

ABSTRACT BOOK

SETAC NORTH AMERICA 44TH ANNUAL MEETING

12–16 November 2023 | Louisville, KY, USA

“One Environment. One Health.”



Abstract Book

SETAC North America 44th Annual Meeting

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This book comprises the abstracts from the 44th annual meeting of the Society of Environmental Toxicology and Chemistry – North America (SETAC North America), conducted from 12–16 November 2023 in Louisville, KY, 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.

If you have training in these or related disciplines and are engaged in the study, use or management of environmental resources, SETAC can fulfill your professional affiliation needs.

All members receive the SETAC Globe newsletter highlighting environmental topics and SETAC activities, reduced fees for meetings and discounts on SETAC books. All members receive online access to *Environmental Toxicology and Chemistry* (ET&C) and *Integrated Environmental Assessment and Management* (IEAM), the peer-reviewed journals of the society. Members may hold office and, with the Emeritus Members, constitute the voting membership.

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Track 1: Environmental Toxicology and Stress Response

1.01.P-Mo Advances in the Photo-Induced Toxicity of Environmental Contaminants

1.01.P-Mo-001 Combined Effects of Crude Oil, Dispersant, and Ultraviolet Radiation on Marine Organisms

Alexis Khursigara¹, Rachel R. Leads¹, Jeffrey M. Morris², Fabrizio Bonatesta², Claire Lay² and Aaron P Roberts¹, (1)University of North Texas, (2)Abt Associates

Oil spills are environmental events that introduce polycyclic aromatic hydrocarbons (PAHs) into aquatic environments. These events can have detrimental impacts on aquatic organisms, with early life stages generally being the most sensitive. In the presence of ultraviolet (UV) radiation, PAHs can become more toxic through the process of phototoxicity. Early life-stage organisms are especially sensitive since they lack UV-blocking pigmentation. Dispersants such as Corexit 9500 can be applied to break up oil slicks and speed up oil degradation. However, dispersants can increase the bioavailability of PAHs and can be toxic on their own. While previous work has examined the effects of dispersants on crude oil toxicity, less is known about the effects of dispersants on phototoxicity. This study examines the effects of crude oil, UV radiation, and dispersants on survival in early-life stage marine organisms. Chemically enhanced water accommodated fractions (CEWAFs) were generated to acutely expose juvenile mysids (*Americamysis bahia*) and larval red drum (*Sciaenops ocellatus*) to crude oil, dispersant, and UV A radiation in a full factorial design. To determine how varying concentrations of dispersant-to-oil ratios can impact bioavailability and toxicity, multiple dispersant loading rates were assessed. Both mysids and red drum showed a significant decrease in survival when co-exposed to crude oil and UV. The addition of dispersants further decreased mysids and red drum survival, with the greatest reductions observed in the presence of UV and at the highest dispersant-to-oil loading rates. This study highlights the need to test the effects of dispersants in the presence of UV and that the impact of dispersants under environmentally realistic exposure scenarios should be used to inform oil spill response.

1.01.P-Mo-002 Factors Affecting Photo-Induced Thin Oil Sheen Toxicity in a Model Early Life Stage (ELS) Fish (*Danio rerio*)

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In the aquatic environment, organisms may be exposed to crude oil in several different ways including dietary exposure, uptake of dissolved constituents, contact with oil droplets in the water column, or contact with thin oil sheens concentrated on the surface of the water. Thin sheens or slicks are thin layers of oil (~1 μm thick) that float on the surface of the water and are formed during oil transport and weathering processes in the environment. For early life stage (ELS) and planktonic organisms that occupy surface waters, thin sheens may be an important and under-studied route of oil exposure. In addition, ultraviolet (UV) light exposure in surface waters may enhance the toxicity of thin sheens to aquatic organisms due to photo-induced toxicity of oil constituents. Laboratory tests indicate that thin oil sheen and UV co-exposure is acutely toxic to several ELS fish and invertebrate species. However, toxicity data are currently limited and exposure mechanisms (e.g., physical contact with the sheen or exposure to underlying water) require further study. To better understand these mechanisms, the present study investigated thin sheen toxicity and UV co-exposure using ELS (4-96 hours post-fertilization) zebrafish (*Danio rerio*) as a model organism. Thin sheens and UV co-exposure significantly decreased survival of ELS zebrafish ($p < 0.0001$). At 96 h, percent survival (mean \pm SE) of ELS zebrafish exposed to thin sheens in the absence and presence of UV was $90.0\% \pm 10.0$ and $10.0\% \pm 5.0$, respectively, indicating an effect of photo-induced toxicity. With thin sheen and UV co-exposure, percent survival also varied significantly with the volume of water underlying the sheen. Percent survival (mean \pm SE) of ELS zebrafish co-exposed to thin sheens and UV in 200 mL of water ($71.0\% \pm 12.5$) was significantly lower than those exposed in 400 mL ($92.0\% \pm 2.0$) or 800 mL ($95.0\% \pm 2.2$) of water ($p = 0.0003$), suggesting that the

concentration of dissolved constituents underlying the sheen influence exposure and toxicity. Together, these results provide novel data on the toxicity of thin sheens and UV to a model fish species as well as new insights into the mechanisms of thin sheen exposure in aquatic organisms.

1.01.P-Mo-003 Surface Water Stressors and a Mechanism of Avoidance in Pelagic Fish Embryos

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Pelagic fish embryos are thought to float in or near surface waters for the majority of their development and are presumed to have little to no control over their mobility. However, we have shown that pelagic embryos of marine fish possess an apparently adaptive UV avoidance response. Stressors occurring in surface waters, such as increased temperature, cardiotoxic oil slicks, and ultraviolet radiation (UVR), pose a serious threat to the fitness and survival of the vulnerable early-life stages of fish. This is especially important when considering the interactive effects of stressors, such as photo-induced toxicity—a phenomenon in which the toxicity of a compound is amplified after exposure to certain wavelengths of light. Therefore, a mechanism in which embryos can alter buoyancy and thus control their vertical position in the water column may be indispensable to the sustainability of these fisheries. Co-exposure to crude oil and other environmentally relevant stressors, such as increased temperatures and UVR, induced an early onset of negative buoyancy in developing fish embryos. Further, recovery of positive buoyancy was observed once the exposure period was completed and all stressors had been terminated, indicating this response is highly dynamic and not solely pathological. To understand more about the energetic requirements of this process, oxygen consumption and yolk sac depletion were measured in developing embryos exposed to multiple stressors known to affect buoyancy. Specific gravity of control versus UVR exposed embryos were measured using density gradient columns. To test the ecological relevance of this mechanism, the Connectivity Modeling System (CMS) was employed to track the transport of mahi-mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*) embryos and investigate how measurements of specific gravity collected in a laboratory setting translate to changes in the vertical position and UVR exposure of embryos in nature. Buoyancy modification due to UVR exposure resulted in embryos positioned significantly deeper in the water column with reduced UVR exposures, illustrating the ecological significance of this mechanism. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

1.01.P-Mo-004 Reduced Survival Rate of Juvenile and Larval Eastern Oyster (*Crassostrea virginica*) and Changes in Cardiac Activity of Cell Cultures Exposed to Photodegraded Anthracene Suggest Phototoxic Effects

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The study of photo-degraded polycyclic aromatic hydrocarbons (PAHs) represents the next step in PAH toxicity research. Solar irradiation at the ultraviolet wavelengths cause PAHs to degrade into various compounds, some of which exhibit higher toxicities than that of the parent PAH. Therefore, a complete toxicity profile of PAHs should include an investigation into the toxicity of photo-exposed PAHs. PAH emulsions were produced using DMSO and ethanol respectively as the solvent and then diluted in deionized water. Subsequent photodegradation was performed using the Suntest CPS+ for 0, 0.5, 1, and 2 hours, with the 0-hr timepoint representing unexposed anthracene. Juveniles (1-4 mm shell length) and larvae (immediate post-fertilization) exposed to the anthracene treatments (1 ppm) experienced higher mortality rates than those in the control group, with raw anthracene being associated with greater mortality than the oysters in both the 1 and 2-hr degraded anthracene treatments. However, those exposed to anthraquinone (1 ppm) experienced the highest mortality of all, being significantly more lethal than any other treatment. Primary cardiomyocyte (heart cell) cultures exposed to 0.1 ppm anthracene or 1 ppm anthraquinone directly in the cell culture medium for 12 hours did not show a significant change in cardiac activity compared to those of the control group, but those exposed to the

anthraquinone treatments exhibited elevated beating rates at the 0, 0.5, and 1-hr degradation time points. GCMS analysis of photo-degraded anthracene confirms the evolution of anthraquinone in the mixture, but at very low concentrations. This suggests that it is not enough to merely consider the individual species of chemicals evolved during photo-degradation because the concentrations of the various compounds post-degradation cause the photo-toxicity of some PAHs to be non-linear and inconsistent.

1.01.T Advances in the Photo-Induced Toxicity of Environmental Contaminants

1.01.T-01 Recommendations for Advancing Test Protocols Examining the Photo-Induced Toxicity of Petroleum and Polycyclic Aromatic Compounds

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Photo-induced toxicity of petroleum compounds and polycyclic aromatic compounds (PACs) is the enhanced toxicity caused by their interaction with radiation and occurs by two distinct mechanisms: photosensitization and photomodification. Laboratory approaches for designing, conducting, and reporting of photo-induced toxicity studies were reviewed and recommended to enhance the original Chemical Response to Oil Spills: Ecological Research Forum (CROSERF) protocols which did not address photo-induced toxicity. Guidance was provided on conducting photo-induced toxicity tests, including test species, endpoints, experimental design and dosing, light sources, irradiance measurement, chemical characterization, and data reporting. Because of distinct mechanisms, aspects of photosensitization (change in compound energy state) and photomodification (change in compound structure) were addressed separately, and practical applications in laboratory and field studies and advances in predictive modeling were discussed. Photo-induced toxicity is not limited to petroleum compounds. The CROSERF modernization recommendations can be and should be adapted to suit the needs of investigations into other environmental contaminants. Photo-induced toxicity has been reported in natural plant compounds (e.g., cercosporin), pesticides (e.g., erthrosin B), nanoparticles (e.g., anatase TiO₂), metalloporphyrins (e.g., tin protoporphyrin), and pharmaceuticals (e.g., ketoprofen). Comparability of studies and gaps in data reporting limit the utility of many studies in the literature and remain an obstacle to inclusion in formal risk assessments of potentially photoactive environmental contaminants. Greater standardization of experimental designs and data reporting can only serve to make studies more comparable, advance the field at a greater rate, and reduce uncertainties in risk assessments.

1.01.T-02 What's Light Got to Do with It? The Combined Impacts of Malathion and Ultraviolet Radiation on Early Life Stage Zebrafish and Purple Urchin

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Malathion is an organophosphate pesticide that is globally utilized protect nearly 100 different agricultural commodities in addition to its use as an industrial pesticide to control populations of mosquitoes, which involves spraying the insecticide directly onto bodies of water to kill aquatic larvae- any aerosolized particles eliminates airborne adults. This study investigates the joint effects of exposure to malathion and ultraviolet radiation (UVR) on early life stage zebrafish and purple urchin. Early life stage aquatic organisms are most vulnerable to phototoxicity effects, due to their limited mobility, transparent bodies, and underdeveloped methods of defense against chemical exposure and environmental stressors. Further, malathion can convert into a more toxic intermediate, malaoxon, through sunlight exposure and subsequent oxidation, and while malathion exposure has been studied in aquatic organisms, studies on the joint effects of malathion and UV radiation exposure are lacking. To address these knowledge gaps, recently fertilized embryos were exposed to environmentally relevant levels of UVR or visible light only combined with malathion for three days and then transferred to ambient conditions. Oxidative damage is assessed through reactive oxygen species (ROS) production and DNA damage using the comet assay, while developmental and cardiac abnormalities are

assessed through microscopy. Finally, to investigate impacts on energy and metabolism, respirometry, yolk sac depletion, and nitrogenous waste excretion trials were conducted.

1.01.T-03 Photo-induced Toxicity of Tire Wear Particle Leachate on Early Life Stage Estuarine Fishes
Kerri Lynn Ackerly, Tamara Rivera, Kathleen Roark, Andrew Esbaug and Kristin Nielsen, University of Texas at Austin

Tire wear particles (TWP) washed into nearby surface waters by storm events have been linked to recurrent large-scale fish kills in highly urbanized watersheds of the Pacific Northwest region of the United States. TWPs leach a complex mixture of chemicals into waterways which may accumulate in aquatic biota. A subset of the chemicals released by TWPs are known or suspected to be photodynamic, indicating that they may become orders of magnitude more toxic in the presence of solar radiation. This mechanism of toxic action is particularly relevant to transparent aquatic organisms (e.g., early life stage fish) in urbanized aquatic environments that experience intense solar radiation, such as estuaries in the Gulf of Mexico (GoM), which provides critical nursery habitats for many economically and ecologically valuable fish species. Data are currently limited on the occurrence of TWP-related contamination within estuaries, especially with regard to their potential to impact survival of early life stage (ELS) fishes via photo-induced toxicity. To fill this gap, we exposed red drum (*Sciaenops ocellatus*) and sheepshead minnow (*Cyprinodon variegatus*), to environmentally relevant concentrations of TWP leachate under ambient indoor lighting or simulated solar radiation (as UV-A). Studies were conducted in a fully factorial manner under controlled laboratory conditions, with impacts on hatch and survival evaluated at 24 and 48 hours. Here, we report our findings with regard to the potential phototoxic impacts of TWP on the survival of two species of ELS estuarine fish found throughout the GoM and Atlantic coast. 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.

1.01.T-04 Lethal and Sub-Lethal Effects of the Photo-Enhanced Toxicity of Diluted Bitumen and Conventional Heavy Crude Oil on *Hyaella azteca* and Wild Fathead Minnows (*Pimephales promelas*)

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Canada is one of the top oil producers globally with approximately 10% of the world's oil reserves. Crude oils and diluted bitumen (bitumen diluted with natural gas condensates) are the major products of the Canadian oil sands region. Potential environmental impacts of oil spills are a concern for the public and the oil industry. Pipelines are statistically the safest transportation method for oil in Canada. The rate of crude oil and diluted bitumen (dilbit) spills has declined over the past decade, however, many pipelines and proposed pipelines cross freshwater, and more knowledge is required about how oil behaves in these systems. Previous studies have evaluated the toxicity of crude oils and dilbit, but many overlook the potential for photo-enhanced toxicity of oil constituents, specifically polycyclic aromatic compounds. Photo-enhanced toxicity is a synergistic interaction between the toxicity of a contaminant and UV radiation. This phenomenon can increase the toxicity of a contaminate anywhere between 2-1000 times. The Freshwater Oil Spill Remediation Study (FOReSt) at the IISD-Experimental Lakes Area was designed to study the effects of oil spills in a shoreline environment and compare the efficiency of non-invasive methods to remediate freshwater shorelines after oil spills. These secondary remediation measures included engineered floating wetlands, nutrient enhanced monitored natural recovery, and COREXIT EC9580A shoreline cleaner. This study was conducted with cold lake blend dilbit in 2019 and conventional heavy crude oil in 2021. To examine the photo-enhanced toxicity of these oils, separate studies were conducted where early-life stages of *Hyaella azteca* were exposed to water accommodated fractions (WAFs) of dilbit and crude oil, and fathead minnows to dilbit from oil weathering experiments and shoreline enclosures from the FOReSt study. The test was then duplicated to expose individuals to low (15%)

and high (90%) UV exposures. Mortality was documented throughout 5-day (*H. azteca*) and 7-day (fathead minnow) exposures, and photographs of living individuals were taken at the end. Using the photos, growth and malformation analyses were conducted to identify and categorize sub-lethal impacts of remediation measures and photo-enhanced toxicity. Overall, both dilbit and crude oil exhibited photo-enhanced toxicity to *Hyalella azteca* as indicated by increased mortality and deformities, and decreased growth.

1.01.T-05 The Mitigating Effect of Photodegradation (Direct and Indirect) on The Photoenhanced Toxicity of Organic Chemicals

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Light can have a significant impact on the fate and effect of chemicals in the environment, both in terms of its role in degradation as well as in photoenhanced toxicity. The role that light co-exposures play is complex and dependent on local site conditions (e.g., light attenuation), light intensity and spectral profiles, as well the light absorption spectra of the substances of interest. Recent development of mechanistic models for photodegradation (APEX) and phototoxicity (photoTLM) provide an improved technical basis for quantitatively evaluating the relative role of photodegradation and phototoxicity in substance risk assessments which served as the aim of this study. Photodegradation was evaluated as the combination of direct (photon absorption and quantum yield), and indirect photolysis (e.g., formation of radicals as light interacts with DOC and major ions in water) as modeled with the APEX tool. The relative impact of light on test substance exposure was compared to the a “dark” condition which included only transformation due to biodegradation, and dilution assuming a default hydraulic residence time associated with large rivers in Europe. The relative impact of light on toxicity was evaluated using the molar photon absorption of the test substance and the assumed empirical modeling parameters specified in the photoTLM. The relative magnitude, of these independent ratios (exposure / toxicity) provide a quantitative basis for evaluating the role that light co-exposure exerts on the estimated risks associated with organic chemicals. This analysis was performed on a range of chemicals with different chemical properties and susceptibility to photoreactivity and biodegradability. Our results show that in general fast photodegradation rates which reduced exposures compensate for the enhanced phototoxicity of light absorbing organic chemicals. Results also show the sensitivity of this general conclusion to seasonal variation in light intensity and spectral profiles, as well the magnitude of short term phototoxicity relative to long term chronic effect thresholds. We conclude that use of chronic toxicity thresholds in generic risk assessments that ignore light interactions and are much simpler to perform in practice will be protective of risks that results when light exposures are taken into account.

1.01.T-06 Determining the Impact of Ultraviolet Light on the Toxicity of Individual Polycyclic Aromatic Compounds and Crude Oil to American Lobster Larvae (*Homarus americanus*)

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Advances in analytical chemistry techniques have allowed for the measurement of oxidized products formed during oil spills following UV exposure. These photo-oxidized products represent a significant weathering pathway, also known as photo-modification, but little is known of their toxicity. Previous studies have focused on pre-exposing organisms to polycyclic aromatic compounds (PACs) or crude oil, and subsequently exposing those organisms to UV light, also known as photo-sensitization. The objective of this study is to better understand the toxicological significance of photo-modification and photo-sensitization for species of cultural and commercial significance. Stage I American lobster larvae (*Homarus americanus*) have a transparent carapace and are found near the waters surface which make them a good candidate species for UV studies. Three individual PACs (fluoranthene, pyrene, and dibenzothiophene), 3 different crude oils, and 14 low-sulfur fuel oils were selected for these studies. UV exposures were conducted using a full solar spectrum lamp, and

UV dose was measured using both chemical actinometry and a radiometer. Exposure concentrations of single PACs were maintained using a PDMS O-ring based passive dosing system, and the PAC and photo-oxidation products were measured using GC/MS. Crude oil exposures were conducted by generating water accommodated fractions (WAFs) of crude oil and exposing select WAFs to UV light while mixing. Changes in WAF chemistry due to UV light exposure were quantified using BE-SPME, GC/MS of PACs in solution, GC/GC of the oils, and TOC measurements. Low, medium, and high UV doses were generated by manipulating irradiation time while keeping the UV light intensity constant. For the photo-sensitization studies, lobster larvae were allocated into the test solution and then were left in the dark to accumulate PACs for 24hrs, after which the larvae were exposed to 0, 3, 6, or 12 hours of UV light, and then assessed for immobilization and mortality during the subsequent 48 hours. The photo-oxidation of single PACs used in this study had no notable impact on the survival of stage I lobster larvae, however photo-oxidation of crude oil WAFs led to increased toxicity. All PACs and WAFs exhibited photosensitization based toxicity regardless of the UV dose. The data generated in this study will be used to validate the phototoxic-target lipid model and to further our understanding of the impact of UV light during a crude oil spill.

1.02.P-Th Alternative Approaches to Animal Testing: Exploring Approaches and Avenues for the Future Ecological Risk Assessments

1.02.P-Th-001 Transcriptomics-Based Points of Departure for *Daphnia magna* Exposed to Per- and Polyfluoroalkyl Substances

Monique Hazemi, Daniel L. Villeneuve, Kendra Bush, Kevin Flynn, Brett R Blackwell, Michelle Le, Emma Stacy, Sarah Kadlec, Joshua Harrill and Felix Harris, U.S. Environmental Protection Agency

Growing evidence suggests short-term transcriptomic responses to chemical exposure can effectively predict concentrations at which much longer term, chronic toxicity can be detected. Consequently, high throughput transcriptomics assays employing human cell lines have been used to screen a library of over 140 Per- and polyfluoroalkyl substances (PFAS) and identify concentrations at which they can produce a concerted molecular change. However, it is uncertain if points of departure derived from mammalian models will be protective of non-mammalian species. In this study, *Daphnia magna* were exposed for 24 hours, in concentration response, to 22 PFAS in a 96-well plate format. Whole bodies were homogenized, and homogenates from five individuals were pooled. RNA was extracted from three pools per treatment and analyzed by RNA sequencing. Concentration-response modeling was applied to the gene expression datasets using BMDExpress to calculate a benchmark dose for each gene for which a concentration-response curve could be fit. Transcriptomic-based points-of-departure (tPODs) were then calculated as the 10th percentile of the distribution of gene-specific benchmark doses. tPODs were able to be derived for 20 out of 22 PFAS tested. When compared to EC50 values derived from *Ceriodaphnia* guideline-like toxicity tests (n=7) and human cell line-derived tPODs (n=13) from same chemical exposures, the tPODs in this *Daphnia magna* study were consistently protective. *Daphnia magna* tPODs were lower than both apical effect concentrations and human cell-line tPODs, suggesting that ecologically-focused, high throughput assays should be conducted independently from mammalian assays to be protective of non-mammalian species. The results from this study suggest that tPODs derived from short-term high throughput assays can effectively predict chronic toxicity and enable more efficient PFAS hazard designations. *This abstract neither constitutes nor necessarily reflects USEPA policy.*

1.02.P-Th-002 Initial Investigation of the Mysid Shrimp Molting System for the Evaluation of Endocrine Disrupting Compounds

Dalton Scott Allen, Marlo K Sellin Jeffries and Becca Bradley, Texas Christian University

There is a need to develop alternative strategies for the assessment of endocrine disrupting compounds (EDCs) in aquatic environments. Though a variety of methods currently exist, many are time intensive, costly, or

employ the use of vertebrate test models. Furthermore, nearly all existing in vivo methods make use of freshwater fish species despite the potential for endocrine disruption in estuarine and marine systems. Thus, the goal of this project was to explore the use of the mysid shrimp (*Americamysis bahia*) for the assessment of endocrine disruption in marine systems. Though this crustacean does not possess vertebrate steroid hormone signaling systems, it does possess its own suite of steroid hormones including ecdysteroids, which regulate growth, molting, and reproduction. Because ecdysteroids are synthesized from cholesterol and have similar chemical structures to vertebrate steroid hormones, we hypothesized that EDCs known to interact with vertebrate steroid hormone receptors also interact with mysid steroid hormone receptors. To address this hypothesis, mysid shrimp were exposed to various concentrations of ponasterone A (a known ecdysteroid receptor agonist), testosterone, and 17 β -estradiol for 14 days. On days 7 and 14 of the exposure, mysid growth and development were assessed and the expression of molt- and reproduction-related genes was measured. The results of this study will be detailed and recommendations regarding the use of mysids as a potential screening tool for both vertebrate and invertebrate endocrine disruption will be discussed.

1.02.P-Th-003 Advancing Alternatives in Marine Toxicity Testing: Can Fish Embryo or Mysids Be Used as Replacements For Fish Larvae?

Katie Solomons, Dalton Scott Allen and Marlo K Sellin Jeffries, Texas Christian University

The EPA mandated toxicity testing of marine effluents mitigates the risk of contamination in marine environments from oil refinery effluents, major oil spills, and wastewater runoff. Acute toxicity of marine effluents is currently evaluated using the larval growth and survival (LGS) test, using either the sheepshead minnow (SHM) or inland silverside (INS). The use of larval fish represents an animal welfare concern, in light of recent legislation and the 3Rs of animal research. Two potential alternatives to the LGS test are the fish embryo toxicity (FET) test (which uses an unprotected life stage thought to experience less pain than larval fish) and the mysid growth and survival (MGS) test (which uses invertebrates). The objective of this study was to determine if the FET and/or MGS tests produce similar toxicity values (e.g., LC₅₀s, EC₂₅s, etc.) to the LGS tests. All tests were run using phenanthrene, an environmentally-relevant component of crude oil. The results showed that the FET tests had significantly higher LC₅₀ values than all of the other test types showing their reduced sensitivity. However, the sensitivity of the INS and SHM FET tests were increased when hatchability was included as a sublethal test metric. The MGS and INS LGS LC₅₀ values were comparable and were both significantly lower than the other test types. This suggests that the MGS test could be a viable replacement for both the LGS tests. Overall, this work provides data supporting the use of the MGS and modified FET tests as possible replacements for marine LGS tests.

1.02.P-Th-004 Developing *Hyalella azteca* Embryo Toxicity Assay for High Throughput Toxicity Tests

Helen C Poynton and Irina Polunina, University of Massachusetts, Boston

Toxicity tests are effective monitoring tools in evaluating site contamination and can be critical for ensuring the health and safety of novel products. 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 that can achieve similar results that are cost-effective and efficient. *Hyalella azteca* is a freshwater amphipod that is used in many ecotoxicology applications to evaluate the effect of pollutants in sediments and waterways. Many different toxicants have been evaluated for their effect on adult and juvenile *H. azteca*; however, little has been studied using *H. azteca* embryos. Using this model organism, I have already characterized the development of *H. azteca* embryos and developed the *H. azteca* Embryo Toxicity Assay to evaluate contaminants for developmental toxicity. The goal of the present study was to further optimize the *H. azteca* Embryo Toxicity Assay using well-known teratogens. Twelve initial teratogens were chosen for screening in a range-finding study to determine appropriate exposure concentrations and evaluated for morphological impacts, such as deformities to the head lobe and digestive tube regions, as well as halted eye development. Three of these teratogens were then chosen for optimization of the assay on

experimental design and the three assay endpoints; morphological, gene expression, and neurobehavioral. At the end of this study, I will have developed a standard operating procedure for the *H. azteca* Embryo Toxicity Assay that will be used to evaluate the developmental toxicity of different environmentally relevant contaminants.

1.02.P-Th-005 Web-based Interspecies Correlation Estimation (Web-ICE) Toxicity Extrapolation Tool v4.0

Shannon Alexis Nelson, Crystal R Lilavois and Sandy Raimondo, U.S. Environmental Protection Agency
Protecting a diversity of species from the adverse effects of chemicals is a significant environmental challenge. Information on the effects of chemicals on species is either limited or lacking entirely, making management and mitigation of environmental contaminants difficult. The United States Environmental Protection Agency (U.S. EPA) developed the Web-based Interspecies Correlation Estimation (Web-ICE) tool to allow toxicity extrapolation from standard test organisms to diverse taxa, including endangered and non-standard test species. This publicly accessible application allows risk assessors and environmental managers from all sectors to estimate acute chemical toxicity to a diversity of fresh and saltwater invertebrates, fish, birds, mammals, and aquatic plants (algae) that may have limited toxicity data. With its update to version 4.0 in 2023, the Web-ICE aquatic database gained new models in nearly all taxa categories and all model-levels (e.g., species, genus, and family), including 2,294 significant species-level models, up from 1,550 in v3.3. The Web-ICE tool serves as a New Approach Methodology useful for future Ecological Risk Assessments as animal testing is reduced and new/existing chemicals continue to be manufactured.

1.02.P-Th-006 A Tiered Bioaccumulation Assessment Framework for Organic Chemicals

Alessandro Sangion¹, James M Armitage², Liisa Toose¹, Trevor N Brown¹ and Jon A. Arnot¹, (1)Arnot Research and Consulting, Inc. (ARC), (2)AES Armitage Environmental Sciences, Inc

Bioaccumulation (B) assessments use various methods, data, and metrics for aquatic and terrestrial species. In absence of reliable measured data, screening-level tools are commonly applied to estimate bioconcentration factors (BCFs) in fish. However, it is now recognized that BCFs in fish may not adequately capture the overall bioaccumulation potential of organic chemicals in the environment. The Bioaccumulation Estimation Tool (BET) and Bioaccumulation Assessment Tool (BAT) provide a consistent, transparent, and tiered method to estimate state-of-the-science B metrics for a broad range of species. The BET uses 1-compartment physiologically-based biokinetic (PBK) models, enabling the calculation of various B metrics such as BCFs, bioaccumulation factors (BAFs), biomagnification factors (BMFs), total elimination half-lives (HL_T) for both aquatic and air-breathing ecological receptors, including invertebrates, fish, birds, and mammals, as well as laboratory test animals such as rats, aquatic invertebrates, and fish. The BET employs a tiered approach for input parameters, offering users the flexibility to consider (i) partitioning in phospholipids, structural proteins and serum albumin as alternatives to relying solely on octanol as a surrogate for lipid, (ii) dissociation for ionogenic organic chemicals (IOCs), and (iii) inclusion of biotransformation rate estimates from in vivo, in vitro and in silico sources. The BET is built into the freely available online Exposure And Safety Estimation (EAS-E) Suite platform providing autoperparameterization of the models requiring only chemical SMILES, name or CAS Registry Number as user input. To complement the BET, the BAT serves as a higher-tiered tool that guides the collection, generation, evaluation, and integration of various lines of evidence (in silico, in vitro, lab, and field studies) for a Weight of Evidence (WOE) approach to support definitive B assessment decision-making. In this presentation, we provide an overview of the BET and the BAT and compare B metrics calculated from the BET to those estimated by traditional B models for several hundred neutral, acidic, and basic compounds. The merits and limitations of both traditional methods and mechanistic PBK models, such as the BET are then discussed.

