hoffmann-La Roche, Switzerland, (7)NiPERA, (8)Shell Global Solutions, Houston, (9)Total Special Fluids Division, France, (10)Rifcon, Germany, (11)School of Biosciences, University of Sheffield, United Kingdom, (12)BASF SE, Germany, (13)European Chemicals Agency, Helsinki, Finland, (14)Aarhus University, Denmark, (15)Unilever, United Kingdom, (16)Exxon Mobil, Cypress, United States, (17)Goethe University Frankfurt, Germany, (18)Corporate Scientific Services, Henkel AG & Co. KGaA, Germany, (19)Syngenta AG, Switzerland*

In accordance with global efforts to reduce the negative impacts of chemicals on biodiversity, the EU Commission has set ambitious goals for mitigating the entry and effects of chemicals in the environment. However, these goals are hindered by unclear definitions, metrics, and relationships of biodiversity with chemical regulation. The European Centre of Ecotoxicology and Toxicology of Chemicals (ECETOC) initiated a Task Force to address some of the listed challenges. Here we report on the outcomes from the Taks Force subgroup that overviewed EU commissioned research projects to better understand how knowledge can be leveraged to inform regulatory frameworks as well as identify research gaps that would become the scope of future funding calls. Preliminary findings indicated that only recently EU Horizon calls start to focus on research questions related to the biodiversity and measures to halt the decline, thus the acknowledgement of their respective research findings in shaping chemical regulation is yet to come. However, while the EU Biodiversity Strategy and the relation to chemical regulation is focussing on a defined regional scale, to effectively address the challenges of biodiversity loss and ecological imbalance efforts need to be coordinated globally and unified regulatory measures implemented to avoid e.g. exporting of environmental footprints. The effective assessment of cross-links between regional frameworks and regulations and their global implications could become an additional research focus area in EU Horizon projects. Designing a pragmatic, implementable holistic impact assessment framework remains a challenge for the foreseeable future.

8.01.P-We-192 Is Environmental DNA-Based Assessment Effective in the Upstream-Downstream Comparison of Macroinvertebrate Communities in a Metal-Contaminated River?

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Environmental DNA (eDNA) is an appealing tool for biomonitoring due to its low sampling effort in the field. Despite its utility, the downstream transport and dispersion of eDNA in river flow, particularly without substantial dilution, can complicate its ability to accurately reflect local biotic communities. In this study, we examined the potential of eDNA-based biological assessment by comparing its results with those of macroinvertebrate sampling in a river influenced by a tributary heavily contaminated with metals such as Cu. For this purpose, we carefully selected study sites to test if the eDNA-based assessment can discern changes in the macroinvertebrate fauna at varying distances from the inflow of the metal-contaminated tributary, without being influenced by upstream communities.

8.01.T Biodiversity Metrics for Improved Chemical Management

8.01.T-01 Assessing Risks to Biodiversity from Exposure to Chemicals: Findings of an ECETOC Task Force on Biodiversity Definitions, Metrics, and Methodologies

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In accordance with global efforts to reduce the negative impacts of chemicals on biodiversity, the EU Commission has set ambitious goals for mitigating the entry and effects of chemicals in the environment. However, these goals are hindered by unclear definitions, metrics, and relationships of biodiversity with chemical regulation. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) initiated a Task Force to address some of the challenges. Here we report on the outcomes from the Task Force subgroup overviewing biodiversity definitions and metrics, as well as methods for assessing and quantifying biodiversity. We used a text-mining approach to survey the peer-reviewed literature, with a primary focus on reviews, perspectives, and meta-analyses. Preliminary results revealed a wide array of both definitions and metrics for assessing biodiversity. Definitions ranged from

descriptive to quantitative and probabilistic. Metrics either focused on a single measurement or integrated multiple measurements into a single index. Proxies for biodiversity (e.g., provision of ecosystem services) were also identified as metrics. The majority of metrics were associated with taxonomic diversity, whereas functional diversity received comparatively less – although increasing attention. More dynamic metrics included temporal and spatial turnover instead of focusing on snapshots of community composition. Metrics varied in their scalability from local to global assessment, and also varied in their ability to be normalized for use across different sectors and applications. As the world moves towards unifying goals of environmental stewardship, emerging frameworks (e.g., The Global Biodiversity Framework, The Essential Biodiversity Variables) are helping to consolidate these definitions and metrics. This will be vital to ensuring that chemical regulations prescribe risk assessment approaches that fully align with those goals.

8.01.T-02 Targeting Umbrella Species to Prioritize Biodiversity in NRDAs

***Rex R Bergamini**, Jill McGrady[2] and Mark Laska[2], (1)Great Ecology*

The overall goal of natural resource damage assessment (NRDA) is to provide public compensation for lost ecosystem services and to restore injured natural resources to baseline condition (i.e., condition prior to the occurrence of injury) or replace with or acquire an equivalent natural resource. Restoration activities include compensatory restoration, whereby the public is compensated for interim losses of natural resources and services from the date of injury to recovery, and primary restoration, or actions that return both injured natural resources and natural resource services (i.e., ecosystem services [ES] that are ecologically or publicly based). Examples of ecologically based ES in NRDA-negotiated settlements include habitat restoration and protection, land parcel acquisition or enhancement, and invasive plant removals; examples of public service-based ES include enhancement or acquisition of recreational amenities, provisioning of seafood and fish-based products, and floodgate replacement. NRDA settlements, due to current regulatory frameworks and a stakeholder-driven focus on anthropocentric ES, may overlook biodiversity conservation, which is important for ecosystem stability, resilience, and connectivity, especially in the context of habitat loss and species translocations resulting from climate change, habitat degradation, and environmental stressors. As biodiversity and conservation are not target priorities of NRDA settlements, how do we bring biodiversity into NRDA settlements, so settlements are more biologically driven? Our thesis is that if ecosystem health is a priority, then mitigation for injured natural resources should be optimized for ecosystem health and resiliency, and not to a baseline condition. The challenge is to identify surrogate species that represent the biodiversity and integrity of the habitats within the ecosystem or ecosystems being assessed. In NRDA, sentinel species typically define environmental and human risk; however, identifying species that meet the criteria of sentinel, flagship, and umbrella species is more appropriate, because protecting such species provides holistic protection for ecosystems and humans, and garners public support. We present NRDA case studies highlighting our successes and lessons learned in using umbrella species as a holistic approach to conservation of biodiversity in upland and aquatic habitats.

8.01.T-03 Documenting the Values of Environmental Mitigation Projects in Transportation and Economic Development

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Environmental mitigation programs consistently utilize nature-based solutions to restore stability and habitat to streams, wetlands, riparian areas, and other areas of ecological significance. Mitigation programs are intended to provide an offset or "replacement" of ecological function and value. Most of these activities are heavily regulated with an unintended consequence and direction of reaching an end performance standard rather than ecosystem benefits. Documentation of project outcomes should not end at regulatory monitoring and performance requirements. Extracting non-regulatory values of environmental mitigation programs such as water quality, water quantity, air quality, biodiversity/habitat, and socio-economic benefit provides documentable environmental and sustainability values from the restoration, enhancement, and preservation of these natural ecosystems. Identifying this suite of benefits supports the communities we all work in by providing a means of comparing values gained against those lost due to impacts. Documenting these efforts also provides inherent value for those attempting to meet sustainable goals that many States have as their objectives. Biodiversity and habitat benefit from restoration/mitigation efforts are receiving greater public interest in resiliency and overall ecosystem services. Yet, biodiversity metrics are seldom evaluated for these projects. Data such as the USGS Gap Analysis Project (GAP) can be utilized to evaluate expected changes in vertebrate species habitat richness, based on land cover analysis. The tool utilized for our analysis can be customized to use site specific species data collected from sites where pre- and post-restoration data are being collected. This presentation will utilize two examples to demonstrate the values of such analyses. The first example is a substantial South Carolina economic development project to evaluate the impacts of the new facility versus the restorative value from the compensatory mitigation. Secondly, is an evaluation to demonstrate the sustainable values provided by a South Carolina Department of Transportation compensatory mitigation project. These examples will provide a review of the metric outputs and opportunities to utilize the results to advance sustainability goals and objectives of a State and associated Departments of Transportation.