1.02.P-Th-007 Diversifying the ECOTOXicology Knowledgebase: Inclusion of In Vitro Toxicity Data to Support Ecological Risk Assessment and Research

Jennifer Olker¹, Michael Hornung¹, Anita Pomplun², Brian Kinzinger², Travis Karschnik², Anne Pilli² and Dale J. Hoff¹, (1)U.S. Environmental Protection Agency, (2)General Dynamics Information Technology

Chemical safety evaluation is shifting to incorporate alternative methods (e.g., *in vitro* assays and *in silico* modeling) to meet demand for rapid and efficient predictions of chemical hazard and toxicity for human health and ecologically relevant species. The USEPA's ECOTOXicology Knowledgebase (ECOTOX) has provided support for ecological chemical assessments for decades through the identification and curation of toxicity results. ECOTOX is the world's largest compilation of curated ecotoxicity data; however, until recently, toxicity results from *in vitro* assays were not considered for inclusion. Presented here is the expansion of ECOTOX to incorporate *in vitro* studies of chemical toxicity in aquatic and terrestrial organisms. The well-established ECOTOX systematic literature review procedures are being applied to identify relevant *in vitro* studies and to extract study details and toxicity data in the structured ECOTOX framework. To date, over 500 publications with *in vitro* ecotoxicity data have been included in the public website (www.epa.gov/ecotox) with studies using assays with cells, tissues, or enzymes from over 300 biological species, including fish, amphibians, reptiles, birds, mammals, plants, algae, and invertebrates. The existing ECOTOX data extraction fields have been flexible enough to capture study details and toxicity results from these *in vitro* studies. Additions to the controlled vocabularies have been made to capture new specific effects and endpoints, with further additions anticipated based on review of published guidelines on *in vitro* study reporting. The incorporation of *in vitro* toxicity results complements the other mechanistic data already included in ECOTOX, such as the molecular, cellular, tissue, and organ level effect measurements from *in vivo* chemical exposure studies. This expansion is anticipated to evolve to adequately support study quality evaluations and analyses for validation of new methods, especially with the on-going progression in consideration of mechanistic and new approach methodologies (NAMs) as supporting information for chemical assessments. The curated toxicity results in ECOTOX can support the evaluation of ecological relevance of mechanistic, *in vitro*, and other non-apical toxicity data to individual or population level adverse outcomes, which is necessary for acceptance of alternative approaches and application in regulatory frameworks. *This abstract does not necessarily reflect US EPA policy.*

1.02.P-Th-008 Towards Establishing a 24-Hour, Microplate-Based, Transcriptomics Assay for Rainbow Trout Embryos

Nil Basu¹, Aylish Marshall, Hugo Marchand, Emily Boulanger, Kritika Mittal and Jessica Head, McGill University

There is interest in the development of early-life stage (ELS) tests with fish embryo models that are high-throughput and can generate transcriptomics point of departure (tPOD) values. The objective of this study was to establish a method in rainbow trout hatchlings that could satisfy both of these interests. We based our pilot method on recent efforts by U.S. EPA researchers to establish a larval fathead minnow high throughput transcriptomics assay. Here, 1-2 day post hatch trout were assayed in 24 well plates in which they were exposed for 24 hours to 12 different concentrations of test chemicals, including a negative control (DMSO, culture water). Test concentrations were based on a tapered design which included an LC50 push zone (10- and 100-fold higher than estimated LC50 from the US EPA EcoTox database), transcriptomics 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 design for malathion was: 30, 3, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001, 0.0003, 0.00003, 0.000003 mg/L (covering 7 orders of magnitude). In pilot study 1 we tested 3,4-dichloroaniline, CuSO₄ (0.3 mg/L), and ethinylestradiol. In pilot study 2 we tested 3,4-dichloroaniline (200 mg/L), CuSO₄ (0.3 mg/L), ethinylestradiol (>10 µg/L), permethrin (>10 µg/L), malathion (1 mg/L), 6PPD quinone (5 µg/L), acetaldehyde (30 mg/L), 4-fluoroaniline (250 mg/L), glyphosate (150 mg/L), ethanol (>1 g/L), thiamethoxam (>300 mg/L), and allyl alcohol (>30 mg/L). In both pilot studies preliminary LC50 values

are provided in parentheses. Repeated studies of CuSO₄ yielded consistent LC₅₀ values. Work is underway to optimize transcriptomics assays from these samples using EcoToxChips and UPXome, with the ultimate goal to be able to derive transcriptomics points of departure. Taken together these results provide a foundation towards establishing a novel testing platform for chemical and environmental risk assessment.

1.02.P-Th-009 Transcriptomic Points of Departure in 24 Hr ELS Tests Of Rainbow Trout Using Ecotoxchips: A Case Study With Ethinyl Estradiol

Emily Boulanger¹, Nil Basu¹, Aylish Marshall¹, Hugo Marchand¹, Kritika Mittal¹, Jessica Ewald¹, Markus Hecker², Jianguo Xia¹ and Jessica Head¹, (1)McGill University, (2)University of Saskatchewan, Canada

To help move away from whole animal acute toxicity testing involving fish, there is interest in the development of early-life stage (ELS) tests that are high-throughput and can also generate quantitative transcriptomics point of departure (tPOD) values. The objective of this study was to evaluate whether we could couple a rapid ELS fish embryo exposure study with EcoToxChip analysis to generate transcriptomic points of departure (tPOD). Briefly, individual rainbow trout hatchlings (1-2 day post-hatch) were placed into single wells (24-well plates) and exposed for 24 hr to a solvent control (0.5% DMSO) and 11 concentrations of ethinylestradiol (EE2): 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 and 100 ng/L (12 individuals per group). After the 24-hr exposure, larvae were pooled (n=4) to create 3 replicates per dose group for gene expression analysis using the 384-gene Rainbow Trout version 0.1 EcoToxChip. Differential gene expression analysis and dose-response analysis methods were performed on EcoToxXplorer (www.ecotoxxplorer.ca). No mortalities or deformities were observed following EE2 exposure. Most differentially expressed genes (DEGs) were found in the 1 ng/L treatment group, and common DEGs included vitellogenin-like and estrogen receptor genes as would be expected with EE2 exposure. A range of statistical tests were conducted to derive tPODs, with the values ranging from 0.8 to 8.3 ng/L depending on input parameters. These values are similar to a recent study in which rainbow trout hatchlings were exposed to EE2 (7 concentrations between 0 to 169 mg/L) from hatch to four days post hatch, following which RNA-seq analysis of whole embryos yielded tPOD values ranging between 0.2 and 3.6 ng/L. Taken together, the findings from this case study lend support to the notion that 24 hr microplate-based bioassays with rainbow trout embryos (1-2 day post-hatch) coupled with EcoToxChips can yield molecular data that provides insights into mechanisms of action and is also conducive to benchmark dose modeling to derive quantitative tPODs.

1.02.P-Th-010 Health Risk of Emerging Contaminants in Pet Hair and Indoor Air: An Integrative Approach of ToxCast Endpoints and AOP Network

Fei Cheng, Huizhen Li and Jing You, Jinan University

Indoor exposome is a growing concern, including a mixture of legacy and emerging contaminants. Recent studies suggest that indoor pollutants may accumulate in pet hair, a part of indoor exposome, increasing health risks to pet owners; however, there is still a lack of evidence for source and hazard of pollutants associated with pet hair. The present study found that indoor hydrophobic pollutants often had higher concentrations than the hydrophilic, and PAHs were the most dominant fractions in indoor exposome while PCMs had the highest concentrations among all contaminant classes in indoor dust and pet hair. The levels of hygiene-related contaminants (PCMs, CUPs, and antibiotics) were accumulated more in pet hair than dust due to direct contact during applications. Health risk assessment using toxicity thresholds from high-throughput screening data showed that human health risks from these five classes of indoor contaminants via inhalation, ingestion, and dermal contact were within acceptable limits, but children may be exposed to a higher risk than adults. The thresholds estimated from the ToxCast data using endpoint sensitivity distribution make the exposome risk assessment feasible in the absence of benchmarks, which is beneficial for including a diversity of emerging pollutants in risk assessment. Both dust and pet hair are typical media for accumulating pollutants in the indoor environment of residents. Chemical analysis was conducted on these two types of indoor samples, by quantitatively characterizing the occurrence of 41 substances belonging to five categories. The causes of

distribution differences of pollutants between dust and pet hair were explained by sources and partitioning model. In addition, nontarget contaminants were identified using HRMS. Finally, an adverse outcome pathway network was developed for risk assessment.

1.02.P-Th-011 Physiologically Based Toxicokinetic Models: Chemical Exposure Simulations Applied to Novel Fish Species

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To model a complete Source To Outcome Pathway (STOP) for fish, it is essential to parameterize the potential environmental concentrations and the pathways that link outside environment concentrations to internal target site concentrations so that an Adverse Outcome Pathway (AOP) can be modeled starting with the Molecular Initiating Event based on target site concentrations. To come to this Aggregate Exposure Pathway model, physiologically based toxicokinetic (PBTK) models can be applied to predict bioconcentration and internal distribution of various chemicals in different species. PBTK models consist of a variety of basic equations that describe the absorption, distribution, metabolism, and excretion (ADME) of a compound in an organism. These equations are linked in a series of ordinary differential equations and parameterized by the physiology (e.g. blood flow to organs, tissue volumes, effective respiratory volume, etc.) of the species being investigated. Currently, there are gaps in literature concerning commercially relevant species of freshwater fish. More specifically, the hybridized striped bass is an important game fish in North America and is commonly stocked in ponds and lakes for recreational fishing and human consumption. Despite this, there are no existing physiologically based toxicokinetic models for this species. As such, this work aims to incorporate newly generated physiological parameter values for tissue specific blood flows and weights for hybridized striped bass into an existing PBTK model structure as a predictive tool in the event of harmful chemical exposure in hybridized striped bass habitats. In addition to generating and incorporating novel data into the existing model structure, this work is a preliminary to a larger undertaking by the EXPECT project in association with the Norwegian Institute for Water Research (NIVA) and Clemson University which is aimed at developing novel PBTK models for marine arctic fish species. The principles and techniques developed for hybridized striped bass will be applied to marine arctic fish to expand the use of PBTK models into undefined territory, exploring how changing environmental conditions and differing physiologies impact ADME in a variety of fish species in differing biomes that are specifically sensitive to climate change and increasing anthropogenic activities.

1.02.P-Th-012 Alternative Approaches to Animal Testing: Exploring Approaches and Avenues for the Future Ecological Risk Assessments

Krittika Mittal¹, Ke Xu¹, Samuel Rulli², Jennifer Jakubowski², Jessica Head¹ and Nil Basu¹, (1) McGill University, (2)Qiagen

Ethical concerns and high costs associated with animal testing have prompted widespread interest in developing cheaper and faster non-animal based alternative testing strategies. There has also been increased interest in using molecular data in risk assessment for benchmark dose response analysis and derivation of transcriptomic points of departure (tPOD). Concurrently, advances in sequencing and ‘omics technologies have led to reduced sequencing costs, making it more feasible to integrate transcriptomics-based test systems into alternative testing strategies. The objective here was to iteratively test, optimize and establish a high throughput *in vitro* transcriptomic-based toxicity test system that couples rainbow trout RTgill-W1 and human Caco-2 cell lines with QIAGEN’s UPXome ultraplex technology so that the combined test system can derive tPODs. The library preparation workflow was first optimized with unexposed RTgill-W1 cells to ensure good quality libraries as verified on an Agilent Bioanalyzer. Subsequently, both cells were exposed for 24 hr. to 6-8 concentrations of solvents (dimethyl sulfoxide and methanol) and positive controls (3,4-dichloroaniline; DCA – RTgill-W1 and hydrogen peroxide – Caco-2). Exposures were based on the OECD fish cell line test guideline 249. RTgill-W1 libraries were assessed on the Bioanalyzer and sequenced on an Illumina MiSeq. The advantage of this

workflow is that the cell lysis step, conducted in microplates, allows for plate-based reverse transcription directly on the cell lysate without isolating RNA from each sample. During the reverse transcription step, a unique sample index for each sample is incorporated into the cDNA which allows for pooling of the cDNA and simplified library preparation than traditional methods. Thus, libraries can be prepared from multiple 96-well plates in one workday, and hundreds of samples can be sequenced in a single lane. No differential gene expression was observed in solvent exposed RTgill-W1 cells compared to unexposed controls. Preliminary analysis showed 40-82 differentially expressed genes in DCA-exposed RTgill-W1 cells (>1.9 mg/L) including genes related to stress, cell signaling, and cell structure, such as *hsp90ba*, *aldoa*, *cavin1b*, *thbs1*, and *flna*, and subsequent data analysis is underway. Analysis of Caco-2 cells and further exposures to evaluate the performance of this platform from environmental samples and chemicals of regulatory concern are ongoing in several case studies.

1.02.P-Th-013 Using Museum Specimens to Document Contamination: A Superfund Case Study within the Tri-State Mining District

Sarah Hileman and Jason B. Belden, Oklahoma State University

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. 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.

1.02.P-Th-014 What is the Value of Standard in vivo Acute Fish Toxicity Tests for Pesticide Products in the US and the EU?

Katherine K. Coady, Audrey Bone, Elena Adams, Sarah Hartmann, Daniel Faber, Yvonne Wolf, Markus Ebeling and Eric Bruns, Bayer AG - Crop Science Division

Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) in the United States and the Commission Regulations (EU) No 283/2013 and No 284/2013 in the EU, the fish acute toxicity test is required for the registration and continued authorization of pesticides. The key use for fish acute toxicity data is to perform acute aquatic risk assessments to determine acceptable use scenarios for pesticide products. Given the initiatives in both regions to reduce the use of vertebrate animals for toxicity testing, it's important to understand the concrete value that a vertebrate toxicity test, such as the fish acute toxicity test, brings to the overall ecological risk assessment. The risk assessments of pesticide products performed by regulatory authorities in both the US and EU were examined to determine the frequency by which the acute fish toxicity endpoint, as opposed to other aquatic organism toxicity endpoints, drives the assessment for the aquatic environment. The results indicated that a very small percentage of the aquatic risk assessments were driven by the acute fish endpoint (e.g., 0.44% in the EU; US assessment ongoing), suggesting that this study is not

necessary for a risk assessment that is protective of aquatic organisms, including fish, in the majority of cases. In addition, alternative tools such as the fish embryo test (FET) and rainbow trout gill cell line assay (RT gill-W1) can provide additional context for fish-specific toxicity if deemed necessary.

1.02.T Alternative Approaches to Animal Testing: Exploring Approaches and Avenues for the Future Ecological Risk Assessments

1.02.T-01 Zebrafish Embryo vs. Mouse – An Alternative to Mammalian Teratogenicity Tests in Assessing Effects of Pharmaceuticals?

Katharina Brotzmann and Thomas Braunbeck, University of Heidelberg

Therapeutic drugs represent one of the greatest benefits, but also challenges to modern society. *Via* improper disposal, industrial waste and wastewater treatment plants, pharmaceuticals are increasingly released into natural ecosystems, potentially expressing effects in non-target organisms as well. To protect the environment and the health of humans, hazard and risk assessments of these chemicals became mandatory in the registration process and ecotoxicological studies. However, the rapid increase of toxicity tests, which were mostly performed in mammalian model organisms, led to ethical concern in the scientific community and the public. Thus, there is a need for the development and implementation of alternative methods for toxicity and ecotoxicity testing. In accordance with this, zebrafish (*Danio rerio*) embryos were investigated for the potential to identify mammalian neuro- and liver toxicity induced by the antiepileptic drug valproic acid and up to 14 analogues. For the identification of neurotoxic potencies, substances were tested with the Fish Embryo Acute Toxicity (FET) test (OECD TG 236); adapting the evaluation by a subsequent comparison of selected FET endpoints to *in vivo* mouse data expressing exencephaly resulted in a good correlation between zebrafish embryos and mice. For liver toxicity, histological evaluations revealed drug-induced liver alterations. Additional comparisons of the deduced hepatotoxic potency to the molecular structure of each compound led to a structure-activity-relationship, which had, so far, only been documented in yeast cells, mosquito larvae, *Xenopus* embryos, mice, rats and human HepG2 cells. Drug-induced accumulation of lipid droplets as a marker of steatosis, however, could not be documented for any substance, although toxicokinetic analysis revealed an accumulation of the parent compounds in zebrafish embryos. In summary, although steatosis as an endpoint in liver toxicity could not be seen with the selected substances, a good correlation between zebrafish and mammals could be observed for valproic acid-induced neurotoxic effects and the structure-activity relationships based on hepatotoxic potencies. Considering the variety of therapeutic drugs, zebrafish embryos present a versatile tool in the evaluation of toxic and teratogenic potencies of pharmaceuticals in vertebrates. Supported by European Union's Horizon 2020 research and innovation program; grant agreement no.681002.

1.02.T-02 In vitro Screening of UV-Stabilizers and UV Filters: Cytotoxicity, CYP1A Activity, and mRNA Expression in an Immortalized Embryonic Double-Crested Cormorant Cell Line

Tasnia Sharin¹, Ramela Arax Koumrouyan², Jessica Head² and Doug Crump¹, (1)Environment and Climate Change Canada, (2) McGill University

Ultraviolet (UV) stabilizers and UV filters are added to industrial and personal care products to absorb UV rays and prevent photo oxidation. Some of these compounds have been listed as substances of concern by the European Chemical Agency due to their persistence in the environment and large production volume. UV stabilizers and UV filters have been detected in a range of environmental matrices, including high trophic level bird species (e.g. double-crested cormorant). Little is known about the toxicological properties of these compounds, especially in birds. Previously, we established and characterized an immortalized embryonic double-crested cormorant hepatic cell line, DCH22, for chemical screening. In the present study, DCH22 cells, cultured as 3-dimensional (3D) spheroids, were exposed to three UV stabilizers (UV-328, UV-329, UV-9) and a UV filter (BP-3) at nominal concentrations of 0.1 to 1000 μ M to determine cell viability, CYP1A activity and changes in mRNA expression. Cell viability and CYP1A activity were measured using intracellular ATP

concentration and ethoxyresorufin-*O*-deethylase (EROD) activity. A customized species-specific PCR array, EcoToxChip, comprising 384 genes, was used to determine changes in mRNA expression. The UV stabilizers and UV filter elicited similar effects on cell viability with LC50 values ranging from 112 to 160µM. UV-9 and BP-3 exposure resulted in a concentration-dependent increase in CYP1A activity with BP3 inducing a greater response than UV-9. UV-328 and UV-329 did not induce CYP1A activity. UV-9 and BP-3 (10µM) altered genes associated with multiple pathways and the common pathways dysregulated were thyroid homeostasis and signal transduction. UV-328 and UV-329 (10µM) gene expression analyses are underway. Concentration-dependent (0.1 to 100µM) changes in mRNA expression will be evaluated for the four compounds and the dose-response modelling program PROAST (RIVM) will be used to determine benchmark doses. The CYP1A activity and gene expression data will provide information on the mechanism of toxicity for each chemical. Given the increased use and prevalence of these compounds, these findings will generate much needed toxicity data in a wildlife avian species.

1.02.T-03 Focused Transcriptomics Arrays (EcoToxChips) to Characterize the Molecular Toxicity Pathways and Transcriptomics Points of Departures (tPODs) of 6PPD-quinone in Rainbow Trout

Markus Hecker¹, Catherine Roberts¹, Alper James Alcaraz¹, Emily Boulanger², Jianguo Xia², Jessica Ewald², Doug Crump³, Nil Basu², Natacha S Hogan¹, Lynn Weber¹, David M Janz¹, Steve Wiseman⁴ and Markus Brinkmann¹, (1) University of Saskatchewan, (2) McGill University, (3) Environment and Climate Change Canada, (4) University of Lethbridge

New approach methods (NAMs), including *in silico*, *in vitro* and high content toxicogenomics technologies, hold significant promise to aid in the transition to more ethical, efficient and reliable chemical safety and environmental risk assessments. This study aims to apply a novel standardized focused transcriptome assay (EcoToxChip system) using short-term embryonic exposures to derive benchmark doses (transcriptomics points of departure [tPODs]) and to identify key molecular toxicity pathways of an emerging chemical of concern, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q). 6PPD-Q is a transformation product of the rubber tire antioxidant 6PPD, which has recently been linked to mass mortalities of coho salmon in the U.S. Rainbow trout (*Oncorhynchus mykiss*) embryos, a species also shown to be sensitive to this rubber tire transformation product, were exposed to 6 incremental concentrations of 6PPD-Q from hatch to 28 days post hatch (dph). At 4 dph, a subset of trout were sampled, and mRNA extracted from whole larvae was subjected to transcriptome analysis using EcoToxChips and Illumina RNAseq analyses. The remaining fish were grown out for 28 dph to assess apical and histological outcomes to anchor molecular responses. Focused transcriptome data was analyzed using EcoToxExplorer.ca, an intuitive online bioinformatics tool. RNAseq data was analyzed using Galaxy and FastBMD.ca. Transcriptome analysis identified several molecular toxicity pathways that may explain the apical effects observed at later exposure time points, including behavioral changes, teratogenicity and mortality. Calibration of focused transcriptome results obtained using EcoToxChips with whole transcriptome data is ongoing. Results will be used to develop a comprehensive toxicity pathway model to support risk assessment of 6PPD-quinone across fishes.

1.02.T-04 A New Approach Methodology for Predictive Developmental and Reproductive Toxicology (DART) with a *C. Elegans*-Based Assay

Sudip Mondal¹, Adam Laing¹, Amber Shen¹, Evan Hegarty¹ and Adela Ben-Yakar^{1,2}, (1)vivoVerse, Inc., (2)University of Texas at Austin

C. elegans has been of great interest to the toxicology community for years as an 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 at a low cost. *C. elegans* have been widely utilized before to study several environmental toxicants, and so are a validated alternative method to assess toxicity *in vivo* without regulatory or ethical concerns. However, high-throughput developmental and reproductive toxicology (DART) studies have previously been restricted to gross

phenotypes such as body size, which limits the sensitivity, specificity, and predictivity of the assay. Here we present a microfluidics-based high-throughput imaging platform that can simultaneously immobilize ~1,000 nematodes and acquire high-resolution time-lapse images of whole worms and their *in-utero* embryos from 24 individual populations per chip in 30 minutes. We analyzed time-lapse images of *C. elegans* exposed to several doses of ecotoxicology-relevant chemicals (triphenyl phosphate, perfluorooctanesulfonic acid, and methylmercury) and quantified subtle, sub-lethal *in vivo* endpoints that are relevant for ecotoxicology DART assessments. We demonstrated that our assay was repeatable across individual technical replicates and multiple independent experiments. Using multiparametric analyses, we were better at identifying lowest observed adverse effect levels (LOAEL) values, especially with the late-stage developing embryo phenotype that was 2-10× more sensitive than the total embryo count for certain toxicants. We also found differing sensitivities to methylmercury in different wild-type genetic backgrounds with a large genetic component to the differences, indicating our assay could recapitulate the stronger effects on vulnerable subpopulations seen in other species. Comparison of LOAELs for our sample toxicants with those from rodents and other model organisms showed that our assay correlates highly with the values obtained in rodents (Spearman Correlation). 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.

1.02.T-06 Genotoxicity of Benzidine-Based Dyes in Chinese Hamster Ovary Cells Expressing Human CYP1A2 and N-Acetyltransferase 1

Mariam R. Habil and David W. Hein, University of Louisville

Benzidine-based dyes are commonly found in paints, textiles, and food colors. Previous studies showed their genotoxic effects using bacterial systems or animal models. However, due to interspecies differences, their data could not reflect human cells regarding metabolism or DNA repair. In the current study we used Chinese Hamster Ovary (CHO) cells that were stably transfected with human CYP1A2 and N-acetyltransferase 1 (NAT1). This model showed stable expression of human CYP1A2 and NAT1. Since the CHO model lacks nucleotide excision repair or (NER), they are hypersensitive to bulky DNA adducts and mutagens. CHO cells were treated with different concentrations of the benzidine-based dyes direct black 38 or direct blue 6 (10-100 µM). Both are known to be carcinogenic in previous animal studies. γ H2AX in-cell western assay was used to measure double strand DNA breaks, that reflects genotoxicity induced by these compounds. Our results showed that direct black 38 did not cause DNA damage significantly ($p > 0.05$) in NER-deficient CHO cells transfected with CYP1A2 and/or NAT1. On the other hand, direct blue 6 induced concentration-dependent increases in DNA damage that was enhanced in CHO cells transfected with CYP1A2 and NAT1 ($p < 0.0001$). Studies are in progress with other benzidine-based dyes. This model incorporates human enzymes and provides information regarding the human metabolic profile for these carcinogens compared to previous studies that were done in bacteria or animal models.

1.02.V Alternative Approaches to Animal Testing: Exploring Approaches and Avenues for the Future Ecological Risk Assessments

1.02.V-033 Focused transcriptomics arrays (EcoToxChips) to characterize the molecular toxicity pathways and transcriptomics points of departures (tPODs) of 6PPD-quinone in rainbow trout

Markus Hecker¹, Catherine Roberts¹, Alper James Alcaraz¹, Emily Boulanger², Jianguo Xia², Jessica Ewald², Doug Crump³, Niladri Basu², Natacha S Hogan¹, Lynn Weber¹, David M. Janz¹, Steve Wiseman⁴ and Markus Brinkmann¹, (1) University of Saskatchewan, (2) McGill University, (3) Environment and Climate Change Canada (ECCC), (4) University of Lethbridge

New approach methods (NAMs), including *in silico*, *in vitro* and high content toxicogenomics technologies, hold significant promise to aid in the transition to more ethical, efficient and reliable chemical safety and

environmental risk assessments. This study aims to apply a novel standardized focused transcriptome assay (EcoToxChip system) using short-term embryonic exposures to derive benchmark doses (transcriptomics points of departure [tPODs]) and to identify key molecular toxicity pathways of an emerging chemical of concern, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q). 6PPD-Q is a transformation product of the rubber tire antioxidant 6PPD, which has recently been linked to mass mortalities of coho salmon in the U.S. Rainbow trout (*Oncorhynchus mykiss*) embryos, a species also shown to be sensitive to this rubber tire transformation product, were exposed to 6 incremental concentrations of 6PPD-Q from hatch to 28 days post hatch (dph). At 4 dph, a subset of trout were sampled, and mRNA extracted from whole larvae was subjected to transcriptome analysis using EcoToxChips and Illumina RNAseq analyses. The remaining fish were grown out for 28 dph to assess apical and histological outcomes to anchor molecular responses. Focused transcriptome data was analyzed using EcoToxExplorer.ca, an intuitive online bioinformatics tool. RNAseq data was analyzed using Galaxy and FastBMD.ca. Transcriptome analysis identified several molecular toxicity pathways that may explain the apical effects observed at later exposure time points, including behavioral changes, teratogenicity and mortality. Calibration of focused transcriptome results obtained using EcoToxChips with whole transcriptome data is ongoing. Results will be used to develop a comprehensive toxicity pathway model to support risk assessment of 6PPD-quinone across fishes.

1.03.P-Tu Characterization of Complex Mixtures with New Approach Methodologies

1.03.P-Tu-001 An Avian 3D Spheroid Hepatic Cell Assay for Monitoring Bioactivity Related to Naphthenic Acid Contamination in Wetlands Near Tailings Ponds in the Athabasca Oil Sands Region

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The Athabasca Oil Sands Region (AOSR) is a major source of oil for Canada and one of the largest bitumen extraction sites in the world. Bitumen extraction produces large volumes of waste called tailings, which have been shown to be toxic to various organisms due to the presence of naphthenic acids (NAs) and polycyclic aromatic hydrocarbons. There is concern that surrounding tributaries and wetlands are susceptible to leaching from the large containment ponds where tailings are stored (i.e. tailings ponds). The objective of the present study was to determine NA concentrations and *in vitro* bioactivity of extracts derived from passive samplers deployed in AOSR wetlands with varying proximity to tailings ponds. NA concentrations were determined using liquid chromatography-tandem mass spectrometry. Bioactivity was determined in 3D spheroid-cultured chicken LMH cells by cytotoxicity, EROD and gene expression assays. We detected elevated levels of NAs in wetlands close to tailings ponds compared to reference wetlands. None of the extracts were found to be cytotoxic. Next steps include determining EROD activity to evaluate CYP1A induction and measuring gene expression using a chicken ToxChip PCR array. The ToxChip contains a curated list of toxicologically relevant genes. Bioactivity results will be used to estimate the relative avian toxicity of the extracts. Further, bioactivity data will be compared to NA concentrations to determine if they are capable of identifying NA contamination. Ultimately, we aim to demonstrate the utility of a non-animal, *in vitro* screening approach to enhance environmental monitoring efforts in a priority Canadian ecosystem.

1.03.P-Tu-002 Using Transcriptomic Points of Departure (tPODs) to Assess the Toxicity of Oils in Atlantic Cod (*Gadus morhua*) Larvae

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Transcriptomics dose-response analysis (TDRA) is a promising approach for predicting the organismal level toxicity of environmental contaminants from transcriptomics data. TDRA has potential applications to the field of environmental risk assessment, but has seldom been used in the context of environmental mixtures. Here, we apply TDRA to early life stage (ELS) Atlantic cod (*Gadus morhua*) exposed to two different types of oil; ultra

low sulfur fuel oil (ULSFO) and cold lake blend diluted bitumen (CLB). In an initial experiment, cod larvae (~200 degree days) were exposed to seawater control or water accommodated fractions (WAFs) made using variable loadings (0.01, 0.1, 1, 10, 25 g/L) of each oil for 24 hrs. Whole larvae were flash-frozen in pools of 5 and preserved for RNAsequencing. Analytical determination of polycyclic aromatic hydrocarbons (PAHs) and other organic chemicals in the WAF revealed that the ULSFO and CLB oils each contained a distinct complex mixture of PAHs that increased with oil loading. At equal oil loadings the ULSFO had a higher concentration of PAHs and was associated with a higher level of mortality in exposed cod. Many genes were dysregulated by both oil types, with concentration-dependent increase in DEGs in fish exposed to CLB (#DEGs per concentration: 61, 80, 195, 240, 298) and ULFSO (#DEGs per concentration: 582, 756, 782, 733, 1011). The strong transcriptomic response across all doses precluded TDRA, so we repeated the exposure in a second experiment using direct loading and additional lower concentrations of the two oils (1 g/L ULSFO or CLB WAF at 0.001%, 0.01%, 0.1%, 0.317%, 1%, 3.17%, 10.01%, 31.7% and 100%). These results are pending. Overall, *in vivo* endpoints, chemical analysis, and the number of DEGs all suggested that the ULSFO oil was more acutely toxic to ELS fish. Our ongoing analysis will evaluate benefits of the TDRA approach and evaluate its potential for assessing the toxicity of environmental mixtures.

1.03.P-Tu-003 Identification of Causative Toxicants in River Waters Using Bioassays with Alga and Daphnid and Multiple-Component Chemical Analysis

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A variety of new chemicals in small volume of production or import, which are required neither toxicity test nor environmental risk assessment in chemical management system (e.g. Chemical Substance Control Law in Japan), have been recently increasing. These various kinds of chemicals released into the environment could cause unknown mixture effect and possibly result in adverse impact on ecosystem. Effect-directed analysis (EDA) is one of the most powerful approaches to identify suspect toxicants in unknown complex mixture, by the combination of bioassays with fractionation and chemical analysis. In this study, 71 samples collected from rivers were subjected to algal growth inhibition test, *Ceriodaphnia dubia* reproduction test, and zebrafish toxicity test on embryo and sac-fry stages and 38 samples were toxic either alga or daphnid. Multiple-component chemical analysis was also conducted using Automated Identification and Quantification System (AIQS) with GC/MS and LC/MS for organic compounds (pesticide, PPCPs, and ICP-MS for metals to identify the toxicants. The predicted mixture toxicity calculated by summing up the individual toxic units (TUs = concentration/NOEC) of detected chemicals in each sample were compared with the measured TUs calculated by IC_x (≡NOEC) of each sample in each toxicity test. Major suspect organic toxicants included an herbicide bromacil and a macrolide antibiotic clarithromycin for alga while several insecticides such as pyridaben, propetamphos, and fenitrothion were identified for daphnid. Nickel and zinc were also identified as primary toxicants in several samples, but the metals' toxicity was possibly overestimated in many samples. The fractionation study revealed that some fractions were toxic to alga or daphnid but the identified chemicals in the fractions could not explain their toxicity. Further chemical analysis and collection of toxicity data are required to identify the other unknown toxicants.

1.03.T Characterization of Complex Mixtures with New Approach Methodologies

1.03.T-01 AI Bridged Bioactivity, Structure and HRMS to Decipher Nontarget Toxicants in Complex Mixtures

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The world is composed of complex mixtures. Identifying causative toxicants is critical for understanding mixture risk but there are many gaps among chemical and bioactivity data. Effect-directed analysis integrates chemistry and biological information to identify toxicants, yet conventional bioassays typically rely on an apical

and/or single endpoint, providing no mechanistic information with limited diagnostic potential to guide chemical identification. These practices are further challenged because chemical screening analyses are commonly restricted to the available mass spectrometry (MS) libraries. This limitation can be overcome by a novel event-driven taxonomy (EDT) framework for mixture risk assessment that relies on high-throughput screening bioassays and chemical predictions integrated by artificial intelligence. The EDT framework was evaluated using complex sediment mixtures eliciting activation of arylhydrocarbon receptor and oxidative stress response. While mixture prediction using expert knowledge-oriented target analysis explained <10% of observed bioactivity in sediment extracts, a recently developed metadata-driven suspect analysis expanded the fraction explained to >80%. Additionally, deep learning models were developed to cluster fingerprints of bioactive candidates and convert these candidates to MS-readable information for non-target screening with GC-qToF-MS. Collectively, we present a “bioactivity-signature-toxicant” mixture deconvolution strategy to connect patchy datasets and guide non-target analysis for diverse chemicals for the first time.

1.03.T-02 The Use of Previous Chemical Analysis Datasets and Predictive Molecular Level Toxicity Signature Workflows In Aquatic Toxicity Testing: A Case Study of the Dallas Metroplex

Emmy Schniederjan, Sarah Nash and Jordan Crago, Texas Tech University

Current aquatic toxicity assessment strategies are heavily focused on targeted analysis and in vitro/in vivo toxicity data to understand the impact on aquatic organisms upon exposure to complex mixtures such as effluent. However, based on this approach alone, it is still difficult to explain observed toxicity symptoms and the hidden impact on biological pathways that were not accounted during an effect analysis. The recent advances in analytical instrumentation (e.g., high-resolution mass spectrometry), as well as in silico modeling of existing data could help us to understand chemical fingerprints of complex mixtures and this information could be integrated with QSAR modeling and existing in vitro databases to understand toxicity signatures of complex mixtures in aquatic organisms. The goal of this study was to incorporate previous environmental monitoring chemical datasets with a workflow that we developed that incorporates QSAR modeling and ToxCast datasets to identify toxicity signatures and expand the concept to four sample sites near wastewater treatment plants along the Trinity River in the Dallas metroplex. Identified molecular signature were confirmed by measuring molecular biomarkers in larval zebrafish exposed to water samples for 5 days. Results from this study indicate that there was a divergence between what was previously identified as biomarkers/AOPs of interest and what QSAR modeling and follow-up studies with exposures. Specifically, pathways associated with psychopharmaceuticals dominated the results, particularly those with serotonin, dopamine and adrenaline/noradrenaline, but were underrepresented in previous studies. ZFET studies using water samples were able to correctly identify alterations to psychopharmaceutical affected genes predicted in the datasets. Results from this study will allow researchers to choose chemical fingerprint guided biological pathways to assess adverse impacts on aquatic organisms upon exposure to complex mixtures.

1.03.T-03 Biomimetic Extraction with Polydimethylsiloxane as a Robust and Sensitive Method For Determination Of Toxicity Of Petroleum Mixtures in Non-Amended and Amended Sediments

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Biomimetic extraction with Polydimethylsiloxane (PDMS) has been demonstrated as a fast and efficient method for determining toxicity of unresolved hydrocarbon mixtures in aquatic systems. The method measures a pooled response of petroleum hydrocarbons (total integrated peak 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. Assessment of total petroleum hydrocarbons from contaminated sites demands routine and reliable measurements at low levels. This includes post-remedy assessments, which are often challenged by limited detection due to high efficiency of amendments used. The primary objective of this study was to evaluate the effect of activated carbon and biochar on reducing bioavailability of unresolved hydrocarbons. The BE data was

further assessed with the CAPSIM model to characterize the potential long-term reduction in bioavailability. Five sediments with varying characteristics and levels of PAHs were exposed for up to 28 days with 30 and 100 μm polydimethylsiloxane (PDMS) coated fibers (Polymicro Technologies, Phoenix, Arizona). The sediments were amended with varying doses of activated carbon (AC) and biochar. Following the equilibration period, fibers were removed, rinsed with distilled water, and blotted dry. The fibers were extracted with 0.25mL acetone-methylene chloride and analyzed using large volume injection GC-FID. C_{pdms} was estimated by translating the total area under the FID curve into molar units using the molar response of 2,3 dimethylnaphthalene as determined by injection of external standards. Results were then normalized by the PDMS volume and converted to $\sum\text{TU}_{\text{pdms}}$ for toxicity evaluation. TU of tested sediments varied from 0.3 to 13 (-) based on EqP. The TU estimates based on BE measurements with PDMS exceeded toxic thresholds for IHS and SF Marina sediments. The BE results for both sediments suggested high toxic potency to organisms and thus were favorable candidate sediments for further evaluation with activated carbon amendments. The reduction in BE following sorbent amendments varied between 50-90% depending on the sediment characteristics. The porewater and BE data for hydrocarbon mixtures before and after AC/biochar addition were further input for CAPSIM modeling, which was used to calculate the effective solid BE concentration without and with AC cap over time under different scenarios.

1.03.T-04 Effects of Paper Mill Effluent on the Reproductive Axis of the Bullhead Minnow, *Pimephales vigilax*

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The United States has the highest concentration of paper mills in the world, with roughly 450 mills currently in operation. Paper mills are a known source of environmental pollutants in proximal ecosystems. These mills produce millions of gallons of liquid waste (effluent) that is treated and released into waterways. The xenobiotic chemicals and natural compounds present in these effluents vary greatly based on the paper product produced and the treatment protocol employed by the mill. Some of the observed effects seen in exposed fish are delayed or altered secondary sex characteristic development, partial or complete sex reversal, slowed gonad development, downregulation of genes that control metabolism, and upregulation of genes responsible for reproductive processes. These effects can cause larger impacts within a population by skewing sex ratios or potentially decreasing population size. In this study, we examined the effects of effluent from two kraft mills on a wild population of the Bullhead Minnow (Leuciscidae: *Pimephales vigilax*), from the Pearl River, in Louisiana and Mississippi, as well as the Bogue Chitto River, a tributary of the Pearl River, which served as a control. Both traditional histological and modern gonadal transcriptomic approaches (QuantSeq) were used to assess the impacts on reproductive parameters. Preliminary GSI analysis suggests that minnows from the Pearl River are allocating energy away from reproductive development. A detailed presentation of the results from the histological and transcriptomic analyses will be discussed.

1.03.T-05 Multigenerational Transcriptomic Changes and Isoform Usage Resulting from Chronic Exposure to Contaminant Mixture Associated with Agricultural Land Use

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A water quality survey performed by the United States Geological Survey of tributaries of the Laurentian Great Lakes between 2010 and 2014 identified a mixture of eight contaminants: alkyl phenols, atrazine, bisphenol A, bromacil, diethyltoluamide (DEET), estrone, metolachlor, and tributoxyethyl phosphate (TBEP), which were associated with agricultural land use. Most of these compounds are known endocrine disruptors and primarily act through the estrogen receptor (ER). To investigate chronic effects of long-term environmental exposure to the mixture, adult *Pimephales promelas* (fathead minnows) were exposed to the agricultural chemical mixture (ten times below, same as, and ten times above the highest measured environmental concentration) in a flow-through exposure system for three months. During this period, F_0 adult fish were able to reproduce and F_1

offspring were reared to adulthood under continuous exposure for six months in the flow-through system. At the end of the exposure period, three months for the F₀ generation and six months for the F₁, apical end points (e.g., length, weight, and secondary sex characteristics) were measured. Blood and tissue samples from fish (e.g., liver, and gonad) were collected for studying epigenetic and gene expression changes. Previously reported results demonstrated that exposure to the agricultural mixture had estrogenic effects on male fathead minnows as evidenced by significant exposure effects: 1) increased plasma vitellogenin concentrations (ANOVA p-value < 0.001), 2) decreased secondary sex characteristics in the F₁ generation (ANOVA p-value < 0.001), and 3) changes in both expression and methylation levels of ER responsive genes in liver. Here we describe transcriptome profiling changes in gonad tissue associated with the exposure along with differential isoform expression in both liver and gonad. We also present our enriched pathway analysis results identified by Ingenuity Pathway Analysis (IPA). Further, we compare differential expression of gene isoforms in liver and gonad in response to mixture exposure with the aim of providing a better picture of exposure effects of the whole organism. Our results suggest it is important to investigate exposure to environmentally relevant mixtures, as opposed to individual chemicals, for assessing biological effects.

1.03.T-06 Toxicity Identification Evaluation for Hydraulic Fracturing Flowback and Produced Water During Shale Gas Exploitation in China: Evidence from Tissue Residues and Gene Expression

Fan Wu and Jing You, Jinan University

Hydraulic fracturing flowback and produced water (HF-FPW) from shale gas extraction processes is a highly complex medium with potential threats to the environment. Current research on the ecological risks of FPW in China is limited and the linkage between major components of FPW and toxicological effects on freshwater organisms is largely unknown. By integrating chemical and biological analyses, toxicity identification evaluation (TIE) was used to reveal causality between toxicity and contaminants, potentially disentangling the complex toxicological nature of FPW. Herein, FPW from different shale gas wells, treated FPW effluent, and a leachate from HF sludge were collected from Southwest China, and TIE was applied to acquire a comprehensive toxicity evaluation in freshwater organisms. Results showed FPW from the same geographic zone could elicit significantly different toxicity. Salinity, solid phase particulates, and organic contaminants were identified as major contributors to FPW toxicity. In addition to water chemistry, internal alkanes, PAHs, and HF additives (e.g., biocides and surfactants) in exposed embryonic fish were quantified using target and non-target tissue analyses. Treated FPW failed to mitigate the toxicity associated with organic contaminants. Transcriptomic results illustrated organic compounds in FPW induced toxicity pathways. Moreover, since similar zebrafish gene ontologies were affected between treated and untreated FPW, results again confirmed that sewage treatment did not effectively remove organic chemicals from FPW. Thus, zebrafish transcriptome analyses reveal organic toxicant-induced adverse outcome pathways and served as evidence for TIE confirmation in complex mixtures under data-poor scenarios.

1.04.P-We-002 Passive Sampling as a Novel Tool for Oil Spill Response in Canada

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Passive sampling has many advantages over conventional sampling methods for quantifying the availability of hydrophobic organic compounds, polar organic compounds, and inorganic compounds in sediment, soil, and surface water in terms of cost and data quality. The ongoing collaboration between Fisheries and Oceans Canada, Geosyntec Consultants, SiREM and the University of Waterloo reviewed the current state of knowledge for the Canadian application of passive sampling to environmentally relevant petroleum chemicals as it pertains to oil spill response in aquatic ecosystems. In this review, sixteen cases where passive sampling has been used for oil spill assessments are summarized. Emphasis will be placed on the use of low-density polyethylene (LDPE) passive sampling devices (an established approach for the measurement of freely

dissolved, bioavailable concentrations) to understand the ecological impact of oil spills. Additionally, passive sampling technology shortcomings, knowledge gaps and developments required for future research will be described, such as applying novel methods to allow LPDE passive samplers to quantify all petroleum hydrocarbons (not just PAHs) as well as shortening the time required to obtain a representative sample to facilitate the rapid response and decision making that is often required in oil spill scenarios. We expect this work to be foundational to the future development of the passive sampling technique and its use in oil spill response operation as well as monitoring of ecosystem recovery. Further, this development is expected to be of particular importance and relevance in the evaluation of exposure and adverse effects to aquatic species listed under the Species at Risk Act (SARA) in Canada under such setting.

1.04.P-We-004 Small Intertidal Microcosm Plant Exposure (SIMPLE) System: Use of a New Microcosm System to Examine the Effect of No. 2 Fuel Oil on Short- and Tall-Forms of Saltmarsh Cordgrass (*Spartina alterniflora*)

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Saltmarshes are ecologically, recreationally, and commercially important environments that can be negatively impacted by numerous contaminants. Mesocosm systems are one tool used to examine the fate and effect of various chemical contaminants within simulated marsh environments; however, they can be expensive and time-consuming to set-up and utilize for testing. A bucket microcosm system named the SIMPLE (Small Intertidal Microcosm Plant Exposure) System was developed to provide a more affordable and faster alternative for conducting tests specifically evaluating the effect of contaminants on saltmarsh vegetation. Each independent system consists of an upper plastic bucket filled with pots of sediment and plants connected to a lower bucket that acts as a water reservoir. With the aid of a timer, seawater can be automatically pumped from the lower to upper bucket to simulate high tide and then allowed to drain back down for low tide. This study utilized 32 SIMPLE systems to examine the effect of No. 2 fuel oil on short- (<0.5 m in height) and tall-forms (>1 m) of saltmarsh cordgrass, *Spartina alterniflora*. The two forms of *S. alterniflora* are genetically distinct and grow in different areas of the marsh with the tall-form being found along the edge of tidal creeks (tall-zone) and the short-form being located further inland (short-zone). This difference in location leads to the two forms experiencing different degrees of tidal inundation. Preliminary results indicate that field collected short-form plants are more sensitive to No. 2 fuel oil than tall-form plants. The purpose of this study is to determine if that difference is due to the genetic differences in the plants or to exposure differences due to differences in tidal inundation. To test this, treatments include systems dosed with No. 2 fuel oil to a 0.01 mm slick thickness vs. unoiled controls. Within each oiling condition we compare two different tidal simulations; a tall-zone simulation and a short-zone simulation. Along with plant growth and survival data, sediment and water samples will be analyzed for hydrocarbon residues. The effects and hydrocarbon chemistry data will help show how SIMPLE systems can be used for marsh contaminant testing.

1.04.P-We-005 Effects of In Ovo Chrysene and Phenanthrene Exposure on Chicken Embryo Development and Cardiac Function: 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 (Σ 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.

1.04.P-We-006 Acute and Chronic Hazard Assessment of 5 PACs to 5 Marine Species

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Polycyclic aromatic compounds (PACs) are moderately to sparingly soluble in water and are present in water-soluble extracts of petroleum products. Even the high molecular weight PACs with 4 and 5 aromatic rings that have low water solubility are detected in trace amounts in water-soluble extracts. Water accommodated fractions (WAF), prepared from water-oil mixtures, typically are used in conducting aquatic toxicity tests. WAFs are subsequently used as the exposure media for organisms since it contains the bioavailable PAC components. In order to be able to predict the toxicity of hydrocarbon mixtures, we must have an understanding of the toxicity of the individual components. Few toxicity tests have been conducted with the individual PAC compounds that make up oil and little to no chronic toxicity test data are available. To this end, five PACs (naphthalene, phenanthrene, dibenzothiophene, fluoranthene, and pyrene) test materials covering a range of molecular weights, degree of aromaticity (from two to five rings), were selected for acute and chronic toxicity testing. These surrogate compounds represent the range of PACs commonly identified in crude oils and crude oil-derived WAFs and are believed to drive the toxicity observed in laboratory oil WAFs exposures. Acute and chronic toxicity testing was performed with five standard marine species; purple sea urchin (*Strongylocentrotus purpuratus*), Mediterranean blue mussel (*Mytilus galloprovincialis*), mysid shrimp (*Americamysis bahia*), Inland silverside (*Menidia beryllina*), and sheepshead minnow (*Cyprinodon variegatus*), following standardized laboratory methods (i.e., OECD and ASTM). Static and flow-through exposure solutions were generated through traditional solvent based dissolution as well as a passive dosing technique (silicone passive diffusive releasers, SPDRs). Test solutions were analytically verified at the start and end of the acute exposures, and at regular intervals during the chronic exposures. All data underwent extensive quality assurance practices, and only tests which met validity criteria were used in the subsequent analysis. The results from the single PAC testing allowed for the calculation of organism specific critical target lipid body burdens (CTLBB; calculated as the intercept of the regression line of log KOW and LC50), and acute to chronic ratios. These metrics can be incorporated into biological effect models to predict the toxicity of complex hydrocarbon mixtures.

1.04.T Collaborative Oil Pollution Research: Successes and Opportunities

1.04.T-01 From Molecules to Morphology: A Multidisciplinary Investigation of the Toxicity of Hydrocarbon Oxidation Products in Pacific Herring Embryos

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Traditionally, oil toxicity to early life fish is typically assessed through water-accommodated or -soluble fraction and reported in PAH concentrations. However, the complex, polar fraction of oil cannot be accurately measured with common gas chromatography, rendering PAH concentration inadequate for assessing oil toxicity. Furthermore, there is a lack of comprehensive knowledge regarding the toxicity implications associated with oil degradation and formation of hydrocarbon oxidation products (HOPs). To address these knowledge gaps, we conducted a multidisciplinary study that integrated analytical chemistry, toxicology, developmental biology, and molecular biology. The study aimed to investigate HOPs generated from

laboratory-simulated spills of Cook Inlet crude oil using a solar simulator. The quantification of HOPs was achieved through nonvolatile dissolved organic carbon (NVDOC) analysis and characterized with fluorescence excitation-emission matrix spectroscopy and high-resolution mass spectrometry. Traditional PAH analysis was also performed for comparison with previous studies. Pacific herring (*Clupea pallasii*) embryos, a species of significant economic importance, were exposed to different concentrations of HOPs (0.5-14 mg/L) using a novel semi-static microscale exposure design. Various morphological endpoints were examined, including pericardial edema, arrhythmia, body length, craniofacial deformities, and spinal deformities in the exposed embryos. Additionally, gene expressions related to xenobiotic metabolism and cardiac health were explored via quantitative polymerase chain reaction. We hypothesize significant differences in NVDOC and PAH concentrations, as NVDOC represents the entire complex mixture of oil, whereas PAHs are only a fraction of the mixture. The newly developed semi-static microscale bioassay offers potential advantages for toxicity experiments involving marine fish, as it does not require specialized equipment or large quantities of test chemicals. This study has implications for understanding the photo-induced toxicity of oil through the less understood photomodification pathway, as compared to photosensitization. Furthermore, we investigate the biological mechanisms of HOPs through the examination of morphological changes and gene expression. Overall, this study provides valuable insights into a multidisciplinary approach for advancing the understanding of oil toxicity.

1.04.T-02 Sensitivity of Six Species of Atlantic Scleractinian Corals to Petroleum Hydrocarbons

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Coral reefs are keystone coastal ecosystems at risk of exposure to petroleum hydrocarbons from a range of sources, including oil spill incidents and chronic runoff, and are one of the highest valued natural resources for protection following a spill. Due in part to the lack of standardized toxicity testing method for corals, previous oil toxicity studies on shallow-water scleractinians have not resulted in a clear characterization of impacts or relative sensitivity compared to other aquatic species. To address this important knowledge gap, a multi-year research program with broad stakeholder collaboration was designed to assess the relative sensitivities of six Atlantic coral species (*Acropora cervicornis*, *Porites astreoides*, *Porites divaricata*, *Siderastrea siderea*, *Stephanocoenia intersepta*, and *Solenastrea bournoni*) to single hydrocarbons (toluene, 1-methylnaphthalene, phenanthrene), MC252 oil, and chemically dispersed oil. Both acute (mortality) and sublethal effect endpoints emphasizing the coral holobiont, including physical/behavioral changes, photosynthetic efficiency, and differential gene expression were assessed with separate 48-h dose response assays. A species-specific range of stress responses were observed, including significant changes in physical condition, with less significant effects on the photosynthetic efficiency of the symbiont. Significant transcriptomic changes, including upregulation of genes associated with detoxification and depuration, occurred at hydrocarbon concentrations 4-8 times lower than exposures causing significant morphological changes. No latent effects were observed, and corals which survived the 48-h exposures recovered to baseline condition within 1-4 weeks. The Atlantic staghorn coral *Acropora cervicornis* was found to be the most sensitive to hydrocarbons, oil, and dispersed oil; the threatened status of this species under the Endangered Species Act is an important consideration in spill response decision-making. Overall, however, the acute and sublethal endpoints examined indicated that healthy corals are relatively resilient to petroleum hydrocarbon exposure compared to other coastal marine species. Although further work is needed to assess the influence of seasonal changes (temperature, light) on coral sensitivity, these results provide a framework for establishing toxicity thresholds in scleractinian corals and support science-based oil spill response decision-making in sensitive coral reef environments.

1.04.T-03 Quantifying Sediment Biodegradation Rates of Hydrocarbons: A Collaboration Between Industry and Academia

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Laboratory derived sediment biodegradation rates that mimic those in the natural environment are lacking in literature. Instead, sediment biodegradation rates are commonly calculated by applying a multiplication factor to water-only test systems or conducting a standard OECD 308: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems, which can be challenging for substrates that may sorb to organic carbon and become non-bioavailable. We designed, modeled and validated a novel sediment biodegradation method that accounts for sorption by incorporating passive dosing to quantify biodegradation rates of hydrocarbons in aerobic freshwater sediment. Natural river sediment was aerated for ~30 days to reduce background sediment oxygen demand and then combined with freshly collected oxygen-saturated river water at a 1:4 volume ratio in 60 mL amber glass vials. Mesocosms had 20 mL of headspace and were placed on a roller table at 20°C in the dark for 128 days. Eighteen individual hydrocarbons spanning 5 classes (C₆ – C₈; log K_{ow}: 3.8 – 7.8) were loaded as a mixture on to silicon rods (1 cm x 1.5 mm). Individual rods were placed in respective treatments and analyzed at 7 time points to determine chemical losses over time using GC-FID. Biodegradation rates and impacts of sorption were determined by comparing the chemical loss in biotic and abiotic treatments with and without sediment. Environmental analyses included oxygen, total nitrogen, total phosphorous, orthophosphate, sulfate, iron, and microbial biomass. Sediment was characterized using pH, total organic carbon, dry weight, and grain size. A chemical fate mass balance model was developed based on literature partitioning studies to predict mass transfer kinetics and then validated with empirical data. Results indicate that sorption kinetics influenced the bioavailability of specific hydrocarbons and their potential to biodegrade in the presence of sediment. This presentation will highlight the measured sediment biodegradation rates, compare observations to model predictions, and lessons learned from the Pilot Tests.

1.04.T-04 Freshwater Sediment Toxicity Evaluation from Meso-Scale Spill Tests of Diluted Bitumen and Conventional Crude

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Oil and gas development and transportation in many areas of the world, such as the oil sands region of Alberta, Canada, are heavily monitored to minimize the environmental impacts of development and the risk of oil spills. However, oil spills to aquatic environments still occur. Although the science of oil spills has received considerable attention of late, uncertainty still remains in evaluating the fate and transport of oil spills as well as the effects of those spills on aquatic biota. Experiments using meso-scale spill tanks were used to examine the physical and chemical behavior of two types of oil, conventional crude (CC) and diluted bitumen (DB), under similar environmentally relevant scenarios (i.e., volume of spill, temperature, duration, wave action, and presence of river sediment). In addition, biological impact assessments via sediment toxicity testing collected from the oil spill tests were conducted. Sediments were evaluated for acute toxicity using three standard sediment test species: *Hyaella azteca*, *Lumbriculus variegatus*, and *Chironomus dilutus*. Sediments collected from the CC simulated spill showed a higher level of acute toxicity than sediments collected from spills with DB. Higher toxicity in the CC-contaminated sediment was supported by higher concentrations of low molecular weight polycyclic aromatic hydrocarbons (PAHs) when compared with the DB-contaminated sediment, while the remaining PAH profile was similar between the contaminated sediments. The use of a meso-scale spill tank in combination with sediment bioassays allowed for the evaluation of oil spills under controlled and

environmentally relevant conditions (e.g., nearshore high sediment loading river), and in turn provides assessors with additional information to identify the appropriate mitigation and remediation efforts needed in the event of future spills.

1.04.T-05 Bridging the Gap Between Laboratory and Field Observations – the *Deepwater Horizon* Oil Spill as a Case Study

Martin Grosell, University of Miami

The 2010 *Deepwater Horizon* (DWH) blowout coincided spatially and temporally with the spawning window of several pelagic top predators. Mahi-mahi *Coryphaena hippurus*, “mahi” in the following) were one of the pelagic fish species likely affected by the DWH and may serve as a model for assessing the effects of oil exposure in pelagic species. A large scale and interdisciplinary effort involving many levels of biological organization allowed for comparison of effects observed at the gene expression level through habitat utilization, survival and reproduction of wild fish. Over the past decade, laboratory based studies of gene expression, physiology and behavior on mahi and other species revealed that oil exposure targets multiple organ systems including the heart explain observations of reduced cardiac output in intact, oil exposed, mahi. These impacts resulted in reduced maximal sustained swim speed (Ucrit) and reduced maximal metabolic rate (MMR) leading to reduced aerobic scope (AAS). In addition to the impacts of oil exposure on heart function, oil exposure affects sensory systems including olfaction and vision and impacts more complex behaviors indicative of central nervous system effects. Cardiovascular impacts, sensory impacts, and effects on more complex behaviors are sublethal under laboratory conditions and relating lab-based impact to wild populations is challenging. To examine if brief sublethal exposures to oil resulted in impacts, pop-up satellite archival tags (PSATs) were employed to examine post exposure behavior of wild mahi in the Gulf of Mexico. Compared to unexposed tagged mahi, reduced survival and spawning activity as well as altered habitat utilization was observed in oil exposed adult mahi. Gene expression data from wild adult mahi following brief oil exposures were in general agreement with data from oil exposed lab reared mahi and suggests impacts to cardiac and neuronal function. In conclusion, lab-based observations of sublethal effects offer predictive power for fitness of wild populations. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).

1.04.T-06 Building Field Effects Models from Toxicity Studies - One Compound at a Time

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Petroleum oils are complex mixtures of organic compounds of varying physical-chemical, and toxicological characteristics, and therefore, varying fates and effects on aquatic organisms. Weathering rates due to evaporation, dissolution, photo-oxidation, and biodegradation vary by compound according to volatility, solubility, compound structure, environmental conditions, and response activities. As a result of these complex processes, the concentrations and relative composition of the compounds in the water column are highly variable in space and time. Oil fate and exposure modeling aims to address the complexities of oil composition, weathering behaviour, and the distributions and behaviors of aquatic biota to estimate exposure histories, i.e., oil component concentrations and environmental conditions experienced over time. Several approaches with increasing levels of complexity have been and continue to be developed to predict adverse effects resulting from these exposures. At Tiers 1 and 2, toxicity-based screening thresholds for assumed representative oil component compositions are used to inform spill response and risk evaluations, requiring limited toxicity data, analytical oil characterizations, and computer resources. Concentration-response relationships are employed in Tier 3 to quantify effects of assumed oil component mixture compositions. Current state-of-the-science Tier 4 analyses combine predictions of spatial and temporal compositional changes during exposure with mixture-based

modeling, relying on summed effects of components using toxic units, to provide more realistic effects predictions. This review provides guidance for toxicological studies to inform the development of, provide input to, and validate Tier 4 aquatic toxicity models for assessing oil spill effects on aquatic biota. Since mixture compositions in toxicity tests do not represent field exposures, modelers rely on studies using single compounds to build toxicity models accounting for the additive effects of dynamic mixture exposures that occur after spills. Well-characterized whole oil bioassay data are used to validate and refine these models.

1.05.P-Tu Complexity of the Immune System and Challenges on the Applicability of Immunotoxicology to Risk Assessment

1.05.P-Tu-004 Identifying Chemical Hazards in Aquatic Systems: Validation of a Small Fish Model to Screen for Immunotoxic Chemicals

Rashidat Jimoh, Catherine Wise and Marlo K Sellin Jeffries, Texas Christian University

Anthropogenic activities that degrade water quality pose a substantial threat to the health of fish and other aquatic wildlife. Though the acute, chronic, and reproductive toxicity of environmentally-relevant chemicals tends to be prioritized for the purposes of environmental risk assessment, chemically-induced immunotoxicity can have adverse population-level consequences. Thus, there is a need to screen chemicals for immunotoxicity. However, appropriate models have not been developed for this purpose. The fathead minnow (FHM) has been identified as a potential model as they are easy to maintain in the laboratory, already used in general toxicity assessments, and have been used to evaluate the immunotoxic potential of nanoparticles, polybrominated diphenyl ethers, and model thyroid disrupting compounds. However, no study to date has shown that FHMs respond to known immunotoxicants in predictable ways, which is an essential step in developing this species as a standardized immunotoxicity model. To support the validation of the FHM as an immunotoxicity model, the objective of this project was to evaluate the impacts of dexamethasone on a variety of immune processes known to be altered following dexamethasone administration in other species (e.g., pathogen resistance, inflammation, etc.) in FHMs. This presentation will highlight the results of this study and provide key information regarding the utility of the FHM as a standardized immunotoxicity model organism.

1.05.P-Tu-005 Assessing Changes in Gene Expression and Immune Function During Immune Aging in the Medaka Model (*Oryzias latipes*)

Elizabeth DiBona, Daniel Duran and Frauke Seemann, Texas A&M University Corpus Christi

Chronic immune diseases increase with age and occurrence may be aggravated by environmental exposures. Inflammatory disease incidence increases as protective immunity decreases during immune aging. To demonstrate the effects of aging on innate immunity, Japanese medaka (*Oryzias latipes*) are used as a model to investigate sex-specific immune aging changes in immune gene expression and profiles of leukocyte populations. Immune bacteria challenge using *Edwardsiella tarda* is used to evaluate immune competence at the organismal level. Flow cytometry (FCM) is used to visualize populations of leukocytes and potential changes in these populations during aging. Changes in expression of immune initiators (CRP, TLR5-soluble, TLR5-membrane, TCRb, MHCII), immune mediators (MYD88, NF-kb, C3, IL1b), and immune effectors (Lysozyme C, C8) are assessed using real time-quantitative polymerase chain reaction (RT-qPCR). Data presented here demonstrate a significant reduction in immune competence of male medaka at 20 months post hatching; no significant changes in female immune competence or between sexes was observed. A reduction of the monocyte population with age is expected from FCM leukocyte population assessment. However, no significant changes have been observed between 7 month and 14 month post hatching (mph) male or female medaka. A critical decrease in expression of immune initiators (CRP, TLR5-soluble, TCRb, MHCII), mediators (MYD88, NF-kb, C3, IL1b), and effector (Lysozyme C) in both females and males after 11 mph. However, after 23 mph expression levels are not different from younger ages (3, 4, 5, 6, 7 mph) in females. Interestingly, both sexes had an upregulation for immune effector, C8, during this older age range (11-13 mph). Together

these data will provide a cellular and molecular profile for immune aging in medaka, a useful tool for future immunological studies considering age as a factor affecting disease susceptibility.