8.01.T-04 Current Uses and Future Applications of Environmental RNA for Community-Level Assessments

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It is well documented that pollution is one of the drivers of biodiversity loss. However, the impacts of contaminants at the community level are rarely evaluated in traditional ecotoxicological assays and assessments. It is important to understand if contaminants cause detrimental population and community-level outcomes to evaluate hazards to ecosystem functions and resilience. Microscopic organisms, such as meiofauna and microfauna, play vital roles in maintaining balanced ecosystems, and they are difficult to identify using conventional morphological methods. Metabarcoding is an emerging method for rapidly and comprehensively identifying organisms using nucleic acids. Environmental DNA (eDNA) is often used as a

template for metabarcoding, but legacy DNA is problematically detected from remnants of organisms no longer active in the community during sampling. Environmental RNA (eRNA) can also be collected and sequenced using the same pipeline as eDNA metabarcoding, but it is only produced by living organisms in the environment contemporaneous with sampling. For these reasons, eRNA metabarcoding is a useful tool for observing community-level changes in response to chemical stressors. Additionally, eRNA has the potential to provide more insight into understanding the molecular mechanisms behind taxa sensitivity or resilience when exposed to contaminants. Here, two case studies will be presented to highlight how eRNA metabarcoding methods can showcase dose responses at the community-level through alterations in community composition and diversity in benthic marine systems. Using field-collected sediment cores as mesocosms in laboratory-controlled exposures, the first case study focuses on the impacts of nanoplastic particles, and the second on nanocopper antifouling paint. Considerations for utilizing eRNA in targeted gene expression analyses will also be presented. Combining eRNA metabarcoding with mesocosm studies allows a greater understanding of community and population-level outcomes by bridging laboratory and field experiments and can be used as a tool in a plethora of applications, including assessing community changes at multiple timepoints. The development of eRNA metabarcoding will ultimately lead to advancements in our knowledge of trophic interactions, ecosystem response to stressors over time, indirect adverse effects of contaminants, and taxonomic identification of understudied organisms.

8.01.T-05 Assessing Risks to Biodiversity from Exposure to Chemicals: Findings of an ECETOC Task Force on the Regulatory Context

Christoph Mayer[1], David A Bohan[2], Marta Baccaro[3], Sian Ellis[4], Franziska Enzmann[5], Anja Gladbach[6], Jutta Hellstern[7], ***Sarah A. Hughes***[8]*, Emily Rogevich Garman[9], Philippe Lemaire[10,11], Jan-Dieter Ludwigs[12], Lorraine Maltby[13], Anna-Maija Nyman[14], Hans Sanderson[15], Simran Sandhu[16], Aaron Stoler[17], Marco Thines[18], Johannes Tolls[19] and Nika Galic[20], (1)BASF SE, Germany, (2)INRAe, France, (3)European Commission - Joint Research Centre, Brussels, Belgium, (4)Corteva Agriscience, United Kingdom, (5)Henkel, Denmark, (6)Bayer AG - Crop Science Division, Germany, (7)F. Hoffmann-La Roche, Switzerland, (8)Shell Global Solutions, Houston, (9)NiPERA, (10)Total Special Fluids Division, France, (11)TotalEnergies, France, (12)Rifcon GmbH, Germany, (13)School of Biosciences, University of Sheffield, United Kingdom, (14)European Chemicals Agency, Helsinki, Finland, (15)Aarhus University, Denmark, (16)Unilever, United Kingdom, (17)Exxon Mobil, Cypress, United States, (18)Goethe University Frankfurt, Germany, (19)Corporate Scientific Services, Henkel AG & Co. KGaA, Germany, (20)Syngenta AG, Switzerland*

In accordance with global efforts to reduce the negative impacts of chemicals on biodiversity, the EU Commission has set ambitious goals for mitigating the entry and effects of chemicals in the environment. However, these goals are hindered by unclear definitions, metrics, and relationships of biodiversity with chemical regulation. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) initiated a Task Force to address some of the challenges. Here we report on the outcomes from the Task Force subgroup focusing on EU chemical regulation and strategic documents, that looked into regulatory context for considering biodiversity in chemical risk assessments. We mapped 42 legislative documents and overarching strategies concerning biodiversity conservation and/or regulation of chemicals, as well as selected global initiatives influential at EU level. A deep dive into the 25 most relevant documents, analyzed aspects such as how biodiversity metrics, protection goals and impact

assessments are defined and how all would link into the EU's political ambitions and strategies. The analysis revealed that legislative documents hardly define biodiversity and if they do it is mainly the vague but overarching definition of the Rio Convention on Biodiversity from 1992.The wide scope is posing a challenge for the definition of clear protection goals and metrics on biodiversity. Accordingly, protection goals are usually provided on a rather general level. Metrics vary based on definition and specification level applied in the regulatory documents, as well as on the scope and purpose of the regulatory documents and the expected site of chemical action in the environment. Biodiversity is indirectly addressed via protection of surrogate groups or functions of the environment. There is a clear need to specify what part of biodiversity is relevant in a certain regulatory context in order to facilitate the definition of protection goals and the choice of useful metrics. The findings provide a starting point for a multi-stakeholder discussion aiming to advance the chemical regulations with respect to biodiversity impacts. The outcome of the work of this ECETOC Task Force will be a short overview/perspectives paper and a workshop involving relevant stakeholders for EU regulations.

8.02.P-Tu Biodiversity Responses to Chemical Pollution: From Species to Services

8.02.P-Tu-183 Impacts of Insecticide Application on Insect Diversity and Abundance in Cocoa Agroecosystems

***Enock Dankyi*[1], *Blessing Umeh*[2], *Silas W Avicor*[3] *and Rosina Kyerematen*[4], *(1)Department of Chemistry, University of Ghana, Ghana, (2)University of Ghana, Ghana, (3)Entomology Division, Cocoa Research Institute of Ghana, Ghana, (4)University of Ghana, Ghana*

Cocoa (*Theobroma cacao* L.) plays a crucial role in the economy of West Africa, contributing significantly to foreign exchange earnings and providing livelihoods for many farmers. Within cocoa agroecosystems, diverse insect species perform essential ecological roles such as pollination, pest control, and nutrient cycling, which are vital for sustainable crop production. However, the widespread use of insecticides poses a threat to insect diversity and the long-term sustainability of cocoa farming. This study investigates the impact of insecticide use on insect abundance and diversity in cocoa farms in Ghana. Through field studies, we employed various sampling techniques to collect data on insects, focusing on two commonly used insecticides: bifenthrin and imidacloprid. Our findings, based on the documentation of 23,605 individual insects representing 374 species across 121 families and 11 orders, reveal significant reductions in insect diversity and richness in treated plots, without a corresponding decrease in pest populations over the long term. Imidacloprid-treated plots exhibited the lowest diversity and showed a more pronounced effect on both pests and non-target insects compared to bifenthrin. In conclusion, transitioning to less chemically intensive practices in cocoa farming not only promotes insect populations, diversity, and ecosystem services, particularly pollination, but also helps mitigate the potential long-term negative effects of frequent insecticide application.

8.02.P-Tu-184 Aquatic Macroinvertebrate Diversity and Water Quality as Indicators of Wetland Health in Natural and Constructed Wetlands of Northeast Arkansas

***Helena Grace Wheeler*[1], *Sarah J Webb*[2] *and Jennifer Bouldin*[3], *(1)Arkansas State University, State University, United States, (2)Ecotoxicology Research Facility, Arkansas State University, (3)Biological Sciences, Arkansas State University*

The Mississippi River Basin (MRB) has experienced drastic loss of natural wetlands due to modern agricultural practices and floodplain engineering, compromising the lands' ability to cycle nutrients and detoxify pollutants. To address this issue the Federal government incentivized landowners to convert portions of their property to wetland systems through the Wetlands Reserve Easement-Agricultural Conservation Easement Program (WRE-ACEP) which led to the reclamation of 11,736 km^2 of wetland habitat. While the program has been implemented for several decades, little research has been done to assess the functional similarity of WRE-ACEP sites to natural wetlands. Macroinvertebrate community structure and water chemistry data will be analyzed from natural and constructed wetlands within the MRB region to determine the health and effectiveness of these constructed WRE-ACEP wetlands. Water samples will be collected quarterly for a year, beginning in February 2024 and ending in November 2024. Macroinvertebrate communities will be sampled semi-annually beginning in March 2024 and ending in August of 2024. Preliminary water quality analysis shows similar dissolved oxygen, conductivity, turbidity and total suspended solid levels between natural wetlands and WRE-ACEP sites. Aquatic macroinvertebrates are diverse organisms with well-known levels of environmental tolerance. Exposure to different abiotic factors results in a shift in macroinvertebrate communities. Analyzing these community dynamics is valuable in determining aquatic ecosystem health. Water and macroinvertebrate samples will be collected from 30 constructed wetlands, five natural wetlands and five agricultural sites. Preliminary results support functional similarity between natural wetlands and constructed WRE-ACEP sites.