1.05.P-Tu-006 Investigating How Per- and Polyfluoroalkyl Substances (PFAS) Suppress Neutrophil Function

Emma Marie Wilber Hepworth, Ashley Connors, Drake W Phelps and Jeffrey A. Yoder, North Carolina State University

Per- and polyfluoroalkyl substances (PFAS) are widespread and persistent pollutants and can be detected in the serum of an overwhelming majority of people in the U.S. There is substantial evidence that PFAS alter immune function. Two particular PFAS, perfluorohexanoic acid (PFHxA) and ammonium perfluoro(2-methyl-3-oxahexanoate) (GenX), have been found to suppress the neutrophil respiratory burst, a critical process in the innate immune response. However, it is not yet known which aspect of neutrophil biology the PFAS are disrupting. One possible explanation is that PFAS exposure is altering cellular metabolism, interfering with the ability of the neutrophils to produce the reactive oxygen species (ROS) necessary to perform the respiratory burst. Compared to some other innate immune cells, the characteristic metabolic profile of neutrophils is primarily glycolytic, in part because glycolysis supplies the cells with the burst of energy necessary to rapidly produce ROS. In addition, RNA sequencing (RNA-Seq) data indicates that neutrophils exposed to PFHxA and GenX have differential expression of genes associated with certain metabolic pathways. Real-time cell metabolic analysis with an Agilent Seahorse Analyzer will be used to investigate if PFAS-exposed primary neutrophils and neutrophil-like HL-60 cells have altered cellular metabolism. These assays will focus on changes to glycolytic rate, oxidative phosphorylation, and mitochondrial dysfunction. Uncovering changes in the metabolism of neutrophils exposed to PFAS will improve understanding of the mechanisms behind PFAS-induced suppression of immune cell function and how this can contribute to increased susceptibility to infection.

1.05.T Complexity of the Immune System and Challenges on the Applicability of Immunotoxicology to Risk Assessment

1.05.T-01 Lethal and Sublethal Effects of 6PPD-quinone on Coastal Cutthroat Trout

Prarthana Shankar, Justin Greer, Ellie Dalsky, Rachael Lane and John Hansen, U.S. Geological Survey

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, a transformation product of 6PPD which is an antioxidant globally used in vehicles to significantly extend tire life and increase safety. Recently, 6PPD-quinone 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-quinone on this species has yet to be studied. Therefore, we are investigating the acute toxicity of coastal cutthroat trout to 6PPD-quinone at three life stages: early-stage alevin, young of the year juveniles, and 1+ year animals. Acute toxicity is measured as concentrations necessary to induce 50% mortality in 24 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 early-stage alevins exposed to 6PPD-quinone concentrations reported in streams impacted by stormwater degradation elicited mortality with a 24-hour LC50 concentration of 320.4 ng/L. Alterations in overall morphology or heart rate were not observed in surviving larvae. These preliminary results suggest that early-stage cutthroat trout could be negatively impacted by 6PPD-quinone exposure in urbanized watersheds. Current work is examining the susceptibility of later life stages in fish from the same population assessed as alevins. In addition to acute mortality, sublethal effects on immune system

function will be assessed including potential effects on innate immunity and disease susceptibility to opportunistic pathogens. Our results will provide data to inform the environmental risk assessment of 6PPD-quinone in the context of an economically and ecologically important fish species.

1.05.T-02 Modulation of Immune-relevant Transcriptional Profiles in Fish Tissues after Largemouth Bass Virus Infection

Tara Sabo-Attwood¹, Candice Lavelle², Sarah Robinson¹, Joseph H Bisesi¹, Amanda Buerger¹ and Nancy D. Denslow¹, (1) University of Florida, (2) U.S. Environmental Protection Agency

Viruses are the most abundant biological entities on the planet that impart both beneficial and detrimental outcomes. The number of identified viruses, particularly in aquatic systems, have soared over the past few decades but our understanding of the molecular mechanisms associated with infections in aquatic species have not kept pace. In addition, a wide array of environmental contaminants, including legacy and emerging compounds, have been shown to modulate the immune system of fishes, which could have significant impacts on viral susceptibility. Largemouth Bass Virus (LMBV) is a double stranded DNA virus that belongs to the family *Iridoviridae*. This virus has been detected in numerous fish species but causes mortality primarily in older and larger Largemouth Bass (LMB). This species can be highly sensitive to the virus based on a number of documented fish kills. Infected fish commonly exhibit lethargy, external and internal hemorrhages, organomegaly, buoyancy or equilibrium problems, and unique swim bladder lesions; however, little is known about how the host immune system responds to infection with LMBV on a molecular level. Unraveling such mechanisms could provide clues about the species specificity and pathogenicity of infection. To address this, we exposed LMB to 10^6 LMBV in the water and after 72 hours the swim bladder and kidney tissues were removed and processed for RNAseq analysis. Results of this experiment showed that 604 and 647 genes were differentially expressed in the kidney and swim bladder, respectively. Of these genes approximately 65% were annotated. In general there was a noted decrease in the expression of innate immune pathways that included interferon-regulated genes in both tissues. This is surprising, as interferon-regulated pathways are typically highly induced in response to a viral infection in order to mount an effective defense. The unexpected repression of key innate immune profiles may account for the sensitivity of LMB in particular to LMBV infection and observed adverse health effects. This study highlights the importance of understanding host immune responses to aquatic viruses that help to explain varied sensitivity across species and sets a foundation for examining viral infections in the context of multiple-stressor exposures that include chemical pollutants.

1.05.T-03 Utilizing Machine Learning to Automate Analysis of White Blood Cell Profiles in Largemouth Bass (*Micropterus salmoides*) and Smallmouth Bass (*Micropterus dolomieu*)

Jessica K Leet, Joseph Bretz, Lillian Bradshaw, Rachel A Claunch, Luke Iwanowicz and Thea Edwards, U.S. Geological Survey

Fish are sensitive to adverse conditions and changes in their environment. Stressors for fish can include disease, contaminants, high temperatures, and other environmental stressors related to climate change and anthropogenic activity. Various parameters can be used to assess changes in fish health resulting from stress, including changes in the relative abundance profiles of different white blood cell (WBC) types. However, the process of analyzing blood smears to determine the WBC profile is time-consuming and requires expertise to distinguish the different types of WBCs. We are developing a tool that automates WBC identification and counting to increase through-put and the ability of non-expert users to gain information about general fish health. A machine learning model has been trained to identify and count WBCs in largemouth bass (LMB) blood smear photos. This tool is being validated using blood smears from a study investigating the physiological and molecular mechanisms of antiviral immune response in smallmouth bass (SMB) compared to LMB. Polyinosinic:polycytidylic acid (poly I:C; $10 \mu\text{l g}^{-1}$) or sterile phosphate-buffered saline (PBS) was administered by intraperitoneal injection to juvenile SMB and LMB to mimic a viral infection and induce an antiviral immune response. Blood smears from ten fish per treatment per species were manually analyzed for WBC

profile and also analyzed by the model to compare the two methods. The results from this study indicated a shift in leukocyte profile in the poly I:C challenged groups in both species. There was a higher neutrophil: lymphocyte ratio compared to the PBS control groups, indicating an immune response was induced. The comparison also suggested a trend toward greater magnitude of the immune response in LMB relative to SMB. To our knowledge this is the first study to compare the antiviral immune response of smallmouth and largemouth bass, as well as the first tool of this nature to automate differential blood cell analysis in bass. The start-to-end workflow developed during the establishment and validation of this tool can be implemented for future automation of additional fish health parameters to further reduce bottlenecks in fish health assessment and increase access to fish health assessment by non-expert users.

1.05.T-04 Developmental Immunotoxicity of Novel and Emerging Per- and Polyfluoroalkyl Substances

Drake Phelps, Nia Moore, Tracey Woodlief, Qing Hu, Krystal Taylor and Jamie DeWitt, East Carolina University

Per- and polyfluoroalkyl substances (PFAS) are a class of more than 14,000 anthropogenic organofluorine compounds. As a class, they are used to create a variety of products, including non-stick cookware, fire-fighting foams, and food packaging, and many others. PFAS are persistent and ubiquitous, but toxicity data for many PFAS are limited. Exposure to PFAS that have been well-studied has been linked to cancer, endocrine disruption, developmental toxicity, liver toxicity, dyslipidemia, and impaired immune function. Our lab has reported that multiple PFAS, including mixtures of PFAS, suppress the T cell-dependent antibody responses (TDAR), an apical adaptive immune endpoint analogous to the vaccine response in humans. In this study, we sought to expand on previous work by asking whether developmental exposure to understudied PFAS in a murine model conferred suppression of the TDAR, as the developing immune system is particularly sensitive to effects of immunotoxicants. C57BL/6 female mice were crossed with C3H/HeJ male mice. Females were dosed from gestational day 1 through gestational day 17 to varying concentrations of different PFAS: perfluoro-2-methoxyacetic acid (PFMOAA), perfluorohexane sulfonamide (FHxSA), or Nafion byproduct 2. Concentrations were chosen to avoid overt maternal and fetal toxicity. After birth, B6C3F1 pups were raised and weaned at postnatal day (PND) 21. Spleen and thymus were collected on PND 28 and 56 for lymphocyte immunophenotyping. On PND 51, mice were immunized to stimulate the TDAR, and serum was collected on PND 56 to measure the TDAR. Preliminary data indicate that PFMOAA exposure altered thymus cellularity and that FHxSA exposure resulted in transient disruptions in development, as measured by postnatal weight gain, when compared to vehicle controls. Nafion byproduct 2 exposure resulted in no observable phenotypes in this model. None of the tested compounds suppressed the TDAR in adult mice after developmental exposures; however, as none of the tested compounds were detectable in serum collected from offspring at PND21, it is possible that internal concentrations during gestation were insufficient to affect the developing immune system. Future studies will work to determine if exposure to other understudied PFAS or PFAS mixtures affects immune parameters after developmental exposures.

1.05.T-05 Per- And Polyfluoroalkyl Substances (PFAS) Impact Macrophage Function in vitro

Ashley Connors, Emma Marie Wilber Hepworth, Jacob Driggers and Jeffrey A. Yoder, North Carolina State University

Immune function can be impaired by environmental contaminants. One class of chemicals recently shown to interfere with the immune system is per- and polyfluoroalkyl substances (PFAS). Earlier work focused on impacts on the adaptive immune system, though disruptions to the innate immune system have also been identified. These studies indicate that PFAS exposure can influence the numbers of innate immune cells, cellular signaling, and functional endpoints. We are evaluating how macrophages are affected by a 2-day *in vitro* exposure to ten PFASs: perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), perfluorooctanesulfonic acid (PFOS), perfluorohexanoic acid (PFHxA), Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), Nafion Byproduct 2, perfluoro-2-

methoxyacetic acid (PFMOAA), and hexafluoropropylene oxide dimer acid (HFPO-DA or GenX). In single-PFAS cytotoxicity studies with human macrophage-like THP-1 cells, exposure to 320 μ M PFDA, PFNA, PFOS, and Nafion Byproduct 2 significantly reduced viability. We observed no changes in cell viability at or below exposures to 80 μ M PFAS. We are now investigating how exposure affects macrophage function. To test the effect of PFAS on phagocytosis, macrophage-like THP-1 cells are exposed to 80 μ M PFAS for 48 hours, then challenged with fluorescent heat-killed *E. coli*. Phagocytic index and number are measured with flow cytometry. Thus far, we have observed that PFOS and PFNA increase the average extent of phagocytosis. To our knowledge, we are the first to see PFAS-induced changes in phagocytosis using an immortalized cell line. Additionally, we are measuring cytokine production by both unstimulated and stimulated THP-1 macrophages. Based on these functional assays, 2-3 PFASs will be selected for further studies to elucidate currently unknown molecular mechanisms of PFAS immunotoxicity. Understanding how PFASs affect innate immunity will help us better understand how these chemicals can alter an organism's ability to recognize and destroy pathogens in its environment as well as infected or transformed cells.

1.05.T-06 Bioactivity Assessment of Environmental Waters Using Immune Cell Lines

Nora Hussain, Isaac Sanchez Montes, Mohamed Gamal El-Din and James Stafford, University of Alberta, Canada

Oil sands process-affected water (OSPW) is generated as a by-product during bitumen extraction. While OSPW is stored in man-made reservoirs on mining sites and is reused in subsequent collections, freshwater must be supplemented in to ensure effective bitumen extraction. This results in a continuously expanding volume of OSPW, which varies in composition based on company protocols, location, or age of the OSPW, for example. Currently, there is no consensus regarding best practices for remediation of OSPW, although government mandates require OSPW to be eventually treated and released into environmental ecosystems. Our lab has employed a cell-based assay to examine immune-activating elements in waters. Macrophages are an immune cell type found in all vertebrates that rapidly respond to foreign challenges in a host and are present at external sites of the body such as the skin, digestive tract, and gills. When applied to *in vitro* research, macrophage cell lines have been shown to reliably and sensitively react to environmental stimuli in rivers or industrially produced treated wastewater. Through the examination of markers associated with pro-inflammatory phenotypes, we demonstrate differential responses of treated and untreated OSPW through antimicrobial and immunomodulatory components. These components provide a range of pro-inflammatory responses per sample, which is referred to holistically as bioactivity. As contemporary treatment of OSPW has focused on the organic fraction, which encompasses toxicologically relevant constituents such as naphthenic acids, we examined treated OSPW using our macrophage cell lines. Interestingly, by comparing two untreated OSPW samples sourced from different oil sands operators, we find that an OSPW containing high naphthenic acids sustains elevated bioactivity across our battery of assays, while an untreated OSPW sample containing low concentrations of classical naphthenic acids (~10mg/L) demonstrates a differential response between antimicrobial production and pro-inflammatory signalling compounds. While further examination into physicochemical components differentiating OSPW samples is required, this preliminary work highlights the importance of approaching *in vitro* toxicity assays from different perspectives to ensure a comprehensive understanding of bioactivity from industry-sourced water.

1.06.P-Mo New Approach Methodologies, Enhanced Strategies and Best Practices for Identifying and Evaluating Endocrine System Adverse Effects

1.06.P-Mo-005 Lessons Learnt from Three Years of Applying the XETA Test Guideline

David Du Pasquier and Gregory F. Lemkine, Watchfrog S.A.

The Organization for Economic Cooperation and Development (OECD) has set up a conceptual framework for the testing and assessment of endocrine disruptors (EDs). Different *in vitro* and *in vivo* standardized methods

(test guidelines, TG) are categorized into five levels of increasing biological complexity. Detection of thyroid active molecules is addressed by mammalian and amphibian testing. The mammalian testing strategy mainly relies on measurements of plasma concentrations of thyroid hormones (THs) as well as on histopathological analysis of the thyroid. Amphibian testing takes advantage of the THs' exclusive control of anuran metamorphosis. In June 2019, the OECD validated the *Xenopus* Eleutheroembryonic Thyroid Assay (XETA) in the test guideline program in order to support the identification of thyroid active chemicals. The XETA utilizes *X. laevis* eleutheroembryos to detect modulation of thyroid signalling by thyroid active chemicals. The assay is transcription-based and uses a transgenic tadpole line expressing the Green Fluorescent Protein (GFP) under the control of a promoter directly regulated by TH. The response measured is fluorescence of embryos. The technical transfer of the assay to contract research organisations (CROs) was a prerequisite to the application of the XETA, the test guideline has been easily implemented in several CROs in a short time frame. To date regulatory studies have been performed to assess the T-modality for approximately 30 chemicals using the XETA. During these studies, some practical points of consideration have appeared including how to consider sub lethal effects, affecting behaviour or obviously testifying of toxicity, in the definition of the Maximum Tolerated Concentration and how to ensure testing concentrations close as possible to the MTC. The objective of the present communication is to highlight these developments that allowed the use of the XETA in chemical assessment since its validation in 2019. The development and rapid implementation of the XETA into the regulatory assessment of chemicals has paved the way to developing assays using embryonic stages of aquatic vertebrates. These are ethical and novel tools that perfectly fit into the strategy of a mechanistic approach for the comprehensive assessment of endocrine activity on targeted EATS.

1.06.P-Mo-006 A Portfolio Screening and Prioritization Framework for Endocrine Disruption

Tim Verslycke¹, Ari S Lewis¹, Tatiana Manidis¹, Delina Lyon², Nicholas Synhaeve², George Hinkal², Leslie J Saunders², Sergio A Villalobos³ and Kat Colvin³, (1)Gradient, (4)Concawe, (3)BP

The European Commission (EC) developed its initial strategy for evaluating endocrine disruptors (EDs) over 20 years ago (COM(1999)706). Currently, the EC is in the process of adopting criteria for identifying EDs under different EU regulations. In 2023, the EC published a delegated regulation amending the EU's Classification, Labelling and Packaging (CLP) Regulation and introducing new ED hazard classes and criteria for the CLP of substances and mixtures; however, guidance on the application of the CLP criteria is not yet available. Therefore, how substances and mixtures will be assigned to these new ED hazard classes and how this might impact other chemical regulations (*e.g.*, REACH) is yet to be determined. Regardless, the potential impact of the CLP amendment to product value chains could be substantial, and there is a critical need for developing technically sound approaches to identify and evaluate ED activity and adversity, particularly across large product and substance portfolios. We developed a framework for efficiently compiling and evaluating information for large product and substance portfolios, considering the 2018 ECHA/EFSA guidance for the identification of EDs under the Biocidal Products Regulation or the Plant Protection Products Regulation. The identification of EDs under this guidance requires a rigorous weight-of-evidence analysis of "all available relevant scientific data," a resource-intensive evaluation that is not feasible to implement across a large portfolio. Therefore, we developed a stepwise, screening and prioritization framework: 1) identifying a list of substances of interest; 2) developing a search strategy to efficiently compile *in vitro*, *in silico*, and *in vivo* information related to estrogenic, androgenic, thyroid, and steroidogenesis pathways from database sources and the scientific literature; 3) employing a "heat map" approach to visualize the compiled information across sources and pathways; and 4) identifying priorities, uncertainties, and data gaps, and recommending next steps. Our framework was applied to a large and complex portfolio of >500 petroleum UVCB substances and hydrocarbon constituents. Our findings illustrate the challenges associated with compiling ED information using the 2018 ECHA/EFSA guidance, especially for complex portfolios. Our framework can be applied to other chemical/product portfolios to support initial ED screening assessments to identify key data gaps and inform decision-making.

1.06.P-Mo-007 Toxicokinetic-Toxicodynamic Modeling of the *Xenopus laevis* Thyroid Axis Using a Subset of Data-Rich Reference Chemicals

Jonathan Haselman and John Nichols, U.S. Environmental Protection Agency

A recently developed computational model of the *Xenopus laevis* hypothalamic-pituitary-thyroid (HPT) axis was used to support the interpretation of existing in vivo effects data for thyroid-disrupting chemicals. The model simulates the normal biology of the HPT axis in *X. laevis* larvae during a developmental period (early pro-metamorphosis) which corresponds to that associated with standardized toxicity testing protocols. Modeled outputs include thyroid hormone (TH) concentrations in plasma and the thyroid gland, gland size (i.e., thyroid follicular cell numbers), and circulating levels of thyroid stimulating hormone (TSH). Included in the model are mathematical relationships that represent several well-documented targets of thyroid disrupting chemicals, thereby providing for toxicodynamic (TD) simulation of chemical effects. The objective of the present study was to evaluate model performance by simulating HPT axis disruption caused by model chemicals known to disrupt the HPT axis through specific mechanisms of action. To address this objective, the HPT axis model was integrated with a toxicokinetic (TK) model that describes chemical uptake and accumulation resulting from a continuous waterborne exposure. The TK model borrows from an existing, physiologically based model of chemical uptake and accumulation in fish, and was informed by physiological information for larval amphibians. The chemicals chosen for this assessment were methimazole and 2-mercaptobenzothiazole, which are known inhibitors of thyroid peroxidase; perchlorate, a known inhibitor of the sodium-iodide symporter (NIS); and iopanoic acid, a known inhibitor of deiodinases (DIOs). Initial simulations were shown to qualitatively reproduce observed in vivo effects associated with these chemicals, including effective compensation mediated by changes in TSH. These results were then used to calibrate several parameters as a means of improving model performance. Given the global emphasis on reducing reliance on animal testing for chemical hazard characterization, the described (TK-TD) model shows excellent promise as a lower tier prioritization tool for endocrine disruptor evaluations. *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.*

1.06.P-Mo-008 The Development of Non-Lethal Methods for the Identification of Endocrine Disruption in Fishes

Emily Kennedy, David M. Janz and Markus Hecker, University of Saskatchewan

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. As these compounds often target steroidogenic pathways, circulating steroid hormone concentrations provide useful information for characterizing the effects of these compounds. Most commonly, quantification is accomplished via blood sampling; however, this is problematic as fish species often used to study endocrine disrupting compounds (EDCs), such as the fathead minnow, do not provide sufficient sample volume for analyses. Ethical concerns have also been raised surrounding the sacrifice of large numbers of animals both in lab and in field-based biomonitoring programs. As such, the objective of my research is to develop lab- and field-based techniques for non-lethal quantification of steroid hormones in fishes. Specifically this will include (1) the development of a liquid chromatography tandem mass spectrometry (LCMS/MS) method for the quantification of steroid hormones excreted into holding tank water, (2) the use of this method for hormone quantification in two additional non-lethal media: mucus and scales, (3) the application of the above methods in-lab for the screening of EDCs, and (4) the application of the above methods in field-based biomonitoring efforts to assess the effects of EDCs on wild fish populations. Overall, my work will highlight the importance of non-lethal approaches in understanding the impact of EDCs on fish populations as well as provide reliable and ethical methods for detecting potential EDCs that contribute to the preservation of aquatic ecosystems.

1.06.T New Approach Methodologies, Enhanced Strategies and Best Practices for Identifying and Evaluating Endocrine System Adverse Effects

1.06.T-01 Evaluation of Regulatory In Vivo Endocrine Assays in Fish and Amphibians: Current Status and Future Needs

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Endocrine pathways play a crucial role in regulating physiological functions in vertebrates, including growth and development, metabolism, tissue function, and reproduction. Exposure to endocrine disrupting chemicals (EDCs) can interfere with normal hormonal function and consequently lead to adverse effects in organisms and/or their offspring. As a result, regulatory testing and assessment requirements have been implemented to identify and/or provide protections from EDCs, but these approaches vary worldwide. Many regulatory entities have adopted the WHO definition for EDCs as those that alter the function of an endocrine system and cause “subsequent adverse effects in an intact organism, its progeny, or (sub)populations”. As such, the current assessment paradigm for EDCs involves initial testing for endocrine activity using *in vitro* and short-term *in vivo* assays, followed by higher-tier definitive *in vivo* testing to confirm endocrine activity and establish any consequent adversity. The Organisation for Economic Co-operation and Development (OECD) and the United States Environment Protection Agency (US EPA), among other entities, have validated and adopted multiple standardized *in vivo* test guidelines for mammals, fish, and amphibians. However, these tests can be highly complex and challenging to perform and many require the use of large numbers of laboratory animals. In this presentation, we summarise the current state of the science for evaluating the endocrine disrupting potential of chemicals in fish and amphibians using *in vivo* test guideline assays across the estrogen, androgen, thyroid, and steroidogenesis (EATS) pathways. Current challenges associated with conducting and interpreting the *in vivo* assays, including laboratory capacity and experience, concentration setting, and the need for better understanding of historical control data will be discussed. Opportunities and potential next steps to strengthen this growing area of testing will be discussed, including their potential to inform the development and application of new approach methodologies (NAMs).

1.06.T-02 The Rapid Estrogen ACTivity In Vivo (REACTIV) assay and the Rapid Androgen Disruption Activity Reporter (RADAR) Assay OECD TG251

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In June 2022 a novel level 3 OECD test guideline was published for the Rapid Androgen Disruption Activity Reporter (RADAR) assay, TG 251. It describes a 72 h *in vivo* assay for the detection of androgen axis active chemicals using transgenic medaka (*Oryzias latipes*) eleutheroembryos as a New Approach Methodology (NAM). A complementary assay for the detection of alterations in estrogen axis signalling is currently under OECD validation. The Rapid Estrogen ACTivity In Vivo (REACTIV) assay is based on the same concept as the RADAR assay, the use of transgenic medaka eleutheroembryos that reveal the level of activity of the hormonal axis of interest by the expression of GFP. In the case of the REACTIV assay, the axis of interest is the estrogen axis and its activity is translated into fluorescence using a portion of the *choriogenin h* gene promoter to drive the expression of GFP coding sequence under the control of liganded estrogen receptors. The use of aquatic

leutheroembryos allows quantification of the effects of test chemicals acting via a range of modes of action (MoA). The chosen reporter, the choriogenin h promoter, as a terminal step in estrogen axis signalling, indicates the net effect of disruption of the estrogen axis via one or multiple mechanisms. Laboratories from six countries took part in the REACTIV assay interlaboratory validation exercise. The chosen test chemicals covered a wide range of MoA. The transferability of the assay and its ability to identify chemicals acting on estrogen axis signalling, either at the receptor level or on downstream steroidogenesis was demonstrated. The six expected inert chemicals were all correctly identified as inactive on the estrogen axis. The results obtained by the lead laboratory for chemicals tested with the REACTIV and RADAR assays during the OECD validation exercises showed that all ten chemicals were correctly identified as active or inactive. Chemicals with different MoA showed differing response profiles in the two assays. The REACTIV assay is a 24 h assay that allows the quantification of estrogen axis activity. It provides crucial data on endocrine activity for a weight of evidence approach. By comparing the results in the presence and absence of testosterone the REACTIV assay can help to elucidate the MoA of a test chemical. A greater precision for identifying the MoA is achieved when the REACTIV assay is performed in parallel with the complimentary RADAR assay.

1.06.T-03 Adaptation of Sperm Quality Characterization Methods to Assess Reproductive Health of Male Mummichog (*Fundulus heteroclitus*)

Sabine Malik, Lance T Yonkos, Tyler Edward Frankel, Carys Louise Mitchelmore and Candice Duncan, University of Maryland

Historically contaminated urban rivers harbor significant loads of legacy persistent organic pollutants (e.g., dioxins, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons) within sediments, so remain a significant hazard to both human and ecosystem health. Of particular concern are endocrine disrupting compounds, as they can exert an undue influence on reproductive success and threaten the viability of native fish populations over time. Impacts to female reproductive competence such as delayed embryonic development and impaired hormone signaling have been found across fish taxa, but descriptions of sperm-related effects are more limited. One of the few persisting species in urban, contaminated ecosystems is *Fundulus heteroclitus*, a killifish often used as a toxicological model for its incredible adaptability and resilience. Using *F. heteroclitus* as a model, we describe a standardized, field-adaptable methodology for measuring aquatic sperm quality using three metrics: 1) *sperm motility* measured by computer-assisted sperm analysis (CASA); 2) *sperm activation capacity* measured by a firefly-luminescence ATP assay; and 3) *sperm DNA damage* measured by the Comet assay. While CASA methods have been used effectively in fish hatchery and aquaculture contexts (e.g., to assess fertilization competence after sperm cryopreservation), adaptation of such methods for purposes of investigating environmental contaminant effects has lagged behind. Similarly, the Comet assay has been used extensively in environmental toxicology, but, to our knowledge, never for fish sperm. Addition of sperm quality assessment methods to other measures of *F. heteroclitus* reproductive competence (e.g., ovarian and testicular histopathology, gonadosomatic index, fecundity) can provide a more holistic measure of endocrine disruptor-mediated adverse effects to aquatic species.

1.06.T-04 Supporting Use of New Approach Methodologies for Endocrine Disrupting Chemicals through Development of Adverse Outcome Pathways

Gerald T. Ankley, Kelvin Santana Rodriguez, Kathleen Jensen, David Miller and Daniel L. Villeneuve, U.S. Environmental Protection Agency

Screening and testing of potential endocrine-disrupting chemicals for ecological effects can directly benefit through use of adverse outcome pathways (AOPs) to establish linkages between alterations in endocrine function measured using new approach methodologies and whole organism- and population-level responses. Of greatest interest from a regulatory perspective are processes controlled by the hypothalamic-pituitary-gonadal/thyroidal axes (HPG/T). However, the availability of suitable AOPs is currently limited in terms of species and life-stage representation relative to the diversity of endpoints influenced by HPG/T function. This

presentation will describe two novel AOPs that comprise a simple AOP network focused on the effects of chemicals on sex differentiation during early development in fish. One AOP initiates with inhibition of cytochrome P450 aromatase (CYP19), resulting in decreased availability of 17 β -estradiol during gonad differentiation, which inhibits the development of female gonads, resulting in a male-biased sex ratio and consequent population-level declines. The second AOP is initiated by activation of the androgen receptor (AR) during sexual differentiation, also resulting in a male-biased sex ratio and population-level impacts. Both AOPs are strongly supported by existing physiological and toxicological evidence, including numerous fish studies with model CYP19 inhibitors and AR agonists. These two AOPs provide a basis for more focused screening and testing of chemicals with the potential to affect HPG function in fish during early development. *The contents of this presentation neither constitute, nor necessarily reflect, official US EPA policy.*

1.06.T-05 Fixed-Stage Termination: Debating the Attributes and Pitfalls in Amphibian-Based Endocrine Disruption Testing

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Presently, endocrine disruption (ED testing programs under the guidance of USEPA and OECD considers two amphibian-based ecotoxicological assays, the Amphibian Metamorphosis Assay (AMA) and the Larval Amphibian Growth and Development Assay (LAGDA). Although it is not yet incorporated into the test guidance, an extended AMA (EAMA) has also been developed and is currently being validated by several sources. The AMA is a 21-d exposure assay initiated with Nieuwkoop and Faber (NF) stage 51 *Xenopus laevis* larvae, and thus offers a non-fixed stage termination. The LAGDA is a 10 weeks pos-metamorphosis assay using *X. laevis* that incorporated two phases of the lifecycle, embryo-larval-metamorphic and juvenile development. Metamorphosis is evaluated in part by assessment of the time required to reach NF stage 62 which is immediately post-metamorphic climax, and thus is a fixed stage terminal measurement in a subset of the developing specimens. The EAMA is performed similarly to the AMA, starting at NF stage 51 and terminating at NF stage 62, and thus also supports a fixed stage termination. Clearly, there is scientific movement toward using a fixed-stage termination in the amphibian-based ED assays primarily due to ease in stage-matching specimens used in histopathological assessment. However, before a consensus approach can be reached, further discussion of the attributes and pitfalls of each approach is needed. As an introduction to these discussions, case studies will be presented that illustrate how physicochemical factors such as feeding and temperature, and biological factors such as genetic variability in stage morphology and rate of development (TTM), can potentially impact thyroid histopathology results.