8.02.T Biodiversity Responses to Chemical Pollution: From Species to Services

8.02.T-01 Temporal Changes in Phytoplankton Biodiversity and Water Quality in the Lower St. Johns River, Florida

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The St. Johns River (SJR) is an ecologically and economically important estuarine river system in Florida undergoing extensive anthropogenic change. In this study, a variety of water quality parameters and a suite of metals were measured in water samples collected from eight sites in the lower SJR, in Florida from 2019 to 2022, continuing from previous work that documented these parameters in the river from 2017 to 2019. Aquatic communities such as phytoplankton can be indicative of river health; therefore, phytoplankton were collected from each site, and the diatom component was identified. The total number of taxa in each sample ranged from 60 to 190, with 25 taxa accounting for the majority (64%). Similar to water chemistry, seasonal fluctuations in phytoplankton abundance and diversity were observed, with a relative abundance of *Skeletonema costatum* and *Skeletonema subsalsum* in times of lowered diversity. Furthermore, decreased phytoplankton diversity correlated with increased metal contamination in the lower SJR. Multivariate analyses highlighted significant interactions among phytoplankton diversity and water chemistry variables. Significant parameters affecting phytoplankton biodiversity included salinity, pH, temperature, copper hazard quotient, and the nickel hazard quotient. This study provides new information about the impact of human disturbance on biotic communities and the complexity in predicting population changes.

8.02.T-02 Interactions Between Arbuscular Mycorrhizal Fungi and Rice

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Rice is a staple for over 50% of the global population, however conventional cultivation practices can have detrimental effects to wildlife and human communities at local and global scales. Ecosystem services are the natural services species provide that benefit humanity and have been cited as a tool to develop more sustainable agricultural cultivation. Arbuscular mycorrhizal fungi (AMF) provide ecosystem services that can promote sustainable rice farming through symbiotic interactions with plants. We hypothesized that rice plants inoculated and colonized by AMF would improve yields and biomass and that any positive effects from AMF on rice growth and yield would be moderated by fungicide application. 60 rice plants were divided among three different soil treatments, that included inocula from commercial rice fields, other agricultural fields, and sterilized soils. Additionally, we applied a commercial fungicide (Stratego©) to determine whether it impacts the interaction between AMF and rice plants. Yields, above ground, and below ground biomass of each pot and treatment were recorded. Root samples were then stained and microscopically inspected to quantify the degree of AMF colonization among all treatments. Difference in soil treatments had no effect on yield and biomass. Application of fungicide, however, resulted in significantly reduced below ground biomass, while above ground biomass and yield were unaffected. Among soil treatments inoculated with live fungal spores, fungicide application decreased AMF colonization within the root samples. Further testing on the effects of fungicide on rice yields on a commercial scale could indicate the benefit of limiting fungicide use, leading to greater ecological and economic sustainability.

8.02.T-04 Legacy Effects of a Large, Historic, Pesticide Application Program on Aquatic Benthic Invertebrate Communities

Amy White[1], Scott Sugden[2], Ilya Dimitrovas[3], Sandra Emry[4], Xiaotian Hua[5], Moira Ijzerman[6], Karen A. Kidd[7], Josh Kurek[3], Jennifer Lento[8], Katlyn Morrow[3], Jessica Ollinik[9], Laura Schnell[9], Marcus Thornmeyer[4] and ***Christopher Edge[10]****, (1)University of Waterloo, Canada, (2)McGill Univeristy, Canada, (3)Mount Allison University, Canada, (4)University of British Columbia, Canada, (5)McGill University, Canada, (6)University of Guelph, Canada, (7)McMaster University, Canada, (8)University of New Brunswick, Canada, (9)University of Regina, Canada, (10)Natural Resources Canada, Ottowa, Canada*

The broad scale application of pesticide began in the 1940's with the advent of non-selective insecticides, airplanes, and methods to apply via foliar applications. Some of the pesticides used historically were efficacious, persistent, and led to serious impacts on the aquatic environment when they were applied, with the most well known and studied being dichlorodiphenyltrichloroethane (DDT). Together, the cumulative application of these pesticides led to impacts on the aquatic communities at the time of application. Impacts that may persist as a legacy effects in contemporary communities. One of the world's largest, and well-studied, aerial spray programs occurred in New Brunswick, Canada from 1952-1993 during which 97 % of 6.2 million ha of the forested lands being treated with at least one, and up to 40 applications of insecticides (12 active ingredients). We investigated potential legacy effects of historical insecticide applications on key lotic communities using digitized historical pesticide records, harmonized benthic macroinvertebrate data (CABIN protocol, 274 sites), and environmental variables that capture limnological measures, landscape attributes, and climate seasonal climate at the scale of sites, reach contributing area, and upstream catchment area. We analyzed 8 biological indices and found that historical pesticide applications had minimal effect on indicator

groups, richness, and diversity indices. Multivariate analysis shows clear separation of sites based on pesticide application at all scales. Sites with high pesticide application had higher amount of forested land in both catchment scales. Environmental variables explained moderate amount of constrained variation in benthic invertebrate communities (RDA1: 25.9%, RDA2: 18.5%). However, measures of cumulative pesticide application predicted a small amount of community variation, with the number of applications being the largest contributing pesticide variable. Results show minimal legacy effects of a large, historic, insecticide application program on modern benthic invertebrate communities 30 years after the program ended. Whether communities have fully recovered requires further work investigating functional diversity and in-depth analyses of pesticide sensitive taxa.

8.02.T-05 PFAS Effects on Organic Matter Processing in Streams

Alison Zachritz[1]*, Abagael N Pruitt*[1]*, Therese M Reisch*[1]*, Brittany Grace Perrotta*[2]*, Daniele de Almeida Miranda*[1]*, Christopher Kotalik*[3]*, Laura Hubbard*[3]*, Dana Kolpin*[3]*, David Walters*[3]*, Jennifer L Tank*[1] *and Gary A Lamberti*[1]*, (1)University of Notre Dame, (2)U.S. Geological Survey (USGS), Columbia, United States, (3)U.S. Geological Survey*

Per- and polyfluoroalkyl substances (PFAS) are emerging as one of the most serious threats to water quality globally. This environmental threat is due to the widespread PFAS use for the past 75 years, their high persistence in the environment, and strong evidence for their adverse effects on human and environmental health. PFAS have been linked to human illness, including cancer, thyroid disease, and low birth weight along with effects to wildlife development, behavior, and reproduction. Studies investigating PFAS in relation to ecological processes, however, are limited. The processing of organic matter in streams is a fundamental ecosystem function and critical to the biogeochemical cycling of both carbon and nutrients but can be strongly influenced by anthropogenic effects, including chemical contamination. We investigated leaf decomposition and microbial respiration rates upstream and downstream from known PFAS point source (i.e., effluent outfall) in a third-order agricultural stream. We deployed 12 pre-weighed leaf packs constructed from senescent maple leaves (*Acer saccharum*) at locations upstream and downstream from the effluent outfall. At 2 weeks and 4 weeks, three packs were retrieved from each location for a final mass measurement to determine litter decomposition and three packs were analyzed for PFAS. We also performed light and dark incubations of leaf disks to quantify microbial respiration. We observed lower decomposition and microbial respiration rates for leaf litter deployed downstream of the effluent outfall. A follow-up laboratory study, however, documented maple leaf exposures to PFOS (perfluorooctanoic sulfonic acid) composition rates did not vary between treatments and differences between microbial respiration were only measured between the high (100 ppb) and control (0 ppb) treatments on day 28. We also found significantly higher dissolved organic carbon (DOC) in treatments that received PFOS versus the control, indicating that PFOS affected microbial use of DOC. PFAS may thus induce previously unrecognized changes in aquatic ecosystem function.

8.04.P-Th Late Breaking Science: Systems Approaches

8.04.P-Th-198 Identification and Analysis of Nanoplastics in Lake Erie Sourced Drinking Water

***Megan Jamison**[1] and John J Lenhart[2], (1)Ohio State University, Columbus, United States, (2)Department of Civil, Environmental, and Geodetic Engineering, Ohio State University*

Microplastics have been found in every surface water body studied, which is concerning as they serve as source water for a number of drinking water treatment plants. Previous studies that have evaluated microplastic removal during drinking water treatment found that plastics >10 um are effectively removed, while plastics <10 um are not effectively removed. This unremoved fraction includes nanoplastics (<1 um) which present a greater health concern due to their ability to cross cellular barriers in the body. Previous studies that have evaluated microplastics in drinking water were unable to include nanoplastics in single particle analysis due to analytical limitations. In this study, a method for isolating nanoplastics for single particle identification was employed to evaluate whether the removal efficiency varied as a function of treatment plant configuration. Samples of raw, post-coagulation, post-sedimentation, and treated water were collected from four conventional drinking water treatment plants that use Lake Erie as source water but do so using different treatment conditions (e.g., different coagulant or filtration media). The samples were oxidized to remove organic matter and concentrated on a filter. These retained particles were resuspended in a dense solution and centrifuged to isolate the plastics from the inorganic particles. Once isolated, the plastics were filtered again before being analyzed via scanning electron microscope to determine size, shape, and number of particles, and optical photothermal infrared spectroscopy to determine polymer type. This method for singe particle analysis of environmental nanoplastics allows for improved insight in to the transport and removal of these plastics in water treatment facilities.