1.06.T-06 Assessing the Predictive Value of Thyroid In Vitro Screening Assays Through Comparisons to Observed Impacts In Vivo

Stephanie Eytcheson¹, Jennifer Olker², Katie Paul Friedman², Michael Hornung² and Sigmund Degitz², (1)Oak Ridge Institute for Science and Education (ORISE), (2)U.S. Environmental Protection Agency

The U.S. EPA's Endocrine Disruptor Screening Program (EDSP) was developed to investigate the potential for chemicals to interact with the vertebrate endocrine system and to characterize adverse effects resulting from these interactions. Since inception of the program, new approach methodologies (NAMs), such as *in vitro* high-throughput screening (HTS) and *in silico* modeling, have become available. One challenge of HTS is translating activity *in vitro* to predict an adverse effect *in vivo*. The objective of this work was to analyze whether the current suite of thyroid relevant HTS assays provides sufficient lines of evidence supporting action on receptors and proteins in the thyroid axis. The fifty-two chemicals screened in EDSP Tier 1 assays were selected for this analysis. The *in vitro* data used in this evaluation were mined from the *in vitro* assay database (invitroDB version 3.4), and the *in vivo* data were pulled from the weight of evidence evaluations prepared by the EDSP and from a literature review. Decision trees were made to classify chemicals as active *in vivo* only, *in vitro* only, *in vivo* and *in vitro*, or inactive to surmise concordance between *in vitro* and *in vivo* activity. With this framework, concordance between *in vitro* activity and *in vivo* impacts ranged from ~60-80%. Limitations of

thyroid HTS were identified including the lack of assays for some known molecular initiating events within the thyroid system (*e.g.*, serum binding proteins), limited availability of orthogonal or confirmatory assays, and lack of a set of reference chemicals. Addressing these limitations would enhance the predictive value of HTS assays and improve our understanding of how well thyroid activity *in vitro* predicts to *in vivo* effects of chemicals for which *in vivo* data are unavailable. *The contents of this abstract neither constitute, nor necessarily reflect, US EPA policy.*

1.07.P-Th Microbial Metagenomics: An Emerging Tool for Predictive Ecotoxicology

1.07.P-Th-015 Increasing Throughput of Full-length 16S Sequencing Utilizing Concatenation

Jeremy Wilkinson¹, Khi Pin Chua¹, Siyuan Zhang¹, Jason Underwood¹, Minning Chin¹, Wei-Shen Cheng¹, Sian Loong Au¹, Primo Baybayan¹, Holly Ganz², Guillaume Jospin², Ye Tao³, Qin Lin³ and Elizabeth Tseng¹, (1)PacBio, (2)AnimalBiome, (3)Biozeron Biotechnology Co., Ltd.

In the past several years, the ability to capture the full-length 16S rRNA gene with PacBio HiFi sequencing has enabled the profiling of microbiomes with significantly higher resolution. Only full-length and highly accurate 16S sequences can robustly identify the broad range of bacteria seen in complex microbial communities at the species level, without bias. To further increase the cost effectiveness of full-length 16S sequencing, we applied the multiplexed array sequencing (MAS-Seq) method (Al'Khafaji et al., 2021, Biorxiv) to 16S amplicons. The MAS-Seq method is a versatile throughput increase method that takes advantage of the longer HiFi read lengths to concatenate smaller amplicons into ordered arrays with programmable array sizes. We demonstrated that MAS-Seq could be applied to 16S amplicons with an ~8 – 12-fold throughput increase. We tested the method on a diverse range (11 types) of samples such as mock communities, feces, guts, soil, and water. We then analyzed the data using a user-friendly bioinformatics pipeline based on Nextflow called “pb-16S-nf” that was developed to provide a FASTQ-to-report analysis solution for full-length 16S HiFi reads (MAS and non-MAS). Comparing the concatenated (16S MAS-Seq) to non-concatenated full-length 16S datasets, we found no bias in community compositions and were able to assign up to 90 – 99 % of reads to species. In addition, on the highly complex ZymoBIOMICS Fecal Reference with TruMatrix Technology (D6323) sample, we found full-length 16S to have high correlation to taxonomic abundances estimated from shotgun metagenomics sequencing using the same sample emphasizing that it's possible to get shotgun metagenome taxonomic resolution at amplicon sequencing costs with full-length 16S HiFi sequencing. Furthermore, with 16S MAS-Seq, researchers may now multiplex more samples to reduce cost/sample or to profile each sample deeper with more reads/sample. The additional reads/sample along with better taxonomic resolution is advantageous for numerous environmental sample types which often are highly diverse, containing many microbial species.

1.07.P-Th-016 Impacts of Erythromycin and an Antibiotic-Mixture on Juvenile Rainbow Trout Gut Microbiome

Phillip Ankley¹, Jonathan Karl Challis¹, Pu Xia¹, Yufeng Gong^{1,2}, Yuwei Xie³, Emily Kennedy¹, Katherine Raes¹, Catherine Roberts¹, Milena Doreen Esser¹, John Giesy¹, Markus Hecker¹ and Markus Brinkmann¹, (1)University of Saskatchewan, (2)University of Toronto, (3)Nanjing Institute of Environmental Sciences

Erythromycin (ERY) is a commonly used antibiotic that can be found in wastewater effluent globally. Due to their mechanism of killing and preventing bacterial growth, antibiotics may have significant unwanted impacts on beneficial microbial communities such as fish gut microbiome. The project was split into two experiments to assess gut microbiome and fish health in response to exposure with ERY alone or in mixture with other common antibiotics. The objectives of experiment 1 were to understand the uptake and depuration of ERY in rainbow trout and profile juvenile rainbow trout (RBT) gut microbiome response to ERY. In brief, juvenile RBT were exposed via diet to three concentrations of ERY and one solvent control in six replicate tanks (5 fish/tank). The exposure period lasted 7-d followed by a 7-d depuration period with mid-gut tissue, bile, and plasma being collected for downstream analysis using 16s rDNA metabarcoding and analyte measurement. In

experiment 2, a follow-up study was conducted to assess the impacts of an antibiotic-mixture (ERY, ampicillin, metronidazole, and ciprofloxacin at 100 µg/g each) using the identical experimental design as described for Experiment 1. Here, three matrices and three datasets were generated, with guts collected for 16s metabarcoding, plasma for metabolomics, and brain for mRNA-seq analysis. For experiment 1, ERY was removed rapidly from the fish, and an elevated amount of ERY metabolite was detected in the plasma at 7-d. ERY-treated fish had an overall increase in Proteobacteria in the 1000 µg/g treatment, and Bifidobacterium shunt pathway was reduced indicating a reduction in fermentation ability. For experiment 2, *Carnobacterium* and *Gallicola* were detected as biomarker genera and remodeling of the gut microbiome and plasma metabolome during growth was detected. Several metabolites and genes were found to be dysregulated in the plasma and brain, with larger impacts due to ERY (1000 µg/g) compared to the antibiotic-mixture. The results of this study will help inform aquaculture and risk assessors when assessing the potential impacts of antibiotics in fish feed and the environment, with implications for host health. Future steps include linking multi-omics datasets through functional analysis to better understand the impacts on host health.

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

1.07.T-01 Evaluating Metagenomic Analyses for Undercharacterized Environments: What's Needed To Light Up The Biological "Dark Matter"?

Kelsey Thompson, William A Nickols, Eric A Franzosa and Curtis Huttenhower, Harvard T.H. Chan School of Public Health

Environmental microbial communities play important roles in nutrient cycling, human disease, and ecosystem health maintenance. However, because they are more diverse and less taxonomically characterized than most host-associated microbial communities, they remain much less understood. Here, we assessed the impact of sequencing depth, lack of previous characterization, and diversity in environmental communities using a variety of typical, state-of-the-art metagenomic analysis methods. While the methods performed similarly at high taxonomic levels, the newest marker-based tools (MetaPhlan 4 and mOTUs 3) performed much better than others at low taxonomic levels, likely due to the inclusion of material from metagenomic assembled genome bins (MAGs) in their reference databases. Assembly-based methods were most impacted by sample complexity and sequencing depth, and accuracy at higher taxonomic levels decreased for all tools when more uncharacterized species were present (even when high-level taxonomies for those species were available). Despite these challenges, reference-based tools still recovered accurate community-level information, including within-sample diversity and between-sample similarity. In real environmental data including soil and ocean samples, taxonomic profiles assigned to the same sample varied tremendously by tool, with very little agreement at lower taxonomic levels using either weighted or unweighted metrics. What similarity did exist depended strongly on the type of algorithm employed, with marker-based, k-mer, and assembly methods showing more agreement with tools of their own type than between types. However, as in simulated data, even disparate methods agreed substantially about between-sample similarity structures. Despite this, taxonomic profiles from different tools yielded very different estimates of the relationships between environmental parameters and microbial profiles in those environments. Although taxonomic assignment tools have become more accurate and informative as their databases have incorporated more environmental genomes, major work still remains in enhancing and harmonizing taxonomic profiling and assignment methods to fully enumerate environmental microbial community membership.

1.07.T-02 Application of Metatranscriptomics to Assess the Role of Gut Microbiota in the Development of Obesity upon Early-life Exposure to Environmental Antibiotics

Monika Mortimer, Zhi Li and Liang-hong Guo, China Jiliang University

Environmental contamination by antibiotics inevitably causes chronic human exposure to low levels of antibiotics. Yet its consequences on the development of metabolic diseases, including obesity, remain unclear.

Experiments in mice have shown that sub-therapeutic antibiotic treatment (STAT) can cause weight gain, alter gut microbiota, and increase adiposity. Specifically, early-life exposure to antibiotics appears to induce the most significant effects both in animals and humans. However, the causality of the STAT effects on microbiota and obesity-related pathophysiology of the host is yet to be established. The development of new-generation sequencing platforms and bioinformatics tools has opened new horizons for elucidating functional changes in microbiota under environmental pollutant stress. Here, metatranscriptomics was applied to establish a relationship between the antibiotic-induced functional changes in the gut microbiota and the obesity-related pathophysiology of the murine hosts after early-life STAT via drinking water. The effects of commonly prescribed therapeutic antibiotics (clindamycin, clarithromycin, penicillin, ciprofloxacin and vancomycin) were compared. The results showed that after 7-week exposure, followed by a 3-week recovery period, none of the five antibiotics had a significant effect on the total body weight of mice but two antibiotics, clindamycin and penicillin, significantly increased the weight of adipose tissue and adipocyte size. All antibiotics caused lipid accumulation and inflammation in the liver. Clindamycin, specifically, increased free fatty acid and lipopolysaccharide content in the serum, hormone peptide YY and adipokine leptin in the intestine, and inflammatory markers (TNF- α , IL-1 β , IL-6 and IL-8) in the liver and intestine. Expectedly, all antibiotics decreased the abundance of gut microbiota, while clindamycin and penicillin also reduced the microbial diversity. Correlation analysis identified several bacterial taxa that were positively or negatively associated with the host obesity and inflammatory markers. The bacterial functions affected by the two antibiotics included lipid and amino acid metabolism, two-component systems, secretion system, and quorum sensing. This study indicated that antibiotics differ in their potency in inducing obesity in mice upon early-life exposure, while clindamycin and penicillin were the most potent in causing adverse effects among the five antibiotics.

1.07.T-03 Impacts of Antimicrobial Exposure on the Gut Microbiome of Early-Life Stage Fish: A Chemical and Species Comparison

Phillip Ankley, Mawuli Amekor, Evan Kohlman, Catherine Roberts, Yutong Zhou, Pu Xia, Alper James Alcaraz, Markus Hecker, Markus Brinkmann and Natacha S Hogan, University of Saskatchewan

Antimicrobials are contaminants of emerging concern as they are continuously discharged via wastewater effluent into aquatic environments where they may negatively affect fish due to their pseudo-persistence. Antimicrobials may alter fish gut microbiomes which serve central roles in the overall health of fishes. This study assessed the impacts of two antimicrobials on early-life stage rainbow trout (RBT) and lake trout (LT) gut microbiomes. Embryos were exposed from hatch to 28 days post-hatch to six graded concentrations of triclosan (TCS) and chloroxylenol (PCMX). Excised whole gut was collected and 16S rDNA amplicon sequencing of gut microbiome was applied to investigate responses to antimicrobials. Gut microbiome of RBT was dominated by phyla *Proteobacteria* and *Bacteroidota* and genera *Aeromonas* and *Acinetobacter*, whereas LT was dominated by phyla *Proteobacteria* and *Firmicutes* and genera *Aeromonas* and *Ahrensia*. Significant changes in Shannon diversity were observed in TCS-exposed RBT, with PCMX-exposed RBT having a marginal (trend but not significant) response. A marginal change in Shannon diversity was also detected for TCS-exposed LT. TCS caused significant changes in both RBT and LT community structure as detected by weighted Unifrac analysis. Three genera correlated with measured concentrations of TCS for RBT while no genera were significantly correlated for LT. Overall, TCS had the largest impact on gut microbiomes of exposed fish and the RBT gut microbiome had a greater response to antimicrobials than that of LT. Results indicate species-specific response of the gut microbiome to contaminants. This study also provides insights into the potential effects of emerging antimicrobials on alevin salmonid gut microbiome with implications on physiological health status of the host, including nutrient utilization and immune defense, using functional prediction of the amplicon data.

1.07.T-04 Deciphering Early-Life Microbial Communities in Childcare Environment Using Long Read Sequencing

Marina Chen¹, Ya Wang¹, Kelsey Thompson¹, Jeremy Wilkinson², Eric A Franzosa¹, John D Spengler¹ and Curtis Huttenhower¹, (1)Harvard T.H. Chan School of Public Health, (2)PacBio

Early-life exposure to microorganisms plays a crucial role in shaping children's health by instructing immune maturation and modulating the risk of disease development. Most preschool-aged children spend seven to ten hours a day in childcare centers, yet the childcare microbiome and how it interacts with the host microbiome have yet to be fully elucidated. A limited number of previous studies have primarily focused on bacterial communities, but often using amplicon-based profiling methods that omit functional or genetic information. Other microbial community members such as fungi have been even more understudied in this context. Additionally, none of the prior research has incorporated host-associated phenotypes and microbial profiles to investigate transmission between host and childcare environment. In this study, we collected a variety of indoor and outdoor environmental samples from two childcare centers, as well as nasal and oral swabs from 34 participating children of age two to four. We profiled these using PacBio full-length amplicon sequencing targeting the 16S rRNA gene for bacterial community members and the internal transcribed spacer (ITS) region for fungi. This provided enhanced resolution to identify previously unknown aspects of the microbial communities in childcare environments. Preliminary results revealed distinct microbial profiles associated with different host-associated and environmental communities and also identified specific microbial taxa that were shared between environmental sites and host communities. These shared elements were detectable based on the precision of full-length amplicon sequence variants, suggesting potential microbial transmission and interactions. Our work thus expands the understanding of microbial ecology in built environments and these communities' relevance to child health, particularly the complex interplay between children and their surrounding environments. The knowledge gained from this study can allow us to identify potential environmental reservoirs of pathogens, track microbial transmission routes, and develop targeted interventions to benefit early-life human health.

1.07.T-05 Low-Level Short-Term Exposure of Mallards to Microcystin-LR Affects Cecal Virome and Microbiome Function

Serguei V Drovetski, Valerie I. Shearn-Bochsler, Erik K. Hofmeister, Kenan Matterson and Robert J. Dusek, U.S. Geological Survey

During recent decades, the frequency of cyanobacterial harmful blooms appears to have increased in fresh and brackish waterbodies globally reportedly due to synergistic effects of anthropogenic eutrophication and global climate change. Microcystins comprise the most frequently detected group of toxins produced by cyanobacterial blooms. They are potent hepatotoxins and with >250 currently known congeners, microcystin-LR (MC-LR) appears to be the most abundant and toxic for wildlife. The knowledge of MC-LR avian toxicity is limited. The 50% Lethal Dose (LD50) concentration have been estimated only for MC-LR intraperitoneal injections (adult mallards *Anas platyrhynchos*; 0.085 mg/kg body weight; 96.4% MC-LR and 3.6% MC-YR) but not for oral exposure (no acute outcome ≤ 17.5 mg/kg of MC-LR). Nonetheless, oral exposure studies have reported sub-lethal effects of MS-LR on various avian organs, including liver, kidney, spleen, and reproductive and immune systems. In laboratory murines, MC-LR exposure further have been shown to induce dysbiotic changes of prokaryotic gut microbiota. No studies of MC-LR effects on avian microbiota have been reported to our knowledge. To identify such potential effects on mallard microbiota, we sampled cecal content of 16 fully grown (0.8 – 1 kg) juvenile female mallards following a week-long oral administration of 0.75 mg MC-LR/day ($n = 8$) or placebo ($n = 8$). RNA extracted from these samples was sequenced on Illumina NovaSeq to a median of 121,129,328 reads/sample. We detected no changes in richness or composition of prokaryotes, fungi, protists, virulence factors, or antibiotic resistance genes. Although there were no differences in virome richness between exposure categories, we detected significant changes in the virome composition (PERMANOVA based on Bray-Curtis dissimilarities, $F_{(1, 14)} = 1.941$, $r^2 = 0.122$, $P = 0.028$). These changes were related to a decline of a

single commensal viral strain and proliferation of two strains, one causing duck hepatitis Aa and the other a common upper respiratory infection in mammals and birds. Furthermore, we also detected decrease in metabolic activity (number of metabolic pathways expressed) of cecal microbiome ($F_{(1, 14)} = 6.967$, $r^2 = 0.285$, $P = 0.019$) despite relatively low dosage of MC-LR and short exposure period. Natural cyanobacterial bloom duration varies between several weeks to a few months, so, future experiments should use longer exposure periods similar to natural ones.

1.07.T-06 Shifts in Insect and Riparian Spider Microbiome Communities Across the Aquatic-Riparian Interface in a Lake with Elevated Copper Concentrations

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Microbial communities living in or on a host comprise an organism's microbiome and play diverse and critical roles in host organism biology. Copper is a well-known antimicrobial agent, and while an essential micronutrient, excessive exposure can alter a host organism's microbiome. Some aquatic insects have extended life stages in the benthos where they may utilize copper contaminated sediments before undergoing metamorphosis and emerging as terrestrial adults. Riparian predators such as tetragnathid and araneid spiders are useful bioindicators of aquatic ecosystem condition because they specialize in the predation of adult aquatic insects. Torch Lake, an Area of Concern in Michigan, USA, has an extensive history of copper mining and, as a result, has elevated copper concentrations in the sediments. Here, we demonstrate a significant alteration of the larval insect, adult insect, and spider microbiome community in organisms living in and adjacent to Torch Lake. The microbial community composition of whole benthic and emerged insects and spiders collected from Torch Lake were significantly different than the same taxa collected from the reference lake. Further, we observed increased relative abundance of endosymbiotic bacteria in aquatic adult insects compared to larvae. Our results demonstrate legacy impacts of historical mining activities on the host microbiomes of aquatic-riparian food webs, which may impact other processes as insects and spiders provide critical subsidies of energy and material fluxes at the land-water interface.

1.08.P-Tu-008 Leveraging Multi-Omics to Elucidate Mechanistic Pathways Associated with Developmental Exposures to Perfluorooctane Sulfonate (PFOS) and Perfluorohexane Sulfonate (PFHxS) in Mummichog

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Many per- and polyfluoroalkyl substances (PFAS) are highly persistent in the environment and have been associated with adverse health effects in a variety of species. However, the mechanistic pathways through which PFAS induce toxicity and the hazards posed to wild fish populations are not yet fully characterized. In this study, we assessed developmental toxicity and measured multi-omic endpoints in mummichogs (*Fundulus heteroclitus*, Atlantic killifish) exposed to perfluorohexane sulfonate (PFHxS) or perfluorooctane sulfonate (PFOS) to investigate the perturbed biological pathways underlying apical organismal effects. An important ecological model, mummichogs are an estuarine fish amenable to morphological and behavioral phenotyping and have a well-annotated genome. Mummichog embryos were aqueously exposed to PFHxS (1.0, 10, and 100 μM) or PFOS (0.93, 9.3, and 93 μM) from one day post fertilization (dpf) until 7 dpf. RNA sequencing (Novaseq platform; Novogene) was conducted at 3 or 4 dpf and at 10 dpf, and metabolomics profiling at 10 dpf. Additionally, developmental endpoints such as morphology, heart rate, growth, swim bladder development and larval light/dark behavior were evaluated between 10-30 dpf. Assessment of multi-omic endpoints preceding or coinciding with apical effects, will contribute to a holistic understanding of PFAS toxicity and the underlying mechanisms. RNA-seq data analysis revealed that exposure to 10 and 100 μM PFHxS induced robust gene expression changes at 3 dpf, and 1 μM at 10 dpf. PFOS exposures elicited fewer differentially expressed genes

at 4 dpf compared to 10 dpf. Biological processes related to metabolism, ion transport, DNA replication, and lipid transport and localization were disrupted. Despite transcriptional changes, neither PFAS induced gross organismal-level effects. PFHxS caused a subtle increase in abnormal heart phenotypes at 10 dpf (9%, 24%, and 26% compared to 3% incidence in controls) and both chemical exposures led to subtle behavior effects at 24 dpf. Abnormal heart and behavior phenotypes concur with previous PFAS studies in freshwater fish species; the lack of gross morphological effects may warrant further investigation into sensitivity differences between species. Multi-omics integration is currently underway and will contribute to our understanding of PFAS ecotoxicity, elucidate modes of action leading to subtle organismal effects, and inform efforts to predict population-level impacts.

1.08.P-Tu-009 Fathead Minnow Omics Resources for Comprehensive Toxicological Assessment

Weichun Huang, David Bencic, Robert Flick and Adam Biales, U.S. Environmental Protection Agency

The fathead minnow (FHM) has become an important model organism for environmental toxicity tests. The public availability of the high-quality FHM genome reference from our group has spurred a variety of toxicogenomic applications for environmental health risk assessment. To further facilitate omics/multi-omics applications for toxicological assessment using FHM, we are building a central deposit of FHM genomic resources essential for toxicological studies. Besides the common genomic reference data available at NCBI/Ensembl, our deposit has the latest version of gene annotation data improved with PacBio Iso-Seq data and newly generated annotation data such as cis-elements, CpG islands, and CpG methylation data. The latest version of gene annotation has been substantially improved in terms of number of full-length transcripts, which can help interpretate RNA-seq transcriptome data better. Particularly, this version has more completed 3' UTRs annotation, enabling to use Quant-Seq, a more cost-efficient transcriptome profiling assay than a regular RNA-seq, for large-scale exploration studies. The newly generated epigenome data (e.g., CpG methylation data) could provide DNA methylation references for gene regulation interpretation and for imputation of missing methylation data, facilitating studies to identify epigenetics markers or detect multigenerational/transgenerational exposure effects using omics technologies such as Methyl-seq or RRBS. Additionally, this central deposit includes resource data in application-specific formats for some popular omics analysis tools such as RnBeads for epigenetic methylation data or BMDexpress for benchmarking dose-response analysis. These tools were mainly designed for studying human health and have built-in omics-resource data for mammalian model species, but they often do not support ecological model species such as FHM. Our deposit can make it easier to use these tools for ecological health assessment. We are continuing to update and expand FHM resources in the deposit, hoping it'll become a free one-stop shop of FHM genomic resources for omics-based toxicological assessment.

1.08.P-Tu-010 Multi-Omics Assessment of Toxicological Responses of Fathead Minnows to 17 α -ethynylestradiol (EE2) Exposure

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Environmental Protection Agency

17 α -ethynylestradiol (EE2) is a widely used estrogenic chemical present almost ubiquitously in aquatic environments throughout the United States and many other countries. EE2, an endocrine disrupting compound, can disrupt hormonal homeostasis, leading to developmental disorders and other diseases. Exposure to EE2 is known to induce in male fish the expression of vitellogenin, a precursor protein of egg yolk normally only expressed in female fish. Though transcriptional responses induced by EE2 have been documented, the underlying genetic and epigenetic mechanisms for exposure responses are not well understood. Using a multi-omics approach, this study aims to provide a comprehensive assessment of the molecular responses across different omics layers and tissues. Male fathead minnows (FHM) were exposed to EE2 at two different doses 2.5ng/L (low) or 10ng/L EE2 (high) for 48 hours. For the high-dose exposure, two additional groups of fathead minnows were depurated for 7 and 14 days, respectively. Two unexposed control groups were included in this

study: the male one for negative control and the female one for positive control. Using RNA-seq we detected changes in gene expression profiles between control and treatment groups including tissue-specific changes in both liver and brain tissues. Additionally, using reduced representation bisulfite sequencing (RRBS), we identified tissue-specific epigenetic changes immediately after exposure and after depuration. Combining both types of data, we assessed overall toxicological responses from EE2 exposure and evaluated if exposure effects were temporary/reversible or potentially long-term/irreversible. The results showed that EE2 exposure generally reduced overall CpG methylation level in male FHM, which was consistent with the observed trend toward increased expression level across genes in treatment groups exposed to EE2. We also saw tissue specific differences in the responses between brain and liver tissue. Tissues differed in the kinetics of their responses both during the exposure and in depuration. Though most response returned to pre-exposure levels during the depuration, several epigenetic changes remained, suggesting a sustained effect.

1.08.P-Tu-011 Bottom-up Proteomics Analysis for Adduction of the Broad Spectrum Herbicide Atrazine to Mammalian Histone Proteins

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Chemical pollution remains a poorly understood human-driven environmental change, and the impact is largely unknown for humans exposed to mixtures of over 350,000 chemicals registered for industrial use. Proteins are the direct mediators of cellular processes and (potential) targets of chemical contaminants. There is estimated to be 20,000 proteins in the human proteome. Proteins can covalently adduct at active amino acid residues with endogenous or exogenous chemicals including drugs, pesticides or their metabolites. Longer term research has demonstrated that these protein-adducts might lead to multiple health issues. Therefore, identification of xenobiotics adducted to key proteins and identification of the sites of adduction within the protein are important for a better understanding of events underlying diseases and chemically-induced adverse reactions. Xenobiotics can react with multiple proteins at multiple sites and identification and characterization of adducted proteins are complicated by analytical challenges. Atrazine (2-chloro-4-[ethylamino]-6-[isopropylamino]-1, 3, 5-triazine) and is commonly and mainly used for control of broadleaf and grassy weeds. Histones are the major proteinaceous component of chromatin in eukaryotic cells and an important part of the epigenome. However, to our knowledge, there has been no research on the formation of atrazine adducts with histone. In this study, a bottom-up proteomics analysis method was optimized and applied to identify histone adduction by atrazine *in vitro*. Whole histones of calf thymus or human histone H3.3 was incubated with atrazine. After solvent-based protein precipitation, the protein was digested by trypsin /Glu-C and the resulting peptides were analyzed by high-performance liquid chromatography–tandem high resolution mass spectrometry (UHPLC-MS/MS). The resulting tryptic/Glu-C peptide of DTNLCAIHAK from calf thymus or human H3.3 was identified with an accurate mass shift of +179.117 Da in atrazine incubated samples. A chemical group with elemental composition of C₈H₁₃N₅ (179.1171 Da) from atrazine adducted with calf thymus or human histone H3.3. MS/MS analysis confirmed that the adduction position was at its cysteine110 residue. Time and concentration dependent assays also confirmed the formation of histone H3.3 covalent adduct with atrazine *in vitro*. Thus, the potential exists that atrazine adduction may lead to the alteration of histones that subsequently disturbs their normal function.

1.08.P-Tu-012 Cmap-ES: Connectivity Mapping with Enrichment and Semantic Analysis

Rong-Lin Wang, David Bencic, Adam Biales, Robert Flick, Morgan Hu and Tom Purucker, U.S. Environmental Protection Agency

Connectivity mapping (Cmap) interrogates a library of whole transcriptomic profiles with a signature query of multiple genes and links their associated chemical or biological conditions based on the similarities in gene expression. Being data-driven, Cmap has many eco-health applications including chemical grouping, read-across, ecological forensics, and discovery of mechanisms of action (MOAs). However, signature queries often yield noisy results of numerous profile hits with seemingly unrelated conditions, which complicates

interpretation. Such an interpretation can be facilitated by post-Cmap enrichment and semantic analysis (Cmap-ES), where significant structural/functional categories (e.g., <https://pubchem.ncbi.nlm.nih.gov/>) or OBO (Open Biological and Biomedical Ontology; <https://obofoundry.org/>) terms previously mapped to profile hits are identified. Of particular interest is the CHEBI (Chemical Entities of Biological Interest) ontology. In its latest release, there are over 160,000 logically related chemical entities in the “Molecular Structure” sub-ontology, and about 24,000 of which are also assigned to the “Role” sub-ontology for their roles in biology, chemistry, or human applications. These “Role” terms are highly informative of MOAs. As such, the Cmap-ES not only clarifies profile hits by framing their associated conditions in a well-established chemical/biological context, but it also provides additional insights by including both the enriched “Role” terms and their semantically similar siblings. More significantly, since these enriched or semantically similar CHEBI terms are also embedded in hundreds of interconnected OBO ontologies covering a wide range of biological domains and levels of organization, they will lead to a broadened understanding of chemical/biological conditions underlying the original Cmap queries. The Cmap-ES approach has been applied to a diverse set of signature queries against the latest Cmap library. Custom categories and CHEBI terms enriched in Cmap hits were found to be highly consistent with and informative of the field and laboratory conditions associated with those queries, both chemically and biologically. Subsequent semantic similarity analysis of enriched CHEBI “Role” terms provided further insights into the MOAs likely implicated in those conditions. Our findings demonstrated the values of leveraging public knowledge bases by enrichment and semantic analysis to augment the widely adopted Cmap method.