8.04.P-Th-199 Richness, Diversity, Productivity and Floristic Quality: How Do We Define "Better"?

***Matthew A. Struckhoff**, U.S. Geological Survey*

This poster will explore a variety of plant biodiversity measures that we have used to assess effects of management and monitor restoration development in terrestrial systems. Using examples from Natural Resource Damage Assessment and Restoration and other cases, the strengths and weaknesses of various assessment tools are explored across different ecosystems with different types of degradation, potential management actions, and restoration endpoints. Many biodiversity measures can document change in plant communities, but their value for injury assessment or long-term monitoring following restoration extends only so far as our knowledge of appropriate targets within a given ecosystem. For example, in some systems, greater species richness does not necessarily represent improvement, particularly if dominated by non-native species or native weeds. Similarly, comprehensive measures of biodiversity intended to quantify “quality” may be less valuable than simple measures that can inform and trigger adaptive management actions such as invasive species removal. In each case, the applicability of any given assessment tool depends upon the defined management goal and the resources available to perform the assessment. No biodiversity measure will be effective if practitioners have not identified clear management endpoints and committed to monitoring the vegetation community characteristics relevant to those endpoints.

8.05.A.T Practical, Effective, and Informative Monitoring and Risk Assessment Strategies for Macro- and Microplastics

8.05.A.T-01 Advancing Frameworks for Monitoring and Assessing the Ecological Risks of Microplastics in the Laurentian Great Lakes

Eden Hataley[1]*, Karen A Kidd*[2]*, Rebecca Rooney*[3]*, Chelsea M Rochman*[4]*, Austin Baldwin*[5]*, Carlie E Herring*[6]*, Dale J Hoff*[7]*, Francois Houde*[8]*, Carl Platz*[9]*, John F Bratton*[10]*, Samir Qadir*[11]*, Lizhu Wang*[12] *and Matthew Child*[12]*, (1)Department of Physical and Environmental Sciences, University of Toronto Scarborough, Canada, (2)McMaster University, Canada, (3)Department of Biology, University of Waterloo, Canada, (4)Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada, (5)U.S. Geological Survey (USGS), (6)Marine Debris Program, National Oceanic and Atmospheric Administration (NOAA), (7)Great Lakes Toxicology and Ecology Division, U.S. Environmental Protection Agency, (8)Québec Ministère de l'Environnement et de la Lutte contre les changements climatiques, de la Faune et des Parcs, Canada, (9)Great Lakes and Ohio River Division, U.S. Army Corps of Engineers, (10)LimnoTech, (11)Potomac-Hudon Engineering, (12)Great Lakes Regional Office, International Joint Commission, Canada*

Plastic pollution, particularly microplastic, is widespread across the Laurentian Great Lakes Basin. The International Joint Commission (IJC) has been working to bring focus to the issue since 2015. Past efforts developed: (1) a *Microplastics Watching Brief* to summarize points of interest related to microplastics science, policy, and media; and (2) recommendations for addressing challenges posed by microplastic pollution across the region, including actions related to science and research, policy and management, and education and outreach. Most recently, in 2022, the IJC's Great Lakes Science Advisory Board convened a work group on microplastic pollution in the Great Lakes comprised of subject matter experts from Canada and the United States. The work group aims to advance coordinated frameworks for monitoring microplastics and assessing and managing their ecological risks for use in the Great Lakes region. To date, the work group has: (1) drafted a literature review to synthesize recent scientific advances in understanding microplastic pollution in the Great Lakes and identify knowledge gaps; (2) updated databases on microplastic environmental contamination and ecological effects; (3) hosted a workshop to develop recommendations for harmonized guidelines for sampling microplastics in water, sediment, and biota and, more broadly, considerations for the design and implementation of a Great Lakes-specific monitoring program; and (4) hosted a workshop to develop recommendations for ecological risk assessment and management frameworks that would contextualize monitoring results and recommend corresponding management actions. Our work group has been fortunate to have had the opportunity to learn from and collaborate with other groups doing similar work on microplastics management from across the United States, including the Southern California Coastal Water Research Project Authority and the Chesapeake Bay Plastic Pollution Action Team. This presentation will provide more information on our work group's objectives and progress toward our deliverables thus far.

8.05.A.T-02 Leveraging Existing Monitoring Efforts to Determine the Extent and Magnitude of Macro- and Microplastic Contamination in the Southern California Bight

Leah Thornton Hampton *and Alvine C Mehinto, Southern California Coastal Water Research Project*

Recently, California became the first state to adopt a Statewide Microplastics Strategy, fulfilling

legislative mandates set in motion in 2018. The strategy outlines a two-track approach to address microplastic pollution. The first track describes a series of "no regrets" actions that may be enacted in the short term to curb plastic pollution in general (e.g., specific product bans). The second track outlines a research plan to gain a more comprehensive understanding of microplastic pollution and its potential impacts on coastal ecosystems. Within this plan, the formation of a statewide monitoring program is highlighted as one of the foundational steps towards understanding the extent and magnitude of microplastic contamination across the state. Rather than initiating a new monitoring program solely dedicated to microplastics, existing programs will be leveraged to 1) take advantage of previously developed infrastructure, coordination, and logistical mechanisms, 2) avoid the duplication of efforts for sample collection, and 3) allow microplastics to be placed within the context of other environmental monitoring data (e.g., bioassessments, legacy contaminants, contaminants of emerging concern). While California boasts multiple regional monitoring programs, The Southern California Bight Regional Monitoring Program, which has included the monitoring of offshore macrodebris since 1994, is the first to incorporate microplastics on a region-wide scale. Planning for the seventh iteration of Bight began in the fall of 2022, bringing together a diverse group of stakeholders from government, academia, and industry as well as environmental managers from both the regulatory and regulated communities. Efforts began with the identification of scientific questions to be answered and the creation of a study design, partly based on the availability of existing and newly identified resources for sample collection, sample processing and analysis, as well as data analysis and interpretation. This monitoring effort will also incorporate newly standardized collection and analytical methods for microplastics to assess microplastic contamination in 90 sediment samples collected across three environmental strata (i.e., estuaries, embayments, and the inner shelf) and mussels collected from 28 sites along the coastline. Though monitoring efforts are ongoing, this presentation will discuss the challenges and advantages associated with this approach to plastics monitoring.

8.05.A.T-03 Microplastic Monitoring Development in the Chesapeake Bay Watershed

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Monitoring of plastic pollution is important to determine the potential impacts and risks to living resources in the Chesapeake Bay. Monitoring can provide critical data such as annual tonnage, high areas of accumulations (hot spots), common plastic types, and long-term status and trends. The Framework for Monitoring Plastic Pollution in the Chesapeake Bay builds upon the foundational work of the Chesapeake Bay Program's Plastic Pollution Action Team (PPAT)'s monitoring subcommittee, where objectives and priorities have been established. Specifically, this framework makes recommendations on monitoring strategies across various media, such as surface water, sediment, and key living resources, as well as scale, frequency, and locations for broad application throughout the Chesapeake Bay and its watershed. The goals for monitoring plastic pollution are many, with each goal influencing the appropriate scale, location, and sampling design. This talk will describe the development of a monitoring framework from which existing monitoring programs can draw on to include microplastics.

8.05.A.T-04 Harmonization of Microplastic Analysis in Europe, the Development of Reference Material

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Microplastic pollution has emerged as a global environmental concern, posing potential risks to human health and ecosystems. Accurate and reliable analytical methods are essential for assessing the extent, fate, and effects of microplastic contamination in the environment. To develop long-term solutions to reduce plastic pollution, it is essential to establish harmonised methodologies. EUROqCHARM, a European Commission H2020 Coordination and Support Action (CSA) project, is addressing this by critically reviewing state-of-the-art analytical methods and validating them through an interlaboratory comparison (ILC) study. Here microplastics reference materials play a crucial role in ensuring the quality and consistency of analytical measurements, enabling researchers to confidently evaluate microplastic concentrations and compare results across studies. Within the EUROqCHARM project several reference materials for micro-, nano-plastics were developed from individual polymers, including PVC, PE, PET, PS, PC, and PP, within the size range of 50 µm to 300 µm using cryogenic milling and multiple sieving steps. The polymer identity and purity of each product were assessed using py-GC/MS and thermogravimetric analysis (TGA), by ISO 17034-certified methods. In addition to the purity, the particle size distribution of the different polymer batches was assessed using laser diffraction. Effervescent tablets containing single or mixed polymers were produced using a mixture of NaHCO3, citric acid (C6H8O7), and lactose (C12H22O11). The neat microplastic polymers were added to the mixture either as a single neat polymer per tablet or as mixed neat blend. The mixtures were combined thoroughly and pressed into shape using a tablet press. Tablets were packed in medical tablet strips to facilitate easy shipping and storage. The reference materials have been used in the 3rd round of the EUROqCHARM/ QUASIMEME/ NORMAN interlaboratory comparison study and are currently being used for the validation of the upcoming ISO standard 16094-2 for water samples. Microplastic effervescent tablets reference materials are now commercially available.

8.05.A.T-05 Leveraging Organismal Traits to Predict Microplastics Environmental Concentrations Within the Context of Quantitative Risk Assessment

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A significant body of ecotoxicological data have been developed for micro- and nanoplastics (MNPs). However, there remain challenges in synthesizing and interpreting these data within the context of ecological risk assessment. While many of these challenges are being addressed, particularly those around study design, QA/QC, and reliability, one significant challenge remains - identification of relevant species for use in the development of ecological protection criteria (i.e., PNECs, HC5). Previous studies have attempted to derive these values, however significant differences in species sensitivity have been observed for micro- vs. nano-sized particles. This results in (1) significantly different PNEC values as a function of the selected particle size / size range, and (2) uncertainty as to the relative sensitivities of different aquatic species to different MNP size, shape, and polymer types. This work summarizes results of a systematic quality

review of over 500 MNP biomonitoring studies using criteria previously developed by Hermsen et al., with minor modification to integrate relevance and reliability more clearly for risk assessment & prioritization of potential future environmental monitoring programs. For studies that met the criteria, trait-based descriptors and particle characteristics and distributions were compiled for all species to provide guidance on (a) species selection and (b) environmentally relevant exposure profiles for informing future biomonitoring and risk assessment studies.
The long-term objectives of this work are three-fold – first, to systematically compile and evaluate physiology and behavioral data for a wide range of freshwater and marine species into a searchable database for use in identification of sentinel species for ecosystem health & quality monitoring, and ecological risk assessment. Second, this work is intended to provide a biologically-relevant framework against which the relevance of existing (and developing) MNP reference materials may be evaluated and additional studies prioritized. This can provide a systematic basis for the inclusion or exclusion of species or materials for the purpose of quantitative risk assessment in various environmental compartments. Third, available information on ingestion and egestion rates as well as behavior and habitat can inform the selection of relevant and efficient species for integrated biomonitoring programs in sea surface, subsurface, estuarine, and sediment environments.

8.05.A.T-06 The RSVP Tool - Representative Sample Volume Predictions for Monitoring Microplastics

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A significant source of uncertainty in environmental exposure and risk assessment for micro- and nanoplastics (MNPs) stems from a lack of standardized methodologies and guidance related to sample collection strategies, extrapolation, and reporting of relevant computed values and their associated statistical variability. This lack of standardization further obfuscates the relevance and reliability of these data for use in the calibration and validation of quantitative exposure assessment models. The purpose of this work is to understand common sampling strategies, identify key parameters which may propagate uncertainty & variability in these techniques, and ultimately to develop a framework for systematically assessing relevance and reliability of these data for use in quantitative risk assessment of MNPs. The development of guidance and best practices presented in this framework focus on several key areas for evaluation and categorization within a standardized rubric / system: (1) Synthesis and evaluation of existing methods for collection, quantifying, extrapolating, and reporting of environmental micro- and nanoplastic (MNP) samples, (2) communication of limitations & uncertainties of sampling strategies with respect to relevance and reliability (e.g., collection of samples by trawl vs. grab samples, sample volumes / trawl lengths, etc.), and their representativeness (e.g., limited geospatial or temporal scales) for informing an exposure assessment for the purposes of assessing risk, and (3) further identification and characterization of ancillary data collection and reporting metrics which are required / desired to increase the reliability and utility of existing and planned environmental MNP data (e.g., ocean current / river flow measurements, water chemistry, sampling depth, particle size range of sampler device, etc.) for use in deriving relevant environmental exposure concentrations for use in assessing risk. The RSVP tool can help researchers better analyze, contextualize, and synthesize existing studies as well as aid in the development of more purposeful and powerful environmental sampling studies with minimal user input. The results can be communicated in a consistent, transparent, and reproducible manner.

8.05.B.T Practical, Effective, and Informative Monitoring and Risk Assessment Strategies for Macro- and Microplastics

8.05.B.T-02 Microplastics Composition and Loadings to Lake Ontario from an Urban Creek via Different Sampling Approaches

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Urban streams are a dominant pathway for transporting land-derived microplastics (MPs) to adjacent waterbodies. Throughout a year of sampling, we assessed concentrations, composition and loads of MPs (>20 µm) under high and low-flow conditions in a highly urbanized creek containing a number of plastics-based industries. Sampling was conducted using different approaches to capture particles across a wise size range, including a drift net (250 µm-mesh; n=35), a pump and 50 µm net (n=50), and standard water quality pumping system (ISCO; n=50). The median concentrations of MPs (>250 µm) during rain and dry events were 1.3 items/L (n=25) and 0.08 items/L (n=10), respectively. The corresponding median loads for rain and dry events were 1.8 x 10^3 and 0.26 x 10^3 items/second, respectively, based on the mean discharge values on the sampling date. MP abundances and loads determined on a particle number basis from the pump/50 µm net were up to 100 times higher than measured via 250 µm mesh net. MP composition was different between rain and dry events. Concentrations of rubber particles >125 µm were strongly and positively correlated with stream flow rate ($p<0.05$, $r>0.7$) and thus abundant during rain events. In contrast, plastic pellet concentrations were associated with the first flush during rain events. Concentrations of fragments from commercial plastics activities were not related to flow volume. These results indicate the considerable loads derived from road wear and litter that are mobilized during rain events, but also industrial inputs entering the creek from, likely, direct discharges. The sampling method chosen influences the loads estimate, MP composition and interpretation of MP sources.

8.05.B.T-03 Unraveling the Characteristics and Risk of Microplastics in Tokyo Bay's Surface Water and Sediment

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In recent years, there has been growing concern about the impact of microplastics (MPs) on marine ecosystems. This has led to increased political and social attention both nationally and internationally. Consequently, the number of studies focusing on MPs in the marine environment has steadily increased, covering areas such as environmental monitoring, understanding MPs' behavior in the environment, developing analytical methods, and studying their ecological effects. However, there is a lack of quantitative assessments of MPs that can inform the development of realistic risk-based management strategies. The concentration of microplastics exhibits significant spatiotemporal variability, making it challenging to determine representative concentrations with limited sampling. Additionally, although the amount of monitoring data is

increasing globally, inconsistent methodologies make direct comparison of concentration levels difficult. To address these issues, we analyzed the concentration of microplastics in water and sediments in Tokyo Bay multiple times in 2023 using the same methodology. Our goal was to understand the characteristics and extent of variation in microplastic pollution in Tokyo Bay and to conduct a screening-level risk assessment based on the monitoring data. We used micro-Fourier-transform infrared spectroscopy (micro-FTIR) to characterize MPs larger than 20 microns in surface water and sediment in Tokyo Bay. Our results showed that the particle size distribution, polymer composition, and shape of MPs varied depending on the sampling location, suggesting differences in MP sources. The detailed characteristics of MPs detected in the water and sediments of Tokyo Bay, the challenges of monitoring, and the screening-level risk assessment using species sensitivity distribution (SSD) will be discussed in our presentation. In addition, some measurement results of MPs ranged from 1 to 20 microns by using imaging technique of micro-Raman spectrometer will be introduced.