1.08.P-Tu-013 High-Throughput Transcriptomic-based Points of Departure for Data Poor Chemicals Detected in the Great Lakes Basin

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Contaminants of emerging concern (CECs) have been detected in surface waters around the globe, including within the Great Lakes (USA) basin, exposing aquatic biota to a diversity of anthropogenic chemical mixtures. Prioritization of CECs for monitoring and management depend on available toxicity data. However, among 550 chemicals detected in Great Lakes tributaries, there were over 140 for which no empirical toxicity data were available to support prioritization. The aim of the present study was to generate protective points of departure (POD) that could be used for screening level prioritization of these compounds. A selection of 12 frequently detected chemicals for which toxicity data were lacking were screened in high-throughput assays employing three species (*Pimephales promelas*, *Daphnia magna*, and *Raphidocelis subcapitata*) representing different trophic levels (secondary consumer, primary consumer, primary producer). Organisms were exposed for 24 h to nominal exposure concentrations ranging from 66.7 to 0.021 μM , tested in a $\frac{1}{2}$ -log dilution series for each chemical. In addition to observations of apical effects such as survival and morphology, whole body transcriptomic response to each chemical was evaluated in *P. promelas* and *D. magna* with targeted analysis and in *R. subcapitata* with full RNA-seq. Benchmark doses (BMDs) for each gene showing a concentration-dependent expression pattern were calculated. The overall transcriptomic POD (tPOD) for each chemical-species combination was defined as the 10th centile of the BMDs. The tPODs ranged from 0.18 to 10.8 μM for *P. promelas*, with the most potent of the chemicals tested being fipronil carboxamide. For *D. magna*, tPODs ranged from 0.002 to 29 μM , with 3,4-dichlorophenyl isocyanate as the most potent. Empirically derived tPODs were compared with concentrations detected in the Great Lakes basin. This work demonstrates the potential utility of emerging ecological high-throughput transcriptomics assays to support timely screening and prioritization of data poor environmental contaminants. *The contents of this presentation neither constitute, nor necessarily reflect, US EPA policy.*

1.08.P-Tu-014 High Throughput Transcriptomic-Based Points of Departure Across Modes of Action in Larval Fathead Minnow (*Pimephales promelas*)

Kevin Flynn, Jenna E Cavallin, Michelle Le, Monique Hazemi, Adam Biales, David Bencic, Brett R Blackwell, Kendra Bush, Robert Flick, John Hoang, John Martinson, Mackenzie Morshead, Kelvin Santana Rodriguez, Emma Stacy and Dan Villeneuve, U.S. Environmental Protection Agency

The US EPA has published a blueprint for computational toxicology focused on the use of untargeted, high content assays, including transcriptomics, in a tiered manner to efficiently characterize hazard for large numbers of chemicals. To help ensure that ecosystems are considered and protected in the tiered screening framework, the US EPA has been developing high throughput transcriptomic-based assays using several aquatic species including larval fathead minnows for the evaluation of chemical hazard. As part of assay evaluation, 12 representative insecticides, estrogen receptor agonists, and herbicides have been tested. Larval fathead minnows (24 h post-hatch) were exposed to ten different concentrations of each test chemical in 1/2-log dilutions, in standard deep-well, 96-well microplates followed by whole-body RNA extraction for sequencing of the transcriptome. Concentration response curves were fit to the resulting transcriptomic data to calculate gene-specific benchmark concentrations. The distribution of 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 the chemical exposure. The resulting tPODs were compared with traditional hazard effect metrics. The rank order of potency (low to high) for the tested chemicals, within mode of action, was fenthion, parathion, then methidathion (acetylcholinesterase inhibitors), bisphenol A, 4-nonylphenol, then estrone (ER agonists), halofenozide, methoxyfenozide, then tebufenozide (ecdysone receptor agonists), and finally simazine, atrazine, then cyanazine (herbicides). The calculated tPODs ranged from 0.1 µg/L for estrone to 0.39 mg/L for fenthion. Upon correction for free concentrations measured in the exposure wells, tPODs for all chemicals, except estrone, were lower than reported apical effect concentrations, suggesting tPODs provide a protective effect threshold for use in risk-based screening and prioritization. *The contents of this abstract neither constitute, nor necessarily reflect, official US EPA policy.*

1.08.P-Tu-015 Stress Biomarker Associations with Phthalate Ester Exposure in Two Species of Captive Delphinids

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Phthalate esters (PAEs) are emerging contaminants of great concern due to their extensive use as plasticizers and additives in consumer products. PAEs are endocrine-disrupting chemicals and have been associated with adverse health effects including anomalies in reproductive, developmental, renal, and immune systems. PAEs can bioaccumulate and biomagnify through the food chain, posing a risk to living organisms, especially long-lived top predators. Therefore, this study investigated exposure to six PAEs during different life stages in two species of captive delphinids (*Tursiops truncatus*; n = 36; 13 individuals, and *Orcinus orca*; n = 42; 14 individuals) located at SeaWorld Parks in Orlando, FL, San Antonio, TX and San Diego, CA, USA. Blood samples were collected from 1994 to 2020 from each individual's central fluke vein using a 19-gauge needle and syringe into vacutainers containing activated thrombin. Serum was separated from the whole blood by centrifugation and stored at -80° C. Information on sex, age, maturity, pregnancy and parturition events were also considered to evaluate PAEs' variability among demographic profiles as well as potential transference from mother to calf. PAEs analyses involved the addition of dichloromethane as the extraction solvent and labeled standards, and a clean-up process with sodium sulfate and florisil. PAEs were determined using gas chromatography-mass spectrometry. Hormones and oxidative stress biomarkers were analyzed using commercial assay kits. Results indicate that all six PAEs were detected in at least one of the delphinids. Mean ΣPAEs were 11-fold higher in killer whales compared to bottlenose dolphins. DEHP and DEP had the highest mean concentrations in both species: 305 and 381 ng.mL⁻¹ in bottlenose dolphins and 2892 and 1482 ng.mL⁻¹ in killer whales, respectively. All PAEs were observed in at least one of the calves after birth, indicating placental

transference. An increase in PAEs concentration after birth also indicates lactational transference in both species. No correlation between PAEs and any of the hormones or stress biomarkers were found, indicating there are likely other factors responsible for biomarker variations in cetaceans. Continued monitoring of PAEs and other emerging contaminants of concern in bioindicator species such as delphinids is crucial for gaining a deeper understanding of contaminant variations over time and demographic states, as well as how it affects individuals physiologically.

1.08.P-Tu-016 Multi-Omics Analysis Pipeline for Toxicological Exposure Data Integration and Visualization

Olivia Torano, Adam Biales, W. Matthew Henderson and Weichun Huang, U.S. Environmental Protection Agency

The measurement of multiple biological endpoints during toxicological exposure studies has become common practice. Integration of multiple-layer omics data including transcriptome, epigenome, and metabolome is essential to comprehensively assess the biological responses of an organism to environmental exposures. While many multi-omics tools have been developed, selecting and applying the optimal tool(s) for data integration and visualization remains a challenging bioinformatics task. To address this, we are developing a multi-omics integration pipeline for integrated analysis of omics data from popular platforms such as RNA-seq, RRBS-seq, and MS-LC. We used both simulated and actual benchmark data to assess performance of multiple methods/tools for each analysis step, including preprocessing and statistical integration. Based on these assessments, we were able to select the best methods/tools for toxicological exposure data analysis. As an example, we successfully tested our integrated analysis approach using *in-house* exposure data from the well-known endocrine disruptor 17 α -ethynylestradiol (EE2). Our results suggest that an integrated analysis is the better approach in assessing exposure effects than individual analyses. Our pipeline also integrates data visualization functions to facilitate rapid data interpretation and hypothesis generation. Our pipeline is being implemented with R and Linux shell script languages and will be freely available to the public.

1.08.P-Tu-017 Delving into the Depths: Illuminating the Microbial Landscape in Abu Dhabi's Wastewater through High-Resolution WGS-based Taxonomic Profiling

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Tracking metagenomic abundance in wastewater is undoubtedly a powerful tool to detect emerging variants and improve community health. However, there are a few factors that limit environmental water-based genomic monitoring: sampling variability, incomplete coverage, genetic fragmentation, degradation, data analysis and interpretation. The decreasing costs of high-throughput sequencing and high-end supercomputers, added to the processing capabilities of G42 Healthcare have greatly increased the use and accuracy of genomic data for wastewater sample detection and monitoring within the region. To better understand the microbial dynamics and to determine the target sequencing depth required to establish taxa that may pose as bio-indicators of an epidemiological outbreak, wastewater samples were collected from different locations within the Emirate of Abu Dhabi, United Arab Emirates using appropriate sampling methods. DNA was extracted using commercially available kits following the manufacturer's protocol. Samples were sequenced for 5Gb and 10Gb using the DNBSEQ G400 genetic sequencer from MGI, China. Kraken's K-mer based approach was used to provide the taxonomic classification of sequencing reads while Bracken was used for computing the relative abundance of microorganisms at a phylum, genus, and species level. The results showed that as little as 15% of data in each sample corresponded to any of ~34,000 known bacterial, viral, fungal, or protozoan species. Despite the relatively high fraction of unclassified data (85%), more than 2,000 species from >800 phyla across >30 genera were detected in each sample. Both 5Gb and 10Gb of sequenced data detected the top ~2,000 species with highest abundance. Doubling the target sequencing depth (i.e., 10Gb vs 5Gb) detected ~500

additional low-abundance species per sample however it did not affect the overall sample composition or translate into higher per-sample species diversity captured. There was a marginal increase in the number of species detected in each sample beyond 0.20Gb of classified data. Overall, the results indicate that sequencing to a 3 Gb depth detects nearly 95% of all species in the samples.

1.08.P-Tu-018 Screening for Emerging Contaminants in Soil, Dust, and Food in Miami Area Using Non-Targeted Analysis and Chemometrics: Implications to Children's Health and Risk Assessment

Luciana Cappellini, Olutobi Daniel Ogunbiyi Sr, Mymuna Monem, Emily Mejias, Piero Gardinali, Florence George, Daniel Bagner and Natalia Quinete, Florida International University

Environmental exposure to contaminants can occur through different routes, such as soil, sediment, water, food, and dust. Because they are present in our daily lives, these matrices can be vectors of contamination, especially in children. Children can be exposed to chemicals by playing outdoors, eating contaminated food, and even indoor house dust. To be able to fingerprint contaminants of emerging concern in environmental matrices, we have applied a non-targeted analysis (NTA) approach previously developed in our lab using liquid chromatography high resolution mass spectrometry (LC-HRMS). NTA allows for the comprehensive screening of a variety of samples without a prior knowledge on chemical composition and/or pollution sources, allowing the detection of a wide range of previously "unknown" chemicals, which might not be usually monitored by traditional targeted analysis methods. In this project, samples of soil, dust, food, water, and urine from children were collected in different regions of the city of Miami-FL from 05/2022 to 05/2024, representing local environmental exposure scenarios. After collections, soil, and dust samples were extracted by ASE (Accelerated Solvent Extraction), an extraction method that uses pressurized solvents to extract compounds from a solid matrix efficiently, and food samples were extracted by QuEChERS (Quick, Easy, Cheap, Effective, Robust and Safe), a sample extraction and cleaning technique widely used in the analysis of residues in food and other complex matrices. Urine and water were processed using online solid phase extraction (SPE)- LC-HRMS, an automated and high throughput sample preparation technique needing with very little pre-treatment steps. Samples were analyzed by LC-MS/HRMS/Orbitrap in positive and negative full scan mode and MS2. Data post-processing was performed in the Compound Discoverer software v3.3, which aligns components across samples, assigning molecular formulas, identifying possible molecular structures and proposing tentative compounds based on available databases and libraries. Our results will bring a better understanding of children's health risks associated with daily exposure to emerging contaminants in the environment and their diet and they are critical information for risk assessments and for the development of environmental protection guidelines.

1.08.P-Tu-019 Targeted and Untargeted Metabolomics For Deriving Benchmark Doses (BMDs) in Fathead Minnows

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Metabolomics is an emerging tool to predict changes in an organism's pathophysiological state prior to the onset of overt toxicity. Although useful for providing insight into biochemical changes in ecological toxicity studies, the use of metabolomics data in the regulatory arena has been limited. Cited pitfalls include, but are not limited to, the inability to derive accepted 'regulatory' benchmarks from untargeted metabolomics data such as LOAELs or NOAELs. In the current study, we utilized both untargeted and targeted gas chromatography high resolution mass spectrometry (GC-HRMS) data to derive benchmark doses (BMDs) in fathead minnows exposed to several contaminants of emerging concern (i.e., pesticides, plasticizers, and pharmaceuticals). Across all contaminants, fathead minnow larvae (5 dpf) were exposed to 10 concentrations identified in range finding studies for 24 hrs. In the non-targeted analyses, the spectral features were used, in the absence of putative metabolite identification, as the input data and using BMDExpress 2 to establish

chemical-specific points of departure (POD). All spectral features were then annotated post-statistical identification and the top metabolites subjected to biochemical pathway analysis. For targeted approaches, an *in-house* library containing >200 metabolites was developed and identified metabolite peak areas were used in a similar method as described above. The metabolites in the curated library spanned >20 biochemical pathways including the urea cycle, gluconeogenesis, glycolysis, and amino acid metabolism. In the targeted approach, BMDs were calculated based on the top biological pathways impacted containing at least three altered metabolite signatures. BMDs calculated across the studied contaminants were all compared to their aquatic life benchmarks, based on traditional organismal endpoints, collected from the US EPA's Ecotoxicology (ECOTOX) Knowledgebase as well as species-specific transcriptomic-based PODs when available. Further, current efforts in our laboratory are aimed at comparing BMDs derived from transcriptomics, metabolomics and behavioral assays. These data highlight the potential integration and use of GC-HRMS untargeted and targeted metabolomics data in ecological risk assessment practices.

1.08.P-Tu-020 Exploring the Developmental Proteome and Life-stage Specific Sensitivities of Larval Zebrafish to a Model Toxicant

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Zebrafish (*Danio rerio*) are a popular model organism in human health, vertebrate development, biomonitoring, and aquatic toxicology with estimates of more than five million used annually for research purposes. Due to their high degree of genetic similarity with humans (>70% of human genes have a zebrafish ortholog), ease of culture, high fecundity, sensitivity, and well characterized genome, zebrafish have become increasingly incorporated within in vivo screening platforms and early life stage studies such as the fish embryo toxicity (FET, OECD no. 236) test. As many governments are not protective of early developmental life stages in non-mammalian vertebrates, embryonic fish models are just one of the many new approach methodologies (NAMs) poised to shoulder the burden created by phasing out mammalian animal testing. Here we leveraged prior research that observed life stage-specific mortality and behavioral sensitivities to diphenhydramine (DPH), a model pharmaceutical toxicant with well conserved molecular targets between humans and zebrafish. Because proteome characterization is limited in zebrafish, proteomic expression differences were first explored across early larval developmental timepoints (4-, 7-, and 10-dpf) using data independent acquisition (DIA) proteomics. When ontology of differentially expressed proteins was performed, genes associated with morphogenesis, angiogenesis, and neural development showed differences in expression across developmental age. Thereafter, dose-dependent responses to DPH were examined, and we identified >3200 proteins (at 1% false discovery rate), resulting in differential expression of proteins implicated in chemoattraction, neuron generation, cardiac development, and ion gated channel activity. By contrasting changes in whole-body protein expression across different developmental and exposure conditions, this study provides a basis to further examine the implications of how temporal sensitivities affect response to toxicants throughout the fish life cycle. Further, as protein interactions can be conserved across species, ongoing work is identifying human orthologues to differentially expressed proteins identified in zebrafish, to identify the suitability of using this model to explore age-specific sensitivity across species.

1.08.P-Tu-021 Gene Expression-Based Dose Response Analysis of Short-Term Rat and Fathead Minnow Exposures to Two Metal Sulfates Indicates Chronic Chemical Potency

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Benchmark dose modeling of gene response from short-term chemical exposures can identify points of departure consistent with chronic adverse effects. Widespread use of this approach could enhance the efficiency of human and ecological risk assessments. Moreover, an understanding of species-specific differences in gene response could aid in risk translation of chemicals. The present effort measured gene response in whole fathead minnow larvae and archival rat liver and lung tissue following 1- or 15-day exposures, respectively, to cupric

sulfate (CuSO₄) and nickel sulfate (NiSO₄). The fathead minnows were exposed to 9 concentrations plus control (n=8) of CuSO₄ (0.00005 to 0.2 mg/L) or NiSO₄ (0.00016 to 1.58 mg/L) in water whereas adult rats (male and female) were exposed to 4 dose-levels plus control (n=5/sex) of CuSO₄ by ingestion (90-770 mg/kg) or NiSO₄ by inhalation (3.5-15 mg/m³). For the rat studies, tissue type was selected based on the National Toxicology Program pathology reports. Gene expression was measured by RNA-sequencing followed by benchmark dose/concentration (BMD/C) analysis in BMDE_{Express2}. Individual dose/concentration responsive genes were then mapped to gene-sets (e.g., associated with pathways or functional annotations) with the median of the most sensitive defining the BMD/C for comparison with traditional, chronic measures of toxicity. BMC analysis of fathead minnow genes revealed CuSO₄ was more potent than NiSO₄ with values at 0.0021 and 0.053 mg/L, by total metal respectively. This corresponded with the chronic no observed effect concentrations for total copper and nickel of 0.0093 and 0.11 mg/L, respectively. The gene-set based BMDs for rat was 158 mg/kg for CuSO₄ and 0.91 mg/m³ for NiSO₄. These values were also consistent, if slightly less sensitive than the most sensitive 2-year BMD values of 33 mg/kg for CuSO₄ (stomach hyperplasia) and 0.11 mg/m³ for NiSO₄ (tooth malformation). These results suggest utility in using gene response to estimate chronic chemical potency. The views in this abstract do not represent the US EPA.

1.08.P-Tu-022 Comparing Sublethal and Lipid Peroxidation Response of *Cerestoderma edule* to Water Accommodated Fractions of Fresh Crude Oil in a Tidal and Stagnant System

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The continuous existence of an organism at the individual level relies on its capacity to screen its environment for stressors. A corresponding response is expected in behaviour, biochemical or physiological effects. This study aims to mechanistically investigate the sublethal response of the marine invertebrate, the common cockle (*Cerestoderma edule*), in acute toxicity exposure to the water-accommodated fractions (WAFs) of crude oil (1: 40, o: w): 0%, 0.41%, 3.7%, 11.11%, 33.33%, and 100%. Experiments were performed under stagnant and tidal conditions at 12°C. The effects of WAFs on the survival and behavioural activities (burrowing, siphoning, and t-half of the filtration rate) were analyzed during a 72 h exposure period at 1,5 h, 24 h, 48 h and 72h. *C. edule* were fed with *Nannochloropsis oceanica* (DW = 28.29%) at 72 h to investigate filtration activity. At the end of the exposure period, no mortality and no significant effect were observed for burrowing in both systems. However, a change in response was observed in the siphoning activity during the first 1.5 h of the tidal and stagnant system. At 1,5 h siphoning activity was poorer for *C.edule* in the higher treatments (11.11% -100 %) WAFs in both systems. *C.edule* in the stagnant system also siphoned less. Filtration activity followed same pattern in both systems however, it was observed that *C.edule* in the highest treatment cleared algae three (3) times lesser than cockles in the control and other treatment. Lipid peroxidation was determined on the gland tissues using a thiobarbituric acid reactive substance (TBARS). Lipid peroxidation in gland tissues of cockles in the tidal system was higher than in the stagnant condition. The outcome of this study suggests that *C. edule* can use avoidance behaviour to escape the acute toxicity of the WAFs of fresh oil in a stagnant system but may become a challenge under a tidal condition.

1.08.P-Tu-023 Metabolomics Study of Effects of Pollutant Mixture Released from Grafted Adipose Tissues on Organs of Mice

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Adipose tissue that has accumulated persistent organic pollutants (POPs) may have an impact on the body's metabolism. To investigate the metabolic alterations brought on by internal exposure to POPs in mice skeletal muscle (soleus, plantaris, and gastrocnemius), kidney, heart, and lungs, a metabolomics investigation was conducted using gas chromatography-mass spectrometry (GC-MS). POP mixtures at the lowest-observed-adverse-effect levels (LOAELs) of 0, 5, and 15 were injected into male donor mice. After grafting the host mice with their adipose tissue (AT), which included the POP, the metabolic changes were seen after 3 or 21 days. A

dysregulation of lactic acid, creatinine, and the amino acids, including alanine, 5-oxoproline, glutamic acid, and glycerides, was seen in the skeletal muscle of mice that had POP-contaminated AT transplanted into them. Dysregulated metabolites like lactic acid, phenylalanine, tyrosine, glutamic acid, and fatty acids have been found in the kidney of mice with kidney grafts. Lactate, phosphate, free fatty acids, and glycerides were down-regulated in the heart, while lactic acid was up-regulated in the lungs and aspartic acid, l-glutamic acid, l-alanine, l-valine, and l-isoleucine were down-regulated. With the aid of multivariate analysis, it was possible to identify how POPs affected the chemical alterations in the transplanted mice. The effect of increasing POP dosage from 5 and 15 did not lessen the regulation of the various metabolite in the tissues for any of the organs. However, there is a shift in the regulation and the kind of the differential metabolites considerably influenced by the POP combination during a longer duration of exposure from 3 days to 21 days. For pairwise comparisons between the control and each of the treated groups, the majority of groups may be easily distinguished from one another, proving that the male mice's skeletal muscle, kidney, heart, and lungs are significantly impacted by the pollutant combination discharged from grafted adipose tissue.

1.08.P-Tu-024 EE2-Induced Differential Isoform Usage in Fathead Minnows

Janine Fetke, Olivia Torano, John Martinson and Adam Biales, U.S. Environmental Protection Agency

The synthetic estrogen, 17 α -ethynylestradiol (EE2), is well-established as an endocrine disrupting chemical (EDC) associated with reproductive problems in fish including decreased fecundity, sperm production, and feminization of males. Estrogen receptor alpha (*esr1*) expression is inducible by EE2 exposure in male fathead minnows (*Pimephales promelas*), however the role of alternative splicing to result in differential gene isoform usage in response to exogenous EDC exposure is less understood. Occurring in >95% of multi-exon genes, alternative splicing leads to differences in isoform usage with potential functional consequences. Differential isoform usage is often specific to tissue type, sex, or developmental stage and has important roles in many biological processes. In this study, we use RNAseq data generated from brain and liver tissues from mature fathead minnows exposed to EE2. We employ isoform analysis tools to predict differential isoform usage and compare usage to control male fish and untreated females. The purpose of this study was to determine the effects of EE2 exposure on isoform usage in *esr1*, as well as to detect sex- and tissue-specific differences in isoform usage and predict potential consequences to protein function. Our results indicate that tissue-specific isoform switching occurs in *esr1* in liver from male fathead minnows treated with EE2. Male fish switched from using an isoform which is missing exon 1 and the A/B domain it encodes (including AF-1) to using an isoform more similarly structured to the isoform dominantly used by female fish.

1.08.V Advanced Omics Applications in Systematic Precision Toxicology

1.08.V-002 Environmental Ribotoxic Insult Compromises Dysbiotic Gut Aging in Worms and Mammals

Yuseok Moon, Pusan National University

Upon exposure to environmental insults, ribosomes stand sentinel. In particular, stress-driven dysregulation of ribosomal homeostasis is a potent trigger of adverse outcomes in mammals. The present study assessed whether the environmental ribosomal insult affects the aging process via the regulation of sentinel organs such as the gut. Analyses of the human aging dataset demonstrated that elevated features of ribosomal stress are inversely linked to barrier maintenance biomarkers during the aging process. Environmental ribotoxic insult-exposed worms displayed reduced lifespan, which was associated with the disruption of gut barriers. Mechanistically, xenobiotic ribosomal stress-activated Sek-1/p38 signaling, a central platform of environmental ribosomal stress responses, counteracted the gut barrier deterioration through the maintenance of the gut barrier, which was consistent with the results in a murine insult model. However, since the gut-protective p38 signaling was attenuated with aging, the environmental ribosomal stress exacerbated the toxic distress in the epithelia and mucosa of the aged animals, subsequently leading to increased bacterial exposure. Moreover, the bacterial community-based evaluation predicted concomitant increases in the abundance of mucosal sugar utilizers and

mucin metabolic enzymes in response to ribosomal insult in the aged host. All of the present evidence on environmental ribosomal insulting against the gut barrier integrity from worms to mammals provides new insights into organelle-associated translational modulation of biological longevity in a one health perspective (This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (2018R1D1A3B05041889), and the framework of international cooperation program managed by the National Research Foundation of Korea (NRF-2022K2A9A1A01098067 and FY2022)).

1.09.P-Mo Extrapolation Across Scales: Using Data and Models to Connect Molecular Data and Individual Level Responses

1.09.P-Mo-009 The Matrix, Reinterpreted

Matthew Etterson, U.S. Environmental Protection Agency

Matrix population models have been recognized for use in Ecotoxicology for over 50 years. They are quick to formulate, parameterize, and analyze. Despite many case studies demonstrating their utility, they remain rarely deployed for regulatory toxicology in the United States. More complex agent-based models, incorporating spatial heterogeneity, temporal variability, and exogenous stressors are superior to the relatively simple matrix projection approach for modeling population processes, though these are also rarely used for risk assessment by USEPA. In this presentation I argue for a reinterpretation of matrix models as individual fitness models that recognizes their unique advantages of parsimony and rapid deployment, without the obvious limitations inherent in their use to represent populations. This interpretation could provide several benefits in the risk assessment tool kit. It provides another, rapid and parsimonious step at which a risk assessment could be terminated after concluding that a chemical use scenario is safe, potentially reducing the number cases requiring a full population model. Many of the oft-cited benefits of population models, including the ability to integrate effects from lower levels of biological organization on vital rates, the ability to analyze sensitivity of model endpoints to changes in vital rates and sub-organismal level processes, and the ability to compare proportional effects among species with different life history strategies would still accrue from such fitness models. The interpretation of matrix models presented herein is consistent with perspectives previously published in the ecological and evolutionary life history literature. 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.

1.09.P-Mo-010 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. These standardized toxicity 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; it is difficult to use standardized test data to predict 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 data on subcellular processes. 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 a suite of concentrations of coal ash through dietary algal

exposure and measured impacts on survival, growth, and reproduction for 28 days. Throughout this exposure, 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). 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 to identify a set of genes predictive of the state variable, sorted and prioritized these genes using machine learning, and selected the model with the best fit to the DEB state variables. 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.

1.09.P-Mo-013 Development of a Neurobehavior Adverse Outcome Pathway in Larval Fish to Facilitate Cross-Species Extrapolations and Assess Population Risk

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To improve risk assessment of neurotoxic chemicals on fish species, we used the adverse outcome framework to predict effects of contaminants with different modes of action on the behavior of larval fish and predicted the subsequent population impacts. We studied three species to determine what adverse outcome pathways are common between species and to get estimates of uncertainty in extrapolations to help determine the acceptable levels of risk to wild populations. We chose the AOP framework because it is modular, allowing us to integrate the classic toxicological paradigm with global analyses across multiple levels of biological organization and across species to provide a more comprehensive systems level understanding. We worked with three different species: zebrafish, yellow perch, and killifish and exposed each to PCB126 and methylmercury (MeHg). We were careful to collect data from several key events that would be comparable across species and developmental stage. Critical genes and pathways predictive of adverse neurobehavior outcomes were identified through network inference methods using RNAsequencing from extracted individual larval fish brains. Impacted pathways and key genes were then compared to behavioral responses; behaviors that were impacted were incorporated into an individual based model for the ecologically relevant species. We found that, while the sublethal impacts of contaminants on swimming speed are subtle, they can still have significant impacts on fish cohort growth and survival and these change with species and across species. Further, we quantified uncertainty in key stressor-demographic linkages and use error-propagation methods to frame simulation outputs in a risk assessment context. We show that monitoring chemical effects on critical genes, pathways, and behaviors on non-animal models such as fish embryos could be indicative of adverse outcomes on fish/animal species at the population level, and that incorporation of uncertainty in population modeling can be protective.

1.09.P-Mo-014 Overview of Data and Models to Connecting Data and Individual Level Responses

Roger Nisbet, *University of California, Santa Barbara*

This presentation is a brief review of previous work that aims to connect data from high throughput assays and other molecular data (especially “-omics”) to metrics characterizing ecological risk. It is intended as an opening to the session. Data from high content studies may or may not not correlate with organismal responses, an illustrative example being the response of marine phytoplankton exposed to metal oxide engineered nanoparticles. Likewise, changes in gene expression may or may not be pointers to toxicity mechanisms. Further data and/or theoretical understanding of mechanistic connection across levels of biological organization is required before molecular data can usefully inform risk assessment. Theory for modeling whole organism

responses to chemical stress ideally involves a triad of dynamic modules describing toxicokinetics (TK), toxicodynamics (TD) and bioenergetics or dynamic energy budgets (DEB). There are seldom sufficient data to adequately parametrize all three modules for any system and modelers typically greatly simplify dynamics at one or more levels. One strategy uses an abstract variable “damage” in place of a multi-dimensional description of toxicodynamics. Likewise, simplified DEB models are used to characterize bioenergetic effects on survival, growth, reproduction, etc. The process of model formulation could be greatly facilitated with access to reliable ways of handling suborganismal information. A working group at the National Institute for Mathematical and Biological Synthesis (NIMBioS) proposed a conceptual model relating adverse outcome pathways (AOP) to the TK-TD-DEB triad and thereby to population and ecosystem process which must be the ultimate focus of most risk assessment. Progress to date on studies attempting to follow that strategy will be described with focus on a recent publication modeling the response of early life stage (embryo + larvae) killifish. Another presentation in the session will offer a second example. The challenges experienced in these studies can inform future work. The approach from the NIMBioS working group is just one possibility. Novel approaches that incorporate molecular/genomic information in mechanistic models of effects at higher levels of biological organization are needed. The goal of this session is to highlight new work being done to bridge these gaps and to potentially foster new collaborations between empirical and theoretical scientists.

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

1.10.P-Tu-025 Assessing the Bioactivity of Surface Waters with Metabolomics Using Multiple Cell Lines

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The presence of anthropogenic contaminants in environmental surface waters has been associated with potential health issues for humans and wildlife. These aquatic contaminants form complicated chemical mixtures, and the composition of these mixtures vary depending on, for example, stream location and adjacent land use. We have used cell culture-based metabolomics to investigate the biological impacts of complex mixtures of contaminants in water samples collected from eight streams across the United States and Puerto Rico. These streams were part of a larger nationwide study and were chosen based on their differing land-use characteristics and contaminant profiles. Two types of human cell lines (liver cell culture HepG2 and brain cell culture LN229) and one fish cell line (zebrafish liver cell culture ZFL) were exposed for 48 hours to water samples from each of the eight streams. We applied high resolution liquid chromatography-mass spectrometry (HR LC-MS) to measure changes in endogenous polar metabolite profiles of the cells (relative to controls). We then used these changes to compare the overall impact of each contaminant mixture on the different cell lines as well as the primary biological pathways that were affected. These analyses revealed differing sensitivities and differing changes in metabolite profiles for the three cell lines following exposure to these contaminated environmental samples. ZFL generally exhibited the greatest sensitivity, while HepG2 exhibited the most robust resistance to environmental stressors, possibly due to the cell line’s innate growth characteristics and detoxification capability. Finally, we identified covariances between metabolite changes and contaminant concentrations across all sites and cell lines. This revealed generally strong covariances between human cell lines and pharmaceutical contaminants. Pesticides covaried most strongly with hepatic-based cell lines (HepG2 and ZFL) regardless of species, while neuroactive contaminants covaried particularly well with the human brain cell line (LN229). These results both demonstrate the diverse and complementary strengths of these cell lines for investigating complex mixtures of contaminants and recommend continued development of new approach methodologies for environmental effects-based monitoring. The contents of this abstract neither constitute nor reflect US EPA policy.

1.10.P-Tu-026 Effects of Short-Chain Per- and Polyfluoroalkyl Substances (PFAS) on Toxicologically Relevant Gene Expression Profiles in a Liver-On-A-Chip Model

Megan Solan, Bennett Schackmuth, Sahar Pradan, Erica Bruce, Christie Sayes and Ramon Lavado, Baylor University

Short-chain per- and polyfluoroalkyl substances (PFAS) are highly stable and widely used environmental contaminants that pose potential health risks to humans. Aggregating reliable mechanistic information for safety assessments necessitates physiologically relevant high-throughput screening approaches. Here, we demonstrated the utility of a liver-on-a-chip model to investigate the effects of five short-chain PFAS at low (1 nM) and high (1 μ M) concentrations on toxicologically relevant gene expression profiles using the QuantiGene Plex Assay. We found that the short-chain PFAS tested in this study modulated the expression of ABCG2, a gene encoding for the breast cancer resistance protein (BCRP), with marked upregulation observed in all but one of the short-chain PFAS tested. PFBS and HFPO-DA repressed SLCO1B3 expression, a gene that encodes for an essential liver-specific organic anion transporter. High concentrations of PFBS, PFHxA, and PFHxS upregulated the expression of genes encoding CYP1A1, CYP2B6, and CYP2C19 with the same treatments resulting in the repression of the expression of the gene encoding CYP1A2. This dysregulation could have consequences for the clearance of endogenous compounds and xenobiotics. However, we acknowledge that increased expression of genes encoding for transporters and biotransformation enzymes may or may not indicate changes to their protein expression or activity. Overall, our study provides important insights into the effects of short-chain PFAS on liver function and their potential implications for human health. The use of the liver-on-a-chip model in combination with the QuantiGene Plex Assay proved to be a valuable tool for high-throughput screening and gene expression profiling in toxicology studies.