8.05.B.T-04 Beyond the Surface: An In-Depth Study of Plastic Pollution in Ghana's Coastal Hotspots

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The presence of marine debris on beaches is an unfortunate consequence of human activity. Synthetic materials, particularly non-biodegradable plastics, are among the most common culprits behind the damage to marine habitats and biota. A study was conducted on the coastal beaches of Ghana to assess the pollution levels of the marine hotspots. The study aimed to examine the distribution, seasonal abundance, types, and effects of mesoplastics and macroplastic debris on two coastal beaches located at Tema Fishing Harbour and Elmina Beach in Ghana. Following the OSPAR guideline for plastic with minor adjustments, the investigation focused on monitoring plastic debris along beaches and fishing activities that had substantial human interaction. The results showed a prevalent plastic pollution problem on both coasts, with an average of 400 debris items per 100 meters of shoreline at Tema and Elmina. The most common forms of debris were plastic bags, single-use plastic (pure water sachets), refrigerator insulators, and hard plastic. Additionally, the study revealed seasonal variations in the types and quantities of meso-and macroplastics. Fragments were the primary physical characteristic of mesoplastics, while styrofoam and film were the primary physical characteristics of macroplastics. White, transparent, and black were the most prevalent colours for both macro- and mesoplastics. The findings further indicated that both beaches were unclean as subjected to the Clean Coast Index (CCI). Additionally, the study revealed buried macroplastics due to continuous sand deposition. The polymer types identified were polystyrene, high-density and low-density polyethylene, and polyurethane. The study recommends a comprehensive approach to addressing plastic pollution in Ghana, which includes enhanced waste management techniques, tools, public education, and policy and regulatory enforcement. Frequent beach cleaning is essential to address the issue of buried debris and mitigate the destructive impact of plastic pollution on Ghana's beaches.

8.05.B.T-05 Trash Traps As Monitoring Tools To Measure Baselines, Trends, and Upstream Sources of Plastic Pollution

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Plastic pollution is ubiquitous around the globe. As such, standardized monitoring frameworks are sorely needed to measure baselines, assess ecological exposure and risk, record trends over time, inform policies to prevent and mitigate plastic pollution, and measure the success of solutions. Cleanup technologies, also known as ‘trash traps’, are increasing in popularity to supplement manual cleanup efforts. Trash traps can function 24 hours a day, seven days a week to clean up pollution from hard-to-reach surface waters and can also capture larger-sized microplastics which are difficult to collect by hand. In addition to cleanup, trash traps provide a unique opportunity for monitoring plastic pollution, providing vital data on both macro and larger-sized microplastic waterway pollution, which is currently lacking from global datasets. In 2021 the International Trash Trap Network (ITTN) was formed through a collaboration between the University of Toronto Trash Team and Ocean Conservancy’s International Coastal Cleanup®. The ITTN aims to support new trash trapping programs, harmonize methods for data collection and compile and share global trash trapping data to motivate and inform upstream solutions to prevent plastic pollution. Here we present how current ITTN data collection protocols can be adapted for macro and microplastic monitoring purposes, and how these methods can be used to quantify plastic pollution baselines, determine statuses and trends of plastic concentrations in surface waters and provide data to inform and monitor the efficacy of solutions.

8.05.P-Th Practical, Effective, and Informative Monitoring and Risk Assessment Strategies for Macro- and Microplastics

8.05.P-Th-088 Priorities to Inform Microplastics Management, Monitoring, and Research: A California Case Study

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The state of California, USA, proposes to establish the first statewide microplastics monitoring program designed to inform management decisions to protect aquatic ecosystems from microplastic pollution. However, current scientific understanding of the presence, fate, and impacts of microplastics (including tire-wear particles) lags behind the urgent information needs environmental policy-makers have to manage microplastic pollution. Therefore, it is critically important for microplastics researchers to understand the priority management questions policy-makers and water quality managers have to inform near and long-term environmental policy decisions, and to prioritize research funding and efforts to address these management questions and associated monitoring data needs. We gathered input from federal, state (California), and local government agencies, as well as local water quality managers to understand their priority management questions and information needs on microplastics. We synthesized this diverse input into a comprehensive framework to articulate clear monitoring goals and objectives for a statewide monitoring program. We also identified priority research needs to address priority

management questions. We invite SETAC members and attendees to provide input as well through an on-line questionnaire. Input received during SETAC will be incorporated into the developing California statewide microplastics monitoring strategy. Lessons learned from California's approach may be applicable widely to other governments embarking on developing monitoring strategies to inform microplastics management.

8.05.P-Th-089 EUROpean Quality Controlled Harmonization Assuring Reproducible Monitoring and Assessment of Plastic Pollution (EUROqCHARM)

Bert van Bavel[1], Stefano Aliani[2], Francois Galgani[3], Joan Fabres[4], ***Huiling Liu**[5]*, *Jon Eigill Johansen[5], Ike van der Veen[6] and Amy Lusher[1], (1)Norwegian Institute for Water Research, Norway, (2)Italian National Research Council, Italy, (3)IFREMER, France, (4)SALT, Norway, (5)Chiron AS, Norway, (6)Vrije University Amsterdam, Netherlands*

Plastic pollution has become a global environmental and societal concern in recent years. Numerous protocols have been developed to monitor plastic debris, but these are rarely comparable. This has hindered gathering of knowledge regarding pollution sources, development of monitoring programmes and risk assessments, and implementation of mitigation measures. EUROqCHARM, a European Commission H2020 Coordination and Support Action (CSA) project has addressed this by critically reviewing state-of-the-art analytical methods and, taking harmonisation one step further, by validating them through an interlaboratory comparison (ILC) study. This has brought together key European laboratories and generated capacity for the analysis of plastic pollution in environmental samples. In addition, reference materials were produced to be marketed for at least three of the four target matrices (water, soil/sediment, biota), during and after project completion. EUROqCHARM has ensured project outputs were under discussion during the ratification of monitoring guidelines on national, regional, and international levels, and supported the capacity building in Europe. Furthermore, EUROqCHARM has made some major breakthroughs to support not only the EUs Green Deal and EUs the Zero Pollution Action Plan but also the Global Plastic Treaty. Together with 15 partners and scientific advisory board consisting of international experts EUROqCHARM has established technical readiness of existing methods to measure plastic pollution, taken the lead in producing references materials for microplastic analysis, organised a large scale interlaboratory intercomparing study for the analysis of microplastic in environmental samples and has put significant effort on capacity building including both online and hands-on workshops. Furthermore, EUROqCHARM has worked closely with standardisation organisations (ISO/CEN) and monitoring initiatives (within MSFD-TGML, OSPAR, ICES, AMAP) to harmonise methods for plastic litter.

8.05.P-Th-090 Risk Assessment of Microplastics and Comparison of Species Sensitivity Distribution Methods

***Sara Hutton**, Kenia Whitehead and Philip E Goodrum, GSI Environmental*

Most papers published on ecological risks of microplastics (MP) are based on laboratory studies with limited exposure profiles or field studies that focus on the occurrence of MPs in aquatic organisms. However, the current literature and knowledge regarding MP toxicity is sparse and often challenging to interpret, which can introduce uncertainty when conducting risk assessments (RAs). Most MP RAs currently published use a species sensitivity distributions (SSDs) approach. SSDs are a commonly used method that compare toxicity across species by taking a single point (e.g., NOEC or EC50) from a dose response analysis, however this approach can also underscore the variability and uncertainty in the data. In the past decade, research and regulatory activity directed at supporting MP RAs that utilize the SSD approach have increased. For example, in 2018 the state of California passed legislation requiring a comprehensive literature review to determine if a RA could be conducted for human health and aquatic organisms. It was concluded there was insufficient data to conduct a formal human health RA, but sufficient data were available to generate SSDs based on NOECs or LOECs. To calculate the SSDs, a database called the Toxicity of Microplastics Explorer (ToMEx) was created and has recently been updated. The SSDs calculated with the original ToMEx data were used to define a 5 percent hazard concentration of 5 particles/liter which is cited in the draft 2024 California Integrated Report: Surface Water Quality Assessments as the threshold used to determine listings waterbodies based on MPs. A refinement to the SSD approach is to use the full dose-response curve (DRC) from each study and integrate these curves into one composite DRC. This approach was developed by USEPA's Office of Pesticide Programs to support ecological RAs of pesticides, and subsequently applied to site assessments of legacy contaminants. This method produces a composite DRC and confidence interval that reflects the variability and uncertainty in each study's DRC. Here we compare the threshold values for MPs when SSDs are constructed using the NOEC/LOEC approach and the DRC approach applied to the same dataset. We find that when all study treatments are incorporated into the composite DRC the resulting confidence intervals span many magnitudes more than the SSD approach, this highlights the extent to which the methods used in RA reflect uncertainty which needs to be considered when conducing RAs.

8.05.P-Th-091 Occurrence of Microplastics in Biscayne Bay, Florida: Characteristics and Analytical Methods

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Biscayne Bay is a vital part of the economic and cultural heritage of South Florida, supporting a variety of wildlife and attracting tourists from all around the world. However, the sustenance of the many benefits the Bay provides is dependent on the good water quality. Microplastics are currently raising global concern as an emerging contaminant due to its ubiquitous and persistent nature in the environment. Due to their properties and their large surface area-to-volume ratio, they can also serve as vectors of many organic pollutants. This study utilizes analytical methods to assesses the presence, abundance and characterization of microplastics from ten sites within Biscayne Bay. This study is a first of its kind that assesses the presence and abundance of microplastics in Biscayne Bay in support of a larger hydrodynamic modeling effort. Water samples were collected within Biscayne bay including Snake Creek, Little River and Biscayne Canal using plankton net (335um). It was further isolated using commonly accepted microplastic techniques; filtration, density separation using potassium formate, digestion using hydrogen peroxide and identification will be done using a Shimadzu AIRsight combined Infrared/Raman microscope. Our preliminary bulk analysis revealed the presence of microplastics with density below 1.4g/cm^3, sizes ranging from 300um to 1mm, and an abundance ranging from 10 to 40 particles per cubic meter within the Bay. The findings of this study provide valuable information about the composition and properties of microplastic fluxes into Biscayne Bay from inland anthropogenic sources and highlighting areas of higher microplastic accumulation within the Bay. Finally, the findings of this study combined with results of the modeling contribute to the efforts of policymakers, government agencies, and regulatory bodies in managing the heavily urbanized watersheds along Biscayne Bay.

8.05.P-Th-092 Microplastic Distribution during the Ice and Ice-Free Period in a Rural Headwater Lake, Muskoka, Ontario

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Microplastics are pervasive contaminants of concern owning to the exponential growth in plastic production coupled with the mismanagement of waste. Initial studies primarily focused on the marine environment; however, there is growing recognition of the need to quantify the microplastic cycle and to understand the influence of climatic conditions on microplastic distribution. Studies have suggested that ice formation in marine environments strongly influences microplastic distribution, with concentrations being substantially higher in ice than in the subglacial waters, but the influence of ice formation on microplastic distribution in freshwater lakes is still relatively unknown. In this study, we examined the distribution of microplastics in a rural headwater lake in Muskoka, Ontario, Canada, during the ice-free and ice-covered periods to assess the role of atmospheric deposition as a pathway for microplastic transport to freshwaters. We hypothesized that atmospheric deposition was the primary source of microplastics and therefore snow and ice would have higher concentrations compared to the lake. Monthly lake samples were collected from the centre of the lake during the ice-free period and during the ice-covered period. In addition to collecting under-ice water samples, snow and ice samples (divided into upper and lower layers) were collected from the surface of the frozen lake. Snow, ice, and under-ice lake water samples were also collected for dissolved organic carbon content to assess the source contribution of atmospheric deposition. Results show that microplastic concentrations were significantly higher in snow and ice compared to lake water but similar concentrations were observed in lake water during the ice-free and ice-covered period. Further, during the ice-free period, microplastic concentrations displayed a vertical distribution down the water column with the highest concentration in the hypolimnion potentially reflecting a water density gradient. FTIR analysis is currently underway, but preliminary results suggest that polyethylene terephthalate, polypropylene and polyethylene are the three main plastic types.

8.05.P-Th-093 Raman, SEM/EDS and ICP-OES Instrumentation for Investigating Adsorption Capacity of Airborne Microplastics on Heavy Metals in Coastal Cities of Portsmouth, UK and Lagos, Nigeria

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As emerging pollutant of growing global concern, airborne microplastics and heavy metals pollution are gaining increasing investigation. However, the influence of varying real-time environmental working conditions on the interaction of different atmospheric microplastics and heavy metals is yet to be well understood. In the ongoing study, we are investigating the adsorption capacity of microplastics for heavy metals. This involves an active field sampling and laboratory experiment, and a comparative simulated condition of the Portsea Island atmosphere of Portsmouth in the United Kingdom and those of Lagos Island in Nigeria, West Africa. Both study areas are of international geographical importance for air quality monitoring and climate sustainability use. Different microplastic polymers including polyethylene (PE), polystrene (PS), polypropylene (PP), polyether sulfone (PES) and polyethylene terephthalate (PET) are being investigated with respect to their adsorption capacity with lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), and Aluminium (Al). Whilst micron Raman and SEM EDS are used for polymer identification, morphological and other physicochemical characterisation of microplastics and identification of elemental chemical composition, ICP-OES is used for heavy metals concentrations during simulated adsorption experiment. This will determine the amount of adsorbed heavy metals onto different types of microplastics. Real-time and simulated experimental results under different working conditions including particle size, temperature, exposure time and concentration of heavy. Adsorption coefficient results are modelled using the Freundlich and Langmuir isotherm models to evaluate the interaction of microplastics and heavy metals with respect to the adsorbent surface layers. Furthermore, the potential of microplastics to act as carries in transport of heavy metals and possible health implications in the ecosystem are investigated.

8.05.P-Th-094 Accurate High Throughput Microplastics Characterization on Aluminium-Coated Filter Using the Agilent 8700 Laser Direct Infrared (LDIR) Chemical Imaging System

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Indeed, microplastic pollution has become a significant environmental concern due to its potential impacts on ecosystems and human health. The Agilent Laser Direct Infrared (LDIR) chemical imaging system represents an innovative approach to imaging and spectral analysis, particularly in the context of microplastic identification and other applications that require chemical characterization of materials. Depending on the level of suspended solids presents, ASTM D8333 describes the steps required for microplastics sample preparation. Regardless of analysis technique used, samples with various levels of microplastics eventually require a filtration step. At present, the Agilent 8700 LDIR microplastics sample introduction can be achieved directly on gold-coated filters. To minimize the cost associated with gold-coated filters while maintaining easy and efficient sample introduction method, accurate microplastics characterization with the 8700 LDIR can be performed on aluminum-coated polyester filters. This study outlines the utilization of the Agilent 8700 LDIR in achieving accurate microplastics characterization on aluminum-coated filters in terms of particles detection, particle count repeatability, particle size accuracy and identification of common microplastics.

8.06.T SETAC-ACLCA Special Session: Nuts and Bolts of LCA

8.06.T-01 The Nuts and Bolts of LCA

***Christoph Koffler**, Sphera Solutions*

8.07.P-We Urban Streams: From Contaminants to Restoring Lost Ecosystem Services

8.07.P-We-193 Nitrogen Source Apportioning in the Tampa Bay Watershed Using Organic Chemical Tracers

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Excessive nutrients are a leading cause of ecosystem impairment in Florida's coastal waters. Nutrients promote algal growth, which can contribute to diminished water clarity, seagrass population decline, marine life deaths, and illness in humans. In nearshore waters off Florida's central Gulf Coast, land-based sources of nutrients help fuel and sustain red tides, resulting in fish kills and asthma-like symptoms in residents. Nutrients originate from a variety of human sources such as wastewater, stormwater runoff, yards, industry, commercial farms, and ranches. This study employed target and nontarget analyses to identify unique chemical tracers useful in apportioning nitrogen back to the specific sources in the Hillsborough River. The Hillsborough River discharges to Hillsborough Bay, one of several bay segments in Tampa Bay, which has one of the highest nitrogen allocations (39%) and in recent years has seen significant reduction in seagrass. Chemical tracers were used in conjunction with nutrients (total nitrogen (TN), nitrate (NO_2^-), nitrite (NO_3^-), ammonia (NH_4^+), total phosphorus (TP), total organic carbon (TOC), dissolved organic carbon (DOC), and silica (SI)) to identify sources of nitrogen entering the river. The chemical tracers were selected to represent different water sources, including treated and untreated wastewater (sucralose, carbamazepine, triclosan, oxybenzone, caffeine, avobenzone, triphenyl phosphate, 4-nonyphenols, etc.) and stormwater and agriculture runoff (6PPD, 6PPD-Quinone, fipronil, atrazine etc.). The analytical method relied on online solid phase extraction (SPE) high-performance liquid chromatography (HPLC) coupled to heated electrospray ionization (HESI) on a TSQ Altis™ triple quadrupole mass spectrometer in selected reaction monitoring mode (SRM). Method detection limits were defined for each compound (<15 ng/L) and quantification was performed on TraceFinder™ EFS 5.1. The concentration of the chemical tracers was determined in surface water samples from 23 locations along the river, with each compound's suitability as tracers assessed. Correlations between the concentrations of chemical tracers and nitrogen (0.2-1.4 mg/L) were explored, and Principal Component Analysis was used to prioritize locations to guide management efforts. The combined datasets offer improved spatial and temporal precision in pinpointing nitrogen source hotspots, aiding in pollution control in the broader Tampa Bay ecosystem restoration efforts.