1.10.P-Tu-027 Metabolic Disruption and Mechanisms of Toxicity Caused by Bisphenol Analogs in Human In Vitro Cell Models

Rafia Afroze Rifa and Ramon Lavado, Baylor University

Bisphenols, widely used in the production of plastics, coatings, and resins, have become the focal point of recent scientific attention due to their potential endocrine-disrupting properties. Bisphenol A (BPA) has been associated with numerous adverse health effects, regulatory actions banning BPA use in several countries have incited the production and use of alternative bisphenol analogs (BPs). These alternatives are often perceived as safer substitutes but can trigger inflammatory responses and may contribute to chronic diseases such as cancer, diabetes, and cardiovascular diseases. This highlights a critical need for more comprehensive studies into the potential toxicity mechanisms of BPs. The proposed research aims to investigate the metabolism disruption and associated toxicity mechanisms 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 two relevant human *in vitro* cell models: the human liver HepaRG cell line and human intestinal CaCo-2 cell line. The preliminary cytotoxicity data indicates that BPP demonstrates the highest toxicity in HepaRG cells, with an EC₅₀ value of 0.65 μ M, while BPS exhibits the lowest toxicity in the same cell line (EC₅₀=1.17 mM). Notably, the EC₅₀ of BPS in intestinal cells was significantly lower, up to 46-fold (EC₅₀=25.5 μ M), suggesting higher toxicity of this bisphenol analog in intestinal cells. Among the bisphenol analogs tested, BPAF showed the highest toxicity in intestinal cells with an EC₅₀ of 7.6 μ M. Ongoing additional toxicity mechanistic investigations involve assessing oxidative stress generation and mitochondrial dysfunction.

1.10.P-Tu-028 Know Your Chemical, Know Your System – Why In Vitro Disposition and Bio-availability Matter

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In vitro bioactivity and toxicity data are increasingly being generated and considered to facilitate hazard and risk assessment activities. One prominent example is the calculation of Administered Equivalent Doses (AEDs)

which can then be compared to traditional *in vivo* points of departure (PODs) or exposure estimates. AEDs are typically calculated as the ratio of an *in vitro* POD (e.g., EC_x , μM) and the estimated steady-state blood or plasma concentration (C_{ss} , μM) corresponding to an oral dose of 1 mg/kg/d (e.g., $AED = EC_x/C_{ss} \cdot 1 \text{ mg/kg/d}$). A key underlying assumption inherent to the calculation of AEDs following this approach is that the *in vitro* POD and steady-state blood or plasma concentration are directly comparable exposure metrics (e.g., identical bioavailability in both media). This assumption can be problematic for several reasons stemming from the fact that *in vitro* PODs are almost always reported on a nominal basis. Because the composition of assay medium can be very different from the composition of plasma and blood and other losses can occur *in vitro* (e.g., volatilization), there may be substantial “mismatches” between *in vitro* PODs and C_{ss} with respect to actual exposure. To assess the potential bias in AED calculations that do not explicitly address *in vitro* disposition and bioavailability, we applied an *in vitro* mass balance model (IV-MBM v2.1) to a set of organic chemicals spanning a wide range of partitioning properties. The model was parameterized to represent *in vitro* test systems with different exposure conditions particularly with respect to the volume fraction of fetal bovine serum (FBS) added. For semi-volatile hydrophobic chemicals, the difference between the AEDs calculated using nominal AC50s and *in vitro* PODs estimated using IV-MBM v2.1 can approach two orders of magnitude. There can also be large differences for volatile chemicals due to excessive losses from the *in vitro* test system. On the other hand, differences between AEDs calculated using nominal AC50s and *in vitro* PODs estimated using IV-MBM v2.1 are relatively small for more hydrophilic chemicals (i.e., less than a factor of two). The model application demonstrates the importance of considering *in vitro* disposition and bioavailability issues when extrapolating *in vitro* bioactivity data to AEDs and then comparing to them to traditional *in vivo* PODs and exposure estimates.

1.10.P-Tu-030 Assessing the Toxicity of the Lampricide 4-nitro-3-(trifluoromethyl) Phenol (TFM) with Gill Cell Lines from Rainbow Trout and Lake Sturgeon Using OECD Test Guideline 249

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For the past six decades, strategies for controlling the invasive sea lamprey (*Petromyzon marinus*) in the Great Lakes have relied heavily on the lampricide TFM [4-nitro-3-(trifluoromethyl) phenol], a protonophore that disrupts oxidative phosphorylation in mitochondrial membranes. The selective toxicity of TFM to sea lamprey compared to nontarget fish has been attributed to the sea lamprey’s relatively poor ability to detoxify the chemical. Recently, concerns over the potential development of resistance mechanisms by the sea lamprey have increased efforts to identify novel chemical lampricides. In line with recent goals of replacing, reducing, and refining animal use in research, *in vitro* cytotoxicity assays with fish cell lines are being developed to screen and prioritize chemicals for to *in vivo* studies. Extrapolations of effective *in vitro* concentrations to *in vivo* lethal concentrations can be affected by the type of cell lines used, assay endpoints selected, media composition, and test chemical mode of action. These complications must be addressed if cytotoxicity assays with fish cell lines are to be used effectively in screening studies. Towards this end, this project is evaluating the Organisation for Economic Co-operation and Development (OECD) Test Guideline 249 for assessing the cytotoxicity of TFM with gill cell line RTgill-W1 from rainbow trout (*Onchorhynchus mykiss*), a species that is relatively resistant to TFM, and a gill cell line from lake sturgeon (*Acipenser fulvescens*), a species that is relatively sensitive to TFM at the juvenile (<100 mm) life stage. Our experimentally measured half-maximal effective concentrations (EC_{50}) will be reported and compared to reported lethal concentrations for whole organisms to determine the potential for the OECD 249 test guideline to serve as an effective *in vitro* tool for screening compounds that may be selectively toxic due to differences in detoxification ability among species.

1.10.P-Tu-031 Combined Effects and Toxicological Interactions of Short-Chain Per- and Polyfluoroalkyl Substances (PFAS) Binary Mixtures in Human Kidney Cells (HEK-293)

Alec Mockros, Megan Solan and Ramon Lavado, Baylor University

Since their discovery several decades ago, per- and polyfluoroalkyl substances (PFAS) have seen widespread use in industry and consumer products due to their chemical stability. The similarities between newer and supposedly less harmful short-chain PFAS and older, phased-out long-chain PFAS present a new topic of interest for researchers. The renal system emerges as a focal point in PFAS toxicity, with mounting evidence indicating a higher propensity for PFAS accumulation within the kidneys than other organs. Previously, our lab has conducted research concerning the cytotoxicity of individual PFAS exposures in HEK-293/hTLR2 cells and found that single exposures of short-chain PFAS had negligible effects on cell viability. In this study, we investigated the cytotoxicity of the following four short-chain PFAS in binary mixtures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to assess viability in binary mixtures of four short-chain PFAS: perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), 6:2 fluorotelomer alcohol (6:2 FTOH) and perfluorohexanesulfonic acid (PFHxS) over a range of 100 pM to 10 μ M for 24 h. Our results suggest that binary mixtures of PFHxS and PFHxA had marked effects on HEK293/hTLR2 viability around 1 μ M, implying that binary mixtures at equimolar concentrations have more significant effects on cell viability observations than single exposures. CellRox DeepRed assay was also used to detect cellular oxidative stress in the HEK293-hTLR2 cells following 24 h exposures to the mixtures of PFAS. The effects of oxidative stress were most apparent in the exposures to binary mixtures containing 1 μ M PFHxS and 6:2 FTOH. Understanding the impacts of PFAS exposure can shed light on the mechanisms of PFAS toxicity and aid in developing effective strategies for managing chemical risks, enabling regulators to make more informed decisions.

1.10.P-Tu-032 Antimycin-a Rainbow Trout Gill Cell (RTGill-W1) Cytotoxicity Compared to Whole Organism Toxicity

Gavin Saari, Blake Sauey, J. Nolan Steiner and M. Camille Kapita, U.S. Geological Survey

Antimycin-a formulations have been used as an effective broad-spectrum toxicant to control nuisance fish species. Historically, the development of new chemical controls required testing on numerous animals. *In vitro* cytotoxicity testing, which minimize the number of animals needed, are becoming more common and allow for high throughput. *In vitro* fish cytotoxicity screening has been initiated to prioritize new candidate chemicals as prospective pesticides to control nuisance fishes. One commercially available cell line is for Rainbow Trout (*Oncorhynchus mykiss*), which is already a common model organism for *in vivo* toxicity studies. However, it remains unknown whether *in vitro* cytotoxicity toxicity values are representative of *in vivo* toxicity values (e.g., lethal concentration to kill 50% of organisms; LC₅₀) for most chemicals. The present study examined the cytotoxicity LC₅₀ values of Antimycin-a using the Rainbow Trout gill cell line (RTGill-W1). Cells were exposed to the complex of Antimycin-a (A1-A4), and each individual pure isomer. The study evaluated the Organisation for Economic Co-operation and Development (OECD) Test Guideline 249 and the CellTiter-Glo 2.0 (Promega, Madison, WI, USA) for assessing RTGill-W1 cytotoxicity. The cytotoxicity LC₅₀ values for the antimycin-a complex and pure isomers varied by assay but demonstrated comparable agreement to Antimycin-a whole animal toxicity. Overall, similarity between Antimycin-a *in vitro* – *in vivo* 24-hour LC₅₀ values would suggest that *in vitro* screening shows promise to effectively predict whole organism toxicity and support the plausible screening of candidate pesticides to develop nuisance fish species controls.

1.10.P-Tu-033 Cytotoxicity and Transcriptomic Points of Departure for 19 Environmentally Relevant Pesticides on Multiple Human and Fish Cell Lines

Krittika Mittal, Sophie Emberley-Korkmaz, Ke Xu, Na'im Temlock, Jessica Head and Nil Basu, McGill University

The widespread use of pesticides is of concern to ecosystem and human health. Animal-based testing of

chemicals like pesticides is challenged as they are resource-intensive, time-consuming, and unethical. These concerns have called for the need to explore New Approach Methods (NAMs) to replace, refine, and reduce animal research. Among various methods, standardizing *in vitro* test systems and derivation of benchmark dose response based transcriptomics point of departure (tPOD) are of particular interest for chemical screening and prioritization. The objectives of this study were to: (A) measure the cytotoxicity (i.e., LC50) of 19 pesticides considered important to Canadian ecosystems on two human (Caco-2 intestinal; HepG2 liver) and three fish (rainbow trout gill RTgill-W1, liver RTL and gut RTgutGC) cell lines; B) characterize molecular responses in both cell lines to derive tPODs; and C) compare the resulting tPOD values with the *in vitro* LC50 values and data from the literature to determine if the tPOD values are protective of adverse outcomes. The OECD fish cell line acute toxicity test guideline 249 was followed to assess cytotoxicity in the rainbow trout cells and was adapted as appropriate for the human cell exposures. Cytotoxicity results based on Alamar Blue tests found that chlorothalonil and imazethapyr were the most cytotoxic pesticides to Caco-2 cells, with LC50 values below 15 μM . HepG2 cells were comparatively less sensitive to the pesticides tested as LC50 values for the most toxic pesticides, chlorothalonil and diquat, ranged from 120 – 180 μM , a magnitude higher than values found in the Caco-2 cells. Chlorothalonil and diquat were also the most cytotoxic pesticides to RTL and RTgutGC cells with LC50 values 6 – 18 μM . In certain cases, the RTgill-W1 cells were comparatively less sensitive than the RTL and RTgutGC cells as the LC50 for the most cytotoxic pesticides, chlorothalonil and carbaryl ranged from 26 - 97 μM . In general, the rainbow trout cells were more sensitive to pesticide exposure than the human cells except for isolated cases including imazethapyr and clothianidin. Exposures to assess differential gene expression have been conducted and will be measured using ultraplex RNA sequencing technology, from which tPODs will be calculated. This research is expected to help support the method development of *in vitro* studies as alternatives to animal testing strategies.

1.10.P-Tu-034 Exposure to “Alternative” Flame Retardants In Vitro Alters Rat Aortic Smooth Muscle Cell Migration, Proliferation, and Growth

Alex G Webb and Christopher G Goodchild, University of Central Oklahoma

Flame retardants are incorporated into commonly used household products (e.g., furniture, car seats, crib mattresses). In 2004, many traditional flame retardants (e.g., polybrominated diphenyl ethers) were removed due to toxicity concerns and replaced with ‘alternative’ flame retardants. However, the toxicity of these alternative flame retardants is unclear. A particular concern is the potential for maternal transfer of alternative flame retardants to the embryo, given flame retardants have been detected in breast milk and placental fluid of pregnant women. *In utero* exposure may lead to adverse developmental outcomes, especially during the early stages of embryogenesis. More specifically, alternative flame retardants may impair cardiac development by inhibiting angiogenesis. To test the cytotoxicity of alternative flame retardants, we exposed rat aortic smooth muscle (RASM) cells to triphenyl phosphate (TPP) and tris 2-chloroethyl phosphate (TCEP) *in vitro* and measured cell migration, proliferation, and growth. Preliminary analysis suggests TPP and TCEP are cytotoxic to RASM, which may have broader implications for cardiac morphogenesis.

1.10.P-Tu-035 In Vitro Cytotoxicity Assays Using RTgill-W1 Cells for Toxicity Identification and Reduction Evaluation (TIE/TRE) Strategies for Wastewater and Ambient Water Analysis

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Current fish toxicity testing methods are important for chemical registration and determination of toxicity of pollutants dissolved in water. Advancements with *in vitro* ecotoxicity techniques have continued to be highlighted for their ethical value and more informative approach (i.e., Mode of Toxic Action - MoA). For instance, the rainbow trout (*Oncorhynchus mykiss*) gill cell line, RTgill-W1, has shown promise for incorporation into Whole Effluent Toxicity (WET) testing. Moreover, while these New Approach Methods (NAMs) offer a promising animal alternative component, they also showed potential for Toxicity Identification/Reduction Evaluation (TIE/TRE) strategies that current *in vivo* assays lack. RTgill-W1 cell

viability was shown to predict mortality of acutely toxic effluents to fish larvae for various wastewater facility types, operations and sample sources (i.e. pulp and paper, agriculture, chemical production, food and beverage, and superfund sites). Additionally, TIE treatments (i.e., chemical addition, mechanical filtration and sample adjustments) were effective in removing toxicity to both fish and cells. Moreover, RTgill-W1 cytotoxicity assays were able to highlight specific MoAs of wastewater samples with specific classes of toxicants based on sample type and subcellular endpoints. Our results have highlighted the ability of RTgill-W1 cells to identify toxicants of concern involved in WET failures and their potential for TIE approaches.

1.10.V Cell-Based Approaches for Ecotoxicity Assessments

1.10.V-027 Assessment of cytotoxic and genotoxic response in *Allium fistulosum* root cells exposed 24h to lindane, chloroform and chlorpyrifos in environmental concentrations in water

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Currently, in the field of ecotoxicology and environmental sciences, it is important to look for alternatives to reduce the use of animals in toxicity bioassays. The *Allium cepa* bioassay is considered a lowcost and easy assay capable of detect the presence a large number of environmental contaminants, it represents an excellent in vivo assay model where it is possible to evaluate the damage produced by a substance or solution of interest on growth of the roots and on cells shape. The basis of the assay is simple, when an *Allium fistulosum* bulb is rehydrated, it stimulates the cells of the root tip and induces their cell division, which is evidenced as an elongation of the roots of the plant. However, when the rehydration process is carried out in the presence of polluting substances, cell division of root apical cells can be blocked, leading to paralysis of the mitotic machinery, inhibiting mitosis and/or causing nuclear or chromosomal abnormalities. To assess the damage on the root cells, *Allium fistulosum* were exposed to 100, 300, 500 µg/L for lindane; 0.1, 1, 10µg /L for chlorpyrifos and 50, 100, 200 mg/L for chloroform. To calculate the frequency in phases of mitosis was applied MAT Index (Metaphase, Anaphase and Telophase) vs IP count (Interphase - Prophase), additionally las abnormalities in nucleus and chromosomal more representatives were counted. Some differences respect to control and between treatments were founded, demonstrated the sensibility of this organism like an excellent option for biomonitoring programs and expand the number of next study sites in the coming months.

1.11.P-We Assessing Environmental Behavior and Effects of Naturally Occurring Radioactive Material (NORM)

1.11.P-We-007 Toxicokinetics and Bioavailability of Radium-226 in the Estuarine Environment

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Anthropogenic industries such as mining, oil and gas extraction, and military activities can contribute to enhanced concentrations of radium-226 (²²⁶Ra), a long-lived naturally-occurring radionuclide, in the coastal and estuarine environments. The existing concentrations and bioavailability of ²²⁶Ra in southeastern US salt marshes is virtually unstudied despite the potential for deleterious biological and ecological consequences from ²²⁶Ra pollution in these critical habitats. We concurrently sampled various abiotic (e.g., sediment, water) and biotic (e.g., mussels, marsh grass) compartments in a South Carolina salt marsh to quantify the concentrations and distributions of ²²⁶Ra and other radionuclides and heavy metals in the system. To understand the bioavailability and toxicokinetics of ²²⁶Ra in an estuarine sentinel species, we also conducted laboratory studies to investigate the influence of exposure duration, concentration, and form (i.e., dissolved, sediment-bound, and dietary) on the uptake and elimination of ²²⁶Ra in *Geukensia demissa*, the Atlantic ribbed mussel. We have shown these estuarine mussels rapidly uptake and eliminate dissolved ²²⁶Ra from their soft tissues but do not accumulate dissolved ²²⁶Ra to any appreciable extent in either the lab or field. Interestingly, these results contrast with

previous studies on freshwater mussels. Work is currently ongoing to quantify the uptake of sediment-bound and dietary (algae) ^{226}Ra and to investigate potential biomarkers and effects after ^{226}Ra exposure. The results from this body of work will be used to inform environmental decision-making and remediation actions in coastal habitats affected by ^{226}Ra .

1.11.P-We-008 Taxonomic Patterns in Polonium-210 uptake in Marine Molluscs

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Polonium-210 (^{210}Po) is one of the most toxic naturally-occurring radioelements found at enhanced concentrations in marine ecosystems affected by industrial NORM discharges. In the UK, a significant anthropogenic source of ^{210}Po was a phosphate ore processing plant near Whitehaven (Cumbria), that operated for several decades until the early 1990's and discharged a large amount of phosphogypsum waste directly into the Irish Sea. This legacy industrial activity is currently the largest contributor to radiation doses from the environment in the UK, with enhanced concentrations of ^{210}Po found in molluscs and crustaceans routinely consumed by the local population. Although routine radiological monitoring is focused on ^{210}Po and its parent radioelement, lead-210 (^{210}Pb), uncertainties remain in the transfer mechanisms involved and the radioecology of the surrounding area, and thus in determining the risk from exposure. Current environmental monitoring approaches rely on direct sampling and measurement of ^{210}Po and ^{210}Pb concentrations, with predictions made based on general transfer factors. Marine molluscs mainly accumulate ^{210}Po in their digestive tract and visceral mass. This, along with many species' being valuable human food sources around the world, mean that they are used as an important indicator for radioactivity in the marine environment. This study investigated the taxonomic differences between ^{210}Po uptake in different species of marine molluscs by modelling pre-existing data plus results from experimental exposure studies, with the aim of expanding existing knowledge of ^{210}Po transfer in marine molluscs. The collation and modelling of pre-existing and published activity concentration data can provide an insight into differences in uptake between taxonomic groups. The use of a REsidual Maximum Likelihood (REML) fitted mixed model minimised differences due to sampling and locational differences, to produce estimated species- and taxon-specific transfer factors. This research built on previous studies using REML to fit a mixed model to transfer of other species of radioecological interest. The identification of taxonomic patterns in ^{210}Po uptake in marine molluscs has the potential to reduce uncertainties in dose estimation by providing more specific transfer factors, enriching the understanding of transfer and helping to improve sampling regimes.

1.11.P-We-010 RNA Sequencing Analysis of Sunflowers Grown in Heavy Metal Salt Media

Jillian Newmyer, Kathryn Higley and Joshua Hargraves, Oregon State University,

RNA sequencing is a way to evaluate plant stressor response on a molecular level and can be helpful to understand the broader implications of genomic responses to environmental stressors. Previous data has shown that plants undergo genetic expression when exposed to radionuclides within the soil, focusing on genes that deal with DNA repair mechanisms, antioxidant defense, and cell cycle progression. Similar expressions can occur in plants undergoing salt stress from heavy metal concentrations within the soil. RNA sequencing for plants grown in radioactive salt mediums can give insight on the genes expressed and determine if similar or varied genes are expressed across similar mediums (i.e., lead and control soils). Uranium mining and milling has resulted in higher concentrations of lead, uranium, radium, and other heavy metals into the vadose zone that has resulted in possible salt stress in endemic biota. The wild sunflower, *Helianthus annuus*, is a common plant found within legacy uranium mining and mill tailing sites. This plant is known for its resistance to alkali salt stress. *H.annuus* was grown in three different soil mediums: radium and uranium laden soil from a legacy uranium site, lead laden soil, and a control soil of a physical structure similar to the exposed groups. The leafy parts of the plant were harvested three weeks after sowing and immediately chilled until analysis to preserve biological material. The samples were sequenced at the Center of Quantitative Life Sciences (CQLS) at Oregon

State University using the Qiagen Rneasy plant mini kit/protocol (50) cat#74904. Quality control was performed using Agilent Bioanalyzer 2100. This presentation will discuss the results from the three test plots and evaluate the confusion matrix between each subset.

1.12.P-Mo Case Studies Using Molecular Tools and New Approach Methodologies for Assessing Toxicity in Non-model Species

1.12.P-Mo-015 Integration of In Silico New Approach Methods (NAMs) to Guide Focused Testing and Inform Pesticide Toxicity Across Species – Case Study with Chlorantraniliprole

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Emphasis has widely been placed on utilizing New Approach Methods (NAMs) and predictive approaches in chemical toxicity evaluations to conserve resources, generate data faster, and reduce the use of animals. Advancements in bioinformatics have led to the development of *in silico* NAMs for addressing species extrapolation, including the U.S. EPA's Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool. The SeqAPASS tool evaluates conservation of protein targets to make chemical susceptibility predictions across thousands of species, including species that will never be tested empirically. Many NAMs are relatively new; therefore, case examples that demonstrate the applicability of the tools through the strategic combination of existing and new knowledge are needed to help build support for their use in generating predictions. Here, a study focusing on chlorantraniliprole (CHL), a diamide insecticide, is described. Diamide insecticides, which act on ryanodine receptors (RyRs), have been gaining popularity for control of lepidopteran agricultural pests. However, there are knowledge gaps regarding their toxicity across species, and detectable concentrations of these chemicals have been found in the environment. We used the SeqAPASS tool to generate susceptibility predictions after querying the RyR. Predictions were used to inform follow-up acute toxicity studies using *Daphnia magna*, *Daphnia pulex*, *Pimephales promelas*, and *Danio rerio*. Multiple endpoints were studied upon exposure to CHL including survival, mobility, and RyR gene expression. Results showed that the *Daphnia* species tested are susceptible to CHL, while the larval fish species were less susceptible, which can be explained in part by their RyR sequence similarity when compared to known targeted pest insects. Together, SeqAPASS evaluations combined with existing toxicity data can inform targeted toxicity testing to gain insight into cross-species chemical susceptibility while increasing confidence in predictive approaches. *The views expressed in this work are those of the authors and do not necessarily reflect the views or policies of the US EPA.*

1.12.P-Mo-017 Visualizing Blood Flow Through the Embryonic Heart Using Optical Coherence Tomography in Chick Embryos Exposed to Various Environmental Contaminants

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Maternal transfer of environmental contaminants from mother birds to their eggs can lead to developmental toxicity for avian embryos. In some cases, *in ovo* exposure to environmental contaminants can impair cardiac development and function (e.g., reduced heart rate, cardiac edema). Some contaminants directly alter cardiac function (e.g., inhibition of cardiac ion channels). However, cardiac function may also be disrupted indirectly by insufficient blood flow through the embryonic heart during 'cardiac looping', which is dependent on shear stress along the cardiac wall caused by increased blood flow. We exposed White Leghorn chicken (*Gallus gallus*) embryos to various polycyclic aromatic hydrocarbons, 'alternative' flame retardants, and algal toxins *in ovo* via egg-injection on embryonic day (ED) 2. On ED 5, we 'windowed' eggs and visualized blood flow

through the embryonic heart using optical coherence tomography (OCT). This study serves as a proof of concept for using OCT to assess cardiotoxicity in avian embryos.

1.12.P-Mo-018 Role of Toxicokinetics in Determining Species Sensitivity to Polycyclic Aromatic Hydrocarbons in Birds

*Jonathan Reid Sangiovanni*¹, *Doug Crump*² and *Jessica Head*¹, (1)McGill University (2) Environment and Climate Change Canada

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. Early-life stage (ELS) exposures have been proposed to help fill this data gap and provide novel data for ecological risk assessment (ERA). Previous results from our group showed a 30-fold difference in sensitivity (LD50) and discordant gene expression profiles in ELS Japanese quail (JQ; *Coturnix japonica*) and double-crested cormorant (DCCO; *Phalacrocorax auritus*) 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. Previous research suggests that the AHR1 genotype is highly predictive of avian sensitivity to dioxins, chemicals with a similar mechanism of action as PAHs. We hypothesized that toxicokinetics may play a role in these differences. The goal of the current project is to characterize time-course and tissue-specific gene expression patterns in developing embryos of JQ and DCCO and monitor the distribution of BaP within egg contents following exposure. Fertilized, unincubated eggs of both species were injected with a solvent control (dimethyl sulfoxide) or 2 nominal doses of BaP (100 and 500 ng/g egg for JQ and 10 and 100 ng/g egg for DCCO, the more sensitive species). RNA was isolated from tissues at different embryonic days (EDs), culminating at mid-incubation for both species. Whole body homogenates (JQ: EDs 5,6,7,8,9; DCCO: EDs 6,8,10,12,14), chorioallantoic membrane (JQ EDs 7,8,9; DCCO EDs 10,12,14) and liver (JQ EDs 8,9; DCCO EDs 12,14) were sampled. BaP will be determined in homogenized embryos and egg contents (yolk, albumen, and allantoic fluid) using Gas-Chromatography/Mass Spectrometry. Preliminary quantitative polymerase chain reaction (qPCR) data indicates that phase I biotransformation genes (CYP1A4/5) peak early in liver formation (JQED7; DCCO ED10) and return to baseline levels of expression by mid-incubation. Expanded gene expression profiles will be analyzed using avian EcoToxChips and compared to chemical data in order to link genomic responses of regulatory concern to quantifiable measures of exposure, and to assess how these responses vary between species.

1.12.P-Mo-019 Effects of PFAS on Model vs. Non-Model Species: A Case Study on the Impacts of Perfluorohexanoic Acid on Zebrafish and Largemouth Bass

Jessica Donaldson, *Amany Sultan*, *Christopher J Martyniuk*, *John A Bowden* and *Joseph H Bisesi*, (1)University of Florida

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. Additionally, the use of these tools across both model and non-model species will help determine whether testing data can be extrapolated across species. In our current study, we exposed a model species (zebrafish) and a non-model species of recreational and economic importance in the state of Florida (largemouth bass) to an emerging PFAS, perfluorohexanoic acid (PFHxA), via the diet. 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. We will analyze the sequenced transcriptome for differential expression in both species to examine novel toxicity pathways as well as similarities and differences across both model and non-model species. 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.

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

1.12.V-021 Enhancing a machine learning approach to predict species sensitivity

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A significant challenge in ecological risk assessment is estimating chemical-induced effects across taxa without species-specific testing. This is especially true for species under legal protection, such as those listed as threatened or endangered under the Endangered Species Act (ESA), which cannot be taken for ecotoxicological testing. In these instances, surrogate animal models are used to estimate potential effects, but it is currently unclear whether the surrogate data are representative, protective, or realistic. Where ecotoxicological data may be more challenging to gather, information on species physiology is more available for a broad range of taxa. Physiology is known to drive species sensitivity, but understanding the relative contribution of specific underlying processes is still elusive. Consequently, there remains a need to understand which physiological processes lead to differences in species sensitivity. The objective of our study was to utilize existing knowledge about organismal physiology to both understand and predict differences in species sensitivity. We trained machine learning models to predict chemical- and species-specific endpoints as a function of both chemical fingerprints/descriptors and physiological variables, including, but not limited to, dynamic energy budget (DEB) properties, life history traits, and potentially taxonomic/phylogenetic relationships. We first used publicly available data from the EnviroTox database, with ToxPrints and DEB parameters as predictor variables explaining chemistry and physiology, respectively. Then, we broaden the applicability of the models by increasing species diversity, using life history traits and taxonomic/phylogenetic relationships, and by supplementing the models with additional ecotoxicology data. We found that a simple random forest model could predict chemical- and species-specific endpoints, and that physiological parameters were relatively important in the model. We also tested the efficacy of the models by derivating species sensitivity distributions (SSD) and comparing them with actual SSDs available for some pesticides. We anticipate that our approach will illuminate how physiological parameters drive species sensitivity, allowing more realistic extrapolations of effects across species without additional animal testing.

1.13.P-Tu Advancing the Use of Behavioral Endpoints and Methods in Assessments of Environmental Contaminants

1.13.P-Tu-036 Frontiers in Quantifying Wildlife Behavioural Responses to Chemical Pollution

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South Wales, Australia, (8)University of Western Australia, Australia, (9)Environment Protection Authority Victoria, (10)University of Exeter, United Kingdom

Animal behaviour is remarkably sensitive to disruption by chemical pollution, with widespread implications for ecological and evolutionary processes in contaminated wildlife populations. However, conventional approaches applied to study the impacts of chemical pollutants on wildlife behaviour seldom address the complexity of natural environments in which contamination occurs. This talk will present a new review by Bertram et al. (2022, *Biological Reviews*, <https://doi.org/10.1111/brv.12844>), which aims to guide the rapidly developing field of behavioural ecotoxicology towards increased environmental realism, ecological complexity, and mechanistic understanding. This review identifies research areas in ecology that to date have been largely overlooked within behavioural ecotoxicology but which promise to yield valuable insights, including within- and among-individual variation, social networks and collective behaviour, and multi-stressor interactions. Further, the review features methodological and technological innovations that enable the collection of data on pollutant-induced behavioural changes at an unprecedented resolution and scale in the laboratory and the field. In an era of rapid environmental change, there is an urgent need to advance our understanding of the real-world impacts of chemical pollution on wildlife behaviour. This review therefore provides a roadmap of the major outstanding questions in behavioural ecotoxicology and highlights the need for increased cross-talk with other disciplines in order to find the answers.

1.13.P-Tu-037 EthoCRED: A Framework to Guide Reporting and Evaluation of the Reliability and Relevance of Behavioural Ecotoxicity Studies

Michael Grant Bertram and Tomas Brodin, Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences

Behavioural analysis has been garnering significant attention as a broad indicator of sub-lethal toxicity, and has secured a place as an important sub-discipline in aquatic 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 behavioral changes, meaning that these, often sensitive, effects are not represented in hazard and risk assessments. Here, we propose a new tool, the EthoCRED evaluation method, for assessing the reliability and relevance 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 behavioral studies in the peer-reviewed literature, and thereby increase their usefulness in chemicals regulation.