8.07.P-We-194 A Novel Tool for In-Stream Continuous Water Quality Monitoring

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Continuous water quality monitoring in streams is challenging due to changes in discharge, accessibility, and disturbance. In this presentation, we describe a modification of an in-situ device that integrates sensors to measure water quality and estimate primary productivity. We modified a mussel silo, a portable cage used to house juvenile freshwater mussels, frequently used to evaluate in situ exposures to contaminants. Mussel silos are used to assist in the habitat quality determination of potential reintroduction sites for restoration and remediation projects. To assess in-stream water quality, we integrated dissolved oxygen, temperature, and conductivity sensors into the silo design so that the sensors would provide continuous water quality data from water at the sediment/water interface. A pressure sensor was integrated to record continuous water level data at each deployment location. Additionally, we used a Bayesian model, using data from the sensor silos, to predict gross primary productivity, ecosystem respiration, and net ecosystem production in the system to provide information about stream quality and energetic input for aquatic invertebrates, including freshwater mussels. In 2023, we deployed four sensor silos in an urban stream near Detroit, Michigan, which historically had high levels of anthropogenic activities and varying water quality issues. We also compared sensor data to water quality data from surface-level water obtained at two-week intervals. Generally, sensor data was similar to surface-level water samples. These sensor silos have been collecting continuous information and will be used to measure stream water quality throughout the year. Furthermore, we are deploying additional sensor silos to examine the water quality of other restoration sites in coal mining sites in Pennsylvania, metal-affected areas in Oklahoma, and mountain streams in Oregon.

8.07.P-We-195 Integrated Tools for Urban Stream Assessment: Evaluating Habitat for Mussel Restoration

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Urban streams have disproportionately received a wide range of stressors that have adversely affected invertebrate habitat. Native freshwater mussels have declined in urban streams due to stressors such as habitat alteration and contaminants. These declines have altered habitat functionality through the loss of ecosystem services they provide, such as water quality improvements, sequestration/processing of nutrients, stream stability, and diverse food web structure. This presentation describes an effort to assess an urban watershed to determine the suitability of areas for mussel restoration. In this study we examined the Clinton River, located within an urban watershed near Detroit, Michigan, USA, with varying water quality issues, strong urban influence, and wastewater input. In the initial phase of the study, we conducted a field study that integrates water quality, habitat conditions, and in situ mussel exposures to assess the potential role of contaminants and habitat stressors on the suitability for mussel restoration and potential uplift. The field study incorporated measures of sediment and water contaminants, water quality analysis, passive samplers, water quality sensors housed within a silo, and monitoring native mussels placed in situ. Fatmucket, *Lampsilis siliquoidea*, (13 mm) were placed in silos in the river for 78 days at five different sites with existing mussel beds to assess their

survival and growth. Additionally, every two weeks, we visited the silos to collect water samples for water quality and particle count analyses. Mussel survival was high, >93%, across all sites, and mussel growth averaged 0.6-2.5 mm, depending on the site. These data will be used to inform the placement of mussels in the river in 2024 and to predict the likelihood of restoration success. Ultimately, we seek to re-establish diverse representative mussel assemblages and evaluate the resulting ecosystem services provided by freshwater mussels including the impacts on water quality and increased macroinvertebrate diversity.

8.07.P-We-196 Monitoring PFAS in the Delaware River and Tributaries to Reduce Loading and Ease Burdens on Drinking Water Systems

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The Delaware River Basin, particularly the urban corridor from Trenton, NJ, to Wilmington, DE, is home to historical and ongoing PFAS research and manufacturing. This river corridor is, therefore, contaminated with PFAS from many dischargers while also being the drinking water source for millions of people. With recently introduced regulations, drinking water systems face the prospect of implementing expensive PFAS treatment technologies to achieve compliance. The Delaware River Basin Commission (DRBC) has been monitoring PFAS in the river and its tributaries for ~20 years, establishing a long-term dataset on its occurrence. Data on PFAS concentrations are typically one-time grab samples from monitoring efforts over the years, providing only a snapshot of concentrations at that point in time. In the summer of 2023, DRBC deployed polar organic chemical integrative samplers (POCIS) at 7 sites from Trenton, NJ, to Marcus Hook, PA, to determine time-weighted averages of PFAS over a one-month deployment. The POCIS samples will enable comparisons with grab samples to better assess their representativeness of PFAS in the river and tributaries. Grab samples show that concentrations of PFAS generally increase as water moves downstream along this stretch of the river, although there can be large variations across sampling events. However, POCIS analysis is not yet complete and will be presented at SETAC. The data generated from this POCIS effort, as well as the ~20 years of previous data, is being used by DRBC to develop a PFAS roadmap to identify sources of these pollutants in the Delaware River and tributaries so that those sources can be mitigated before the full implementation of regulations on PFAS in drinking water.

8.07.P-We-197 Environmental Fate of SSRIs in Urban Environments that Receive Municipal Wastewater Effluents

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Municipal wastewater treatment plants (WWTPs) have been identified as a source of a broad suite of substances including pharmaceuticals and personal care products (PPCPs) to aquatic ecosystems. One class of pharmaceuticals of concern are the widely prescribed antidepressants known as selective serotonin reuptake inhibitors (SSRIs). Increased usage, continuous consumption, and release have contributed to their pervasiveness such that SSRIs have been reported in wastewater effluents (WWE) and receiving waters globally. However, much less is known regarding whether SSRIs affect wild biota chronically exposed to WWE. During the 2023 field season, >180 samples, including WWE, biota from multiple trophic levels (plants to fish) and habitats (pelagic & benthic) as well as environmental samples were collected from an upstream habitat and areas immediately downstream of 2 WWTPs. Selected SSRIs (and breakdown products) including Citalopram (Desmethyl-Citalopram, Didesmethyl-Citalopram) Sertraline (Desmethyl-Sertraline), Fluoxetine (Norfluoxetine), Paroxetine, as well as Venlafaxine were quantified (n=5/type/site) in surface water, suspended particles, sediments, periphyton, macrophytes, benthic invertebrates, crayfish, freshwater mussels, and 2 small-bodied fishes. Extracted samples were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). A C18 column was used for separation with detection by electrospray ionization MS and calibration employed isotopically labelled standards. Overall, PPCP concentrations were higher in water, suspended particles, and sediments collected downstream of WWTPs than upstream. For instance, water concentrations of the 2 most prevalent SSRIs, Citalopram and Sertraline were 0.21 (±0.06) and 0.07 (±0.02) µg/L, respectively and Venlafaxine was 0.41 (±0.09) µg/L downstream of a WWTP, and all upstream samples were below the limit of quantification. Similarly, Citalopram and Sertraline associated with suspended particles were 87.8 (±17.4) and 32.5 (±7.21) ng/g, respectively and Venlafaxine was 42.3 (±14.5) ng/g downstream of a WWTP, and all upstream samples were <1 ng/g. The concentrations of SSRIs and Venlafaxine in biota are pending. Levels of PPCPs in biota will be examined with respect to their stable isotope signatures to explore relationships between trophic levels and ecosystem components. This study's findings will expand our understanding of the environmental fate of SSRIs and Venlafaxine in aquatic ecosystems.

8.08.P-We What Is Regenerative Agriculture and How Can Soil Health Be Improved in Agricultural Landscapes?

8.08.P-We-198 Assessing the Suitability of Alternative Organic Matter Materials for Peat Replacement in Standard Artificial Soil Formulations

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Artificial soil prepared from well-standardized and easy-to-obtain materials has been used in regulatory ecotoxicology and in general chemical toxicity testing with soil organisms since 1984. Such standardization allowed the comparability of toxicity data among multiple chemicals and test species. Formulated standard artificial soil (SAS) consisting of 10% finely ground Sphagnum sp. peat, 20% kaolinite clay, approx. 69% quartz sand and approximately 1% of $CaCO_3$ to adjust pH to approximately 6.0 is often used for assessing the toxicity of chemicals to earthworms (OECD 222 or ISO 11268-2), enchytraeids (OECD 220 or ISO 16387) and Collembola (OECD 232 or ISO 11267). Recently, however, the use of peat in the SAS formulation has become less advisable for a variety of reasons, including the need to reduce peat exploitation and the degradation of peatlands, and to decrease anthropogenic carbon dioxide emissions from degraded peatlands. Therefore, we are conducting toxicity studies with the earthworm *Eisenia andrei* to assess the utility of two potential organic matter alternatives to peat, including rye (*Secale cereale*) straw and timothy grass (*Phleum pratense*) hay. These studies include exposing earthworms to a reference chemical, boric acid, in SAS (containing 5% peat) and in formulated soils that use 5% rye straw or timothy hay as organic matter components.

When completed, these studies will provide critical information on the utility of the two peat replacement alternatives in soil toxicological assessments.

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