1.13.P-Tu-038 Choosing the Right Organism for Use in Sediment Avoidance Behavior Tests; How Other Behaviors Affect This Choice

Roger Yeardeley, Marc Mills and James M. Lazorchak, U.S. Environmental Protection Agency

Soil avoidance tests have been developed for earthworms, Collembola, and other soil organisms. Many organisms are sensitive to toxic contaminants and have the ability to avoid areas of contamination. Correlations between avoidance behavior and acute and other toxicities have been found by our lab and others. Earthworm soil avoidance tests have been increasingly used in risk assessment since their development circa 1996, until in 2008 an international standard method (ISO 17512-1) was published. So, the potential for a sediment avoidance

test is there. We assessed the potential for use of the freshwater aquatic macroinvertebrates (*Chironomus dilutus* larvae, *Hyalella azteca*, and *Lumbriculus variegatus*) used in standard USEPA sediment toxicity and bioaccumulation methods (Methods 100.1, 100.2, and 100.3), for use in sediment avoidance testing. Behaviors other than avoidance of contaminants - feeding habits, predator avoidance, mating, phototaxis, thigmotaxis, and especially mobility - affect avoidance testing. So, we have been evaluating these candidate organisms with respect to these behaviors. Presence or absence of sediment affected phototactic behavior. With no sediment present, both *Hyalella* and *Lumbriculus* showed a preference for the dark sides of chambers. In tests with sediment present, *Hyalella* showed a preference for light, while *Lumbriculus* showed no preference. 24-hour mobility tests with reference sediment, with a null hypothesis that the organisms will not remain on the same half of the chamber where they were placed at test start, showed that *Chironomus* ($p=0.002$) did not exhibit sufficient mobility to be useful in avoidance testing. *Hyalella* ($p=0.327$) showed good mobility. *Lumbriculus* ($p=0.236$) fared better than *Chironomus* in these mobility tests, but *Lumbriculus* did not spread themselves relatively evenly in chambers with only reference sediment. *Lumbriculus* would be a good candidate due to ecological relevance, and being in contact with sediment both internally and externally. However, due to issues like the one mentioned above, which calls into question independent movement of individuals, and others we've seen like an 'edge' effect (possibly due to thigmotactic behavior), and a significant number being on the sediment surface at test end, the right parameters have not yet been found to make this organism a good candidate for sediment avoidance tests.

1.13.P-Tu-039 Behavioral Impacts of Three Acute Phthalate Exposures on Larval Fathead Minnows

Meagan Bell, Robert Flick, Tom Purucker, Brett R Blackwell, David Bencic and Adam Biales, U.S.

Environmental Protection Agency

Commercial industries use various phthalates to generate consumer and industrial products. Phthalates are commonly used as plasticizers for plumbing materials, flooring, and other household items, in addition to personal care products and childrens' toys. However, some governing agencies, including the European Union, have started placing restrictions and regulations on phthalate use to limit human and environmental exposure. Phthalates have become an environmental concern because of their increasing presence in aquatic ecosystems because of widespread use. As part of a larger effort to evaluate metabolomic, transcriptomic, and behavioral responses to phthalate exposure, we performed acute exposures of larval fathead minnows (FHM, *Pimephales promelas*) to three phthalates: benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), and diisobutyl phthalate (DIBP). Herein, we present results of our study focused on the behavioral responses to phthalate exposures. Behavior is non-lethal and more sensitive than the traditional endpoints: mortality, reproduction, and growth. FHM larvae (5-days post fertilization) were exposed to 9 concentrations of each of the three phthalates with a moderately hard reconstituted water (MHRW) control, and a MHRW solvent control with silicon rings. Concentrations were confirmed through analytical chemistry at the 24-hour timepoint. Exposures were performed in triplicate per chemical. Silicon rings were used as a vehicle for passive dose control with a methanol carrier because of low solubility of phthalates. Following a 24-hour exposure, 9 individual larvae (3 per replicate exposure) from the 6 highest concentrations without mortality, and both controls, were separated for behavioral studies. Behavior assays were performed to compare FHM activity between varying concentrations of phthalates, as well as responses to a photoperiodicity stimulus. FHM larvae were video-recorded, and individual movement was monitored. Behavioral responses were evaluated using various metrics of movement (distance moved, speed, etc.), along with individual response to photoperiod stimulus. Results from our behavioral study will be combined with the metabolomic and transcriptomic results to create a multi-layered dataset of toxicological effects of phthalates on larval FHM. This complete dataset will be used to inform regulatory risk assessors on the potential harm and effects of phthalates in aquatic ecosystems.

1.13.P-Tu-040 Plastic Leachates Stimulate Chemosensory Responses in Sea Anemones

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The chemosensory cues leading to marine animal plastic consumption are hypothesized to be mixtures of additives and contaminants that leach from plastics. We examined the impact of aqueous plastic leachates of polystyrene pre-production pellets and post-production polystyrene knives on sea anemones. Leachates were dried on precombusted glass fiber filters. Precombusted filters with dried HPLC grade water were controls. We found that both pre- and post-production plastic leachates led to 3.5 to over 4 fold longer feeding retention times than controls. In feeding trials, new anemones were offered egested filters, and retention times for pre- and post-production plastics were similar and significantly different from refeed control filters. To verify the type of plastic used in the experiments, we conducted fourier transform infrared spectroscopy. We will use analytical chemistry techniques, inductively coupled plasma mass spectrometry, and gas chromatography–mass spectrometry, to determine leachate chemistry. Our data provide clear evidence that molecules leaching from pre- and post-production polystyrene used to make disposable utensils contain feeding stimulants. We look forward to collaborative efforts to identify plastic flavors with the goal of altering flavors to deter plastic consumption and reduce toxicity.

1.13.P-Tu-043 Selection Preference in *Hyalella azteca*: A Behavioral Assay for Ecotoxicology

Sam Nutile, *Miranda Johns*, *Adam M. Simpson*, *Lynne Beaty* and *Kyle Deloe*, Pennsylvania State University (The Behrend College)

Non-target organisms in aquatic environments are not only affected by lethal toxicity of contaminants, but also sublethal effects. Continuing to study the effects that sublethal contaminant concentrations can have on non-target species, specifically behavioral changes, can introduce new protocols and regulations into the field of ecotoxicology. The objective of this study was to utilize a two-choice behavioral assay to monitor the avoidance behavior of *Hyalella azteca* when exposed to three different contaminants, bifenthrin, chlorpyrifos, and permethrin, at lethal and sublethal concentrations. A two-choice behavioral assay was used for each chemical to observe if *H. azteca* avoid sediment containing contaminant concentrations at the lethal concentration 50 (LC₅₀), LC₂₅, and LC₁₀ determined from 48 h toxicity tests. For all three contaminants, *H. azteca* demonstrated a statistically significant preference for the uncontaminated sediment over the contaminated sediment at the LC₅₀, LC₂₅, and LC₁₀, spending significantly more time in the uncontaminated versus contaminated sediment. For the LC₁₀ bifenthrin exposures, the *H. azteca* were more active during behavioral assays than any other exposure scenario, suggesting the LC₁₀ may have been at the lower limit of detectable concentrations for this species. These findings suggest that *H. azteca* avoid sediments that are contaminated with common pesticides at concentrations below those associated with traditional toxicological endpoints (i.e., lethality), demonstrating that avoidance behavior is a sensitive endpoint in the context of contaminant exposure. Furthermore, as only naïve individuals were used in behavioral assays, the results of this study indicate avoidance behavior is an immediate response in this species. While more research is needed to understand the population level effects of such avoidance behavior, this study demonstrates the potential impacts that may occur through contamination at sublethal concentrations.

1.13.P-Tu-044 Behavioral Endpoints as Lines of Evidence: Current Use in Regulations and Considerations for Assessment of Toxicity

Alexandra Steele and *William L. Goodfellow*, (1)Exponent, Inc.

Regulatory toxicity studies traditionally use endpoints such as mortality, growth, and reproduction. While these traditional endpoints provide reliable evidence in toxicity testing and provide a connection to toxicity effects on populations, interest in use of behavioral endpoints in toxicity testing has been growing. This expanded interest has led to an increase in the number of studies and expansion of knowledge in relating behavior to ecotoxicology. Aquatic organisms have developed specific mechanisms such as identifying prey, avoiding

predators, courtship displays, etc., important behaviors for an individual's fitness and for population dynamics and ecosystem function. Chemicals in the environment can impact aquatic organisms by disrupting or masking necessary behaviors. While interest in behavioral endpoints is driven by the sensitivity, technical developments creating increasingly automated frameworks to measure behavior, and the potential to offer evidence on the mode of action of chemical contaminants provided by behavioral toxicity tests; regulatory bodies have produced guidance which influences the use of these measures. A common theme throughout this guidance is the selection of endpoints that demonstrate ecological relevance. Behavioral endpoints can show ecological relevance and provide a connection to population level processes; however, the usefulness of these measures is dependent on several factors including the appropriateness of the test organism selected, the behavioral endpoint used, and how the endpoint relates to a population-level effect. This presentation will discuss how current regulations define the use of behavioral endpoints and population level effects and in addition will explore strategies for using behavioral measures in toxicity assessments and provide examples of behavioral toxicity testing data with comparisons to traditional toxicity test measures.

1.13.P-Tu-045 Linking Conventional- and Behavioural Endpoints: Impact of Sediment-Associated Pharmaceuticals in Deposit-Feeders

Henriette Selck, Roskilde University

Prospective environmental risk assessment (ERA) of chemicals has traditionally assessed potential impacts of individual chemicals *a priori* to their use, based on short-term effects measured (primarily for survival, growth and reproduction) in a few species using standard tests and retrospective assessment conducted for the worst contaminated sites. However, recent work for biologically active molecules (pesticides, biocide, pharmaceuticals) has identified how chronic impacts and novel modes of action (e.g., behavioral, immune modulation and genotoxicity) can lead to population change in certain species. Neurotoxic contaminants (e.g., pharmaceuticals) inhibiting acetylcholinesterase (AChE) impair nerve impulses affecting behavioral traits, such as escape efficiency from predation, burrowing, capability for feeding and irrigation activity. Effects on these traits can have impacts on population density by affecting predation rates and feeding efficiency. Observations exist that endpoints, such as behavior, are more sensitive to pharmaceuticals than conventional toxicological endpoints. The EU H2020 Marie Skłodowska-Curie actions (MSCA) Innovative Training Network (ITN) "Chronic exposure scenarios driving environmental risk of chemicals – CHRONIC" aims to develop tools and approaches to identify relevant nonstandard modes of toxicity for low chronic chemical exposure and integrate these with environmental stressors. This presentation will provide insights into approached to identify behavioral impact of sediment-associated pharmaceuticals in selected sediment-dwelling organisms and discuss how conventional (i.e., mortality, growth, reproduction) and non-conventional (i.e., behavioral) endpoints relate.

1.14.P-Tu Detection, Toxicity and Environmental Risk of Sunscreens, Cosmetic Products and OTC Drugs

1.14.P-Tu-046 Spatial and Temporal Relationships of Organic UV Filter Concentrations in Seawater from The Florida Keys, USA

Carys Louise Mitchelmore, Andrew Heyes and Michael Gonsior, University of Maryland Center for Environmental Science

Limited data exists on the concentrations of organic UV filters in the Florida Keys despite concerns regarding their potential impact to corals. Previous studies from other reef locations have found spatial and temporal variations in concentrations, for example, lower concentrations with depth in the water column and distance from the shoreline. Researchers have hypothesized that concentrations are higher in the microlayer yet there is limited or no data to support this. To address these knowledge gaps seawater samples from multiple sites (i.e., beach and reef locations) in the Florida Keys, USA were analyzed for up to 13 organic UV filters. At

recreational beach locations multiple sites (and replicated discrete samples) were assessed for concentrations in the microlayer and surface seawater at two distances from the shoreline. At one beach location a temporal assessment was conducted with samples collected early in the morning, mid-afternoon and late in the evening. At reef locations both surface and at coral depth seawater samples were analyzed. UV-filters were measured using LC-ESI-MS/MS techniques in both the dissolved and particulate fractions. Numerous UV filters were detected in seawater with the highest concentrations present in the sites closest to the beach correlating with the level of recreational activity. UV filters were present at significantly lower concentrations in samples taken further from the beach and in early morning and late evening samples. A number of UV filters were present in higher concentrations in the microlayer samples compared with the corresponding surface water samples. A number of UV filters were detected in seawater collected both at coral depth and the surface, although both were at much lower concentrations compared to beach samples. This study provides insight as to the environmentally relevant concentrations in seawater samples at beaches and coral reefs in the Florida Keys. Furthermore, the spatial and temporal assessments provide data that are essential for developing probabilistic marine exposure models and environmental risk assessments.

1.14.P-Tu-047 Thyroid Disrupting Potential and Related Toxicological Effects of BEMT in Embryo-Larval Zebrafish (*Danio rerio*)

Ba Reum Kwon, Kyungho Choi and Yujin Park, Seoul National University

Bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT) is a widely used UV stabilizer added in sunscreen products. Recently, the presence of BEMT in aquatic environment has been reported, prompting studies to evaluate its toxicity. One particular concern regarding potential toxic effects of BEMT is its ability to disrupt thyroid function, similar to other organic UV filters such as benzophenone-3 and octocrylene. In order to evaluate the adverse effects of BEMT on thyroid function, embryo-larval zebrafish (*Danio rerio*) were exposed to BEMT for 5 days post fertilization, and measured for thyroid hormones and related gene transcripts. Zebrafish larvae exposed to varying concentrations of BEMT (0, 0.01, 0.1, and 1 μ M) exhibited an inverse correlation with whole-body free thyroxine (fT4), free triiodothyronine (fT3) and thyroid stimulating hormone (TSH). The concentration-dependent decrease in fT4 level was particularly notable, with a significant reduction observed at the highest BEMT concentration (1 μ M). Furthermore, genes associated with thyroid function were generally down-regulated, supporting the observation of decreased thyroid hormone levels. Significant down-regulations of genes in thyroid hormone synthesis in brain and thyroid axis, such as *trh*, *tsh*, and *tg* genes, were observed. In addition, transcription of the *dio2* gene, responsible for the conversion of T4 to T3, was significantly reduced, suggesting decrease of T4 to T3 bioconversion. Moreover, an up-regulation tendency of the *ttr* gene, which codes for a thyroid hormone binding protein, was observed, potentially further decreasing free T3 concentrations. These findings suggest that BEMT possesses thyroid-disrupting potential similar to other organic UV filters. While current studies on BEMT toxicity in aquatic environments primarily focus on thyroid disrupting toxicity at larval stage, further investigation of its endocrine-disrupting effects is necessary, considering its widespread use and high predicted lipophilicity.

1.14.P-Tu-048 Distributions of UV Absorbents in the Surface Water of Habitats for the Endangered St. Lawrence Estuary Beluga and Southern Resident Killer Whale in Canada

Amina Ben Chaaben¹, Mathieu Babin¹, Frank Wania², Hayley Hung³, Magali Houde³, Liisa Jantunen³, Kelsey Lee⁴, Frank Gobas⁴, Huixiang Xie¹ and Zhe Lu¹, (1)University of Quebec at Rimouski, (2)University of Toronto, (3)Environment and Climate Change Canada, (4)Simon Fraser University

St. Lawrence (SL) Estuary beluga whales (*Delphinapterus leucas*) and Southern Resident killer whales (*Orcinus orca*) are endangered cetacean populations in Canada. Exposure to various contaminants is a major stressor affecting the health and recovery of these whale populations. Nonetheless, the prevalence and distribution of many emerging contaminants in the water of their habitats are poorly understood. UV absorbents (UVAs) are additives to sunscreens, personal care products, plastics, and other products to protect skin from

sunburn or prevent the materials from UV-induced color change or degradation. UVAs have been detected in the tissues of SL Estuary beluga whales and their food web. This demonstrates the presence of UVAs in the habitat of SL Estuary beluga and the potential input of these contaminants from the rivers draining into this system, such as the SL River and the Saguenay River (SR). In contrast, the occurrence of UVAs in killer whales and their habitat is still unclear. This study aims to investigate the distribution of two groups of UVAs, UV filters (UVFs) and benzotriazole UV stabilizers (BZT-UVs), in the dissolved phase and the suspended particulate matter (SPM) of the surface water in the habitats of these two whale populations. Water samples were collected from 60 locations in the beluga habitat (the SL Estuary, SL River, and SR; 2019-2020) (Quebec) and 9 sites in the killer whale habitat (the Salish Sea; 2021) (British Columbia). UVFs concentrations (3-1570 ng/L) were generally greater than BZT-UVs (<MDL-118 ng/L) in the water of both habitats. The total BZT-UVs and UVFs concentrations in the dissolved phase of the St. Lawrence system were significantly higher than in the Salish Sea, suggesting that the SL Estuary belugas may be exposed to more UVAs via surrounding water than the killer whales. Compared to the SL Estuary, the river samples showed higher detection frequency and concentrations of four BZT-UVs and three UVFs in the dissolved phase. For killer whales' habitat in the Salish Sea, water close to Victoria Harbor had greater UVAs levels than other locations. High abundances of UVAs on suspended particulate matter were found in both habitats, suggesting that their ingestion could be an important exposure pathway to BZT-UVs and UVFs for aquatic organisms in these two habitats. This study establishes baseline concentrations of UVAs in St. Lawrence beluga and Southern Resident killer whales' habitats so future trends can be assessed.

1.14.P-Tu-049 Trophodynamics of Organic UV Filters, Benzotriazole UV Stabilizers and Aromatic Secondary Amines in the Food Web of the St. Lawrence Estuary Belugas

Alexis Eric Adrien Trinquet¹, Mathieu Babin¹, Alexis Agathine¹, Jonathan Verreault², Véronique Lesage³, Jory Cabrol³, Stephane Lair⁴, Gerald Tetreault⁵ and Zhe Lu¹, (1) University of Quebec, Rimouski, (2) University of Quebec, Montréal, (3) Fisheries and Oceans Canada, (4) University of Montreal, (5) Environment and Climate Change Canada

Organic UV filters (UVFs), benzotriazole UV stabilizers (BTZ-UVs), and aromatic secondary amines (Ar-SAs) are contaminants of emerging concern in the environment that may cause adverse health effects in organisms. A previous study from our laboratory detected some of these contaminants in the blubber and liver samples collected from beluga carcasses of the St. Lawrence Estuary (SLE), a population that is considered endangered in Canada. However, the trophodynamics of these contaminants in the food web of SLE beluga is currently unknown. To fill this knowledge gap, tissues were collected from 17 known and potential prey of SLE beluga in 2019 and 2020 to investigate the biomagnification patterns of selected UVFs, BZT-UVs and Ar-SAs in the SLE beluga's food web. Carbon sources and trophic levels of all beluga prey items were determined using stable isotopes to investigate relationships between prey ecology and contaminant concentrations to identify the most probable dietary sources for these contaminants. Contaminants were analyzed using a gas chromatograph coupled to a triple quadrupole mass spectrometer (GC-MS/MS). Our results indicate that the level of target contaminants followed the order of UVFs>BZT-UVs>Ar-SAs. All UVFs were frequently detected (>50%) except for BP in the 17 species. Five UVFs (BP3, EHMC, EHS, HMS, OC) and four BZT-UVs (UV326, UV320, UV328 and UV327) were the most common compounds in the food web samples. The trophodynamics of the target contaminants with 100% detection were evaluated. UVP, UV328, total BZT-UVs, HMS, BP3, and total UVFs were positively associated with $d^{15}N$, demonstrating that these contaminants were biomagnified in the SLE food web. Although the mean of the analyzed species' EHS and OC levels were not associated with $d^{15}N$, positive correlations were identified when considering all individuals in the analysis. Thus, EHS and OC may also biomagnify in the food web of the SLE. UV327 and EHMC were found in all samples; however, they did not correlate with $d^{15}N$. This study provides a baseline for monitoring these contaminants in the SLE food web and contribute to a better understanding of the environmental fate of these contaminants in the estuarine environment.

1.14.P-Tu-050 Understanding the Behavior of Common Ultraviolet Filtering Compounds Under Simulated Environmental Conditions

Carter Coleman, Donna A. Glinski, Shannon Alexis Nelson, Cheryl Hankins, Sandy Raimondo and W. Matthew Henderson, U.S. Environmental Protection Agency

Ultraviolet (UV) filters have recently become contaminants of emerging concern due to their global detection in seawater, sediments, and biota. These compounds are ubiquitously used in personal care products to prevent UV spectra infiltration, as well as in commercial products to prevent photodegradation. As usage increases, these contaminants are being detected more frequently in the aquatic environment likely after being rinsed off after application and subsequent leaching into the ecosystem. However, a knowledge gap exists in understanding how these compounds interact with aquatic organisms as research into their fate and stability in the environment is limited. This study aims to 1) assess the stability of five UV filters (avobenzone, homosalate, octisalate, octocrylene, and oxybenzone) across a range of temperatures and salinity; 2) refine extraction procedures across a range of salinity, pH, and chlorination, and 3) investigate the stability of these compounds under simulated solar irradiation as individuals and in combination. To achieve this, the stability of these five compounds were monitored for 28 days, across three temperatures, and in both common analytical solvents and in aquatic environments (i.e., freshwater, marine water, and pool water). Method refinement for the extraction of these contaminants using solid phase extraction was compared following pH adjustment across all matrices. Assessing the fate and stability of common UV filters will provide data applicable to the analytical determinations of these and other class constituents in various aquatic environments. Further, data presented will inform the analytical considerations necessary for standardizing testing for coral exposure experiments currently being conducted by the U.S. Environmental Protection Agency that is needed to accurately assess risk of UV filters to these marine invertebrates.

1.14.P-Tu-052 Freshwater Environmental Safety Assessment of UV filters in the United States

Emily E. A. Burns and Iain Davies, PCPC (Personal Care Products Council)

Organic ultraviolet (UV) filters are used in cosmetic and personal care products (CPCPs), including over-the-counter sunscreens, due to their ability to absorb solar radiation. CPCP ingredients can be washed down the drain, enter wastewater treatment and be released to the freshwater environment with treated effluents. In this presentation, an environmental risk assessment framework to conduct down-the-drain environmental safety assessments for CPCP ingredients is applied to six UV filters (avobenzone, octinoxate, octisalate, oxybenzone, homosalate and octocrylene). Exposure was characterized using the spatially resolved aquatic exposure model, iSTREEM. iSTREEM provides a comprehensive exposure assessment through the consideration of spatial variability in emissions, wastewater treatment, flow, and loads transported from upstream to estimate CPCP concentrations in receiving waters across the conterminous United States. The result is a predicted environmental concentration (PEC) distribution representative of spatial variability in conditions across the region. A review of standard toxicity data was used to derive a predicted no-effect concentration (PNEC) using appropriate assessment factors. A safety assessment was conducted by comparing the PEC distribution with the PNEC. The results demonstrate freshwater environmental safety as the PNEC does not exceed 90th percentile PEC for each of the UV filters. These results are instrumental in demonstrating the environmental safety of key organic UV filters in the U.S. freshwater environment and will help prioritize future work.

1.14.P-Tu-053 Occurrence of Organic UV filters in Coastal Waters and the Coral *Acropora cervicornis* from the Florida Reef Tract

Dorothy-Ellen Abigail Renegar¹, Andrew Heyes², Michael Gonsior², Ellen R Skelton¹, Megan Kennedy¹ and Carys Louise Mitchelmore², (1)Nova Southeastern University, (2)University of Maryland Center for Environmental Science

Organic UV filters, which include the active ingredients in sunscreens, have been detected in both seawater and in marine biota, highlighting the potential for uptake and environmental risk from exposure. However, despite

scientific and societal concern, a limited number of studies have measured UV filter concentrations in seawater near coral reefs, and even fewer have examined the presence of UV filters in coral tissues. While some laboratory studies have suggested detrimental effects of UV filters on coral reproduction, studies which tested UV filters in field-collected coral tissues did not provide information on the health of sampled corals or the overall health status of corals at collection sites. This study was designed to address the knowledge gap between environmentally relevant UV filter exposures and potential effects on coral reproductive capacity, a key element of coral health. Up to 12 organic UV filter concentrations were assessed in matched seawater (shallow and coral depth) and coral tissue samples from four coral nursery sites (three offshore and one onshore) in Florida, and correlated with coral fecundity in the endangered *Acropora cervicornis*, a keystone species throughout the Caribbean. Some UV filters were either not detected or detected in very low (ng/L) concentrations at the offshore nurseries, and no UV filters were detected in the onshore nursery. Six of the 12 UV filters were detected in shallow and coral-depth waters at the offshore nurseries located near Ft. Lauderdale and near Tavernier in the Florida Keys. Two of the 12 organic UV filters were detected in shallow and coral-depth water near Carysfort Reef in the Florida Keys. Octocrylene and avobenzone were the most commonly detected. Coral fecundity was overall lower at the Broward nursery and highest at the Carysfort nursery. Overall, both organic UV filter concentrations and coral fecundity are consistent with expected levels of anthropogenic contamination at each site although the specific chemical contaminant(s) drivers are unknown. This is the first study to provide data regarding UV filter concentrations in corals in the continental United States, and the first to examine the potential effects of environmental UV filter exposure on coral fecundity. The insights gained provide critical support for regulatory policies and management decisions concerning UV filters, and create a foundation for future research in this new and emerging field of coral toxicology.

1.14.P-Tu-054 Investigating Sunscreen Rinse-off: in vivo and in vitro Differences of Formula Attributes and the Implications for Environmental Emissions of UV Filters

Andrea Carrao and Celine N Schmitt, Kao USA, Inc.

Sunscreens protect people from harmful ultraviolet (UV) radiation using chemical ingredients known as UV filters in formulated products. Often sunscreens are applied near outdoor locations such as beaches. When people who have applied sunscreen to their skin enter the water, direct emission of UV filters to the environment occurs. While the effects of UV filters in the aquatic environment are well researched, environmental exposures are often understudied. Very often, when estimating environmental emissions of UV filters, it is assumed that 100% of the sunscreen will be rinsed off into the environment. However, this is likely an overestimation. Sunscreen water resistance testing requires the product to maintain the labelled SPF (sun protection factor) after being submerged in water for up to 80 minutes. Therefore, further research is needed in the potential rinse-off of sunscreens. To date, there has been limited investigation into the potential rinse-off of UV release from dermally applied sunscreen into the environment and what factors may influence rinse-off rates. For this study, two experiments were conducted investigating the sunscreen rinse-off of a commercially available water-resistant sunscreen and a moisturizer with SPF protection. Both products were formulated with the same UV filters: avobenzone (AVO), homosalate (HS), octisalate (OS), octocrylene (OC). The first experiment used 14 human volunteers and was modelled after the United States Food and Drug Administration (US FDA) water resistance test method. The target application thickness for each sunscreen was 2.0 mg/cm² in line with the US FDA recommendation. Water and rinsate samples were taken to analyze for UV filter presence in the aqueous phase as well as potential sorption to the test beakers. A second experiment was also conducted but instead used Vitro-skin as an *in vitro* alternative to using human volunteers. The method was set-up to mimic the *in vivo* study and used the same products and application thickness. The work of this study will investigate the differences observed in sunscreen rinse-off from human volunteers as well as an *in vitro* alternative. The results will compare the differences between a water-resistant sunscreen and one that is not.

1.14.P-Tu-055 The Development of the MERCI Modeling Framework to Evaluate the Exposure of Sunscreen and Cosmetic Ingredients to Marine and Freshwater Ecosystems

W. Martin Williams¹, Scott Dyer², Todd Gouin³, Nicola Hefner⁴, Eva Klingelmann⁵, Amelie Ott⁶, Sascha Pawlowski⁷ and Maura Roberts¹, (1)Waterborne Environmental, Inc., (2)LeTourneau University, (3)TG Environmental Research, (4)DSM Nutritional Products, Ltd., (5)Symrise AG, (6)Cosmetics Europe, (7)BASF SE

A tiered modeling framework called MERCI (Models to Evaluate Direct Release of Cosmetic Ingredients Into Natural Waters) has been proposed and tested to evaluate the potential environmental exposure of sunscreen and cosmetic ingredients to marine and freshwater organisms. The framework consists of several levels of assessment ranging from simple dilution-based estimates to complex 3-dimensional circulation models. A tiered system permits an initial conservative screen to determine if a chemical may pose a potential risk with minimal effort and often with sparse information. Progressive tiers introduce additional processes and complexity to improve the accuracy of predictions. Specific models are proposed 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. Model testing involved comparing model predictions of chemical concentrations to measured values reported in three UV filter monitoring studies. The studies were selected based on criteria that included the robustness of monitoring data, the availability of information on beach attendance and UV filter use, and availability of information on bathymetry, currents and water/sediment properties. This presentation describes the model framework, the results of model testing, a sensitivity analyses conducted to identify the relative importance of model inputs, guidance for input parameter selection, and descriptions of model scenarios that are being considered for the implementation of the MERCI framework.

1.15.P-Mo Investigating the Ecological Effects of Nanoplastics in Aquatic Environments

1.15.P-Mo-020 Establishing Ecotoxicology Testing Strategies for Comprehensive Governance of Micro(nano)plastics

Andrew Barrick^{1,2}, Amelie Chatel¹ and Nicolas Manier³, (1)Western Catholic University, (2) Auburn University, (3)Institut National de l'Environnement Industriel et des Risques (INERIS)

Plastics are prolific anthropogenic stressors with vast amounts entering the biosphere through multiple routes, leading to high levels of accumulation in receptor ecosystems. Ecotoxicology research has primarily focused plastics polymers and micro(nano)plastics (MNPs), particles less than 5mm and less than 1µm in size respectively, with inconclusive results regarding ecotoxicological potential. As our understanding of hazards associated with MNPs advances, it is evident that plastics are complex stressors due to 1) variations how polymer types and other physical properties influence environmental hazards, 2) how physicochemical properties of MNPs increase bioavailability of co-contaminants (additives and ambient contaminants) and 3) how MNPs and co-contaminants intersect on ecotoxicity. There is also an emergent need to investigate the fate and ecotoxicology of MNPs and associated hazardous chemicals across environmental compartments (soil to freshwater and freshwater to sea). Effective management of plastic contamination requires effective governance and mitigation options. Lack of communication across research groups has severely hampered regulatory perspectives for MNP research with only 15-20% of publications providing sufficient information to establish effective governance. As plastic wastes are an international issue, it is necessary to take a global perspective on ecotoxicology and identify how local research can address the plastic challenge. This presentation provides a synopsis of research efforts in France and New Zealand to characterize hazards associated with plastic pollution. The research focuses on lessons learned and highlights emerging research needs to establish standardized ecotoxicological testing required for regulation. This platform presentation highlights considerations that need to be made to make MNP research regulatory ready.

1.15.P-Mo-021 Developmental Exposure of Zebrafish to Nanoplastics Affects Cardiovascular Structure and Function but Does Not Disrupt Blood Brain-Barrier Permeability

Jordan Avery Pitt, Neel Aluru and Mark Hahn, Biology, Woods Hole Oceanographic Institution

Microplastics and nanoplastics can be found ubiquitously in the natural environment and are a source of aquatic life exposure. The health impacts of these exposures are not yet fully understood. Previous research has indicated that nanoplastic exposure potentially compromises the blood-brain barrier (BBB). The objective of this study was to investigate the impacts of a nanoplastic exposure on cardiovascular function in the developing brain. Using 50 nm polystyrene nanoplastics (PS NP), we conducted exposure experiments with the transgenic zebrafish line Tg(*kdrl:DsRed*), in which the vasculature is labeled. Embryos or larvae were exposed to 0, 1, 10, 50, or 100 ppm PS NPs during two different developmental periods (2 days post fertilization (dpf) – 6 dpf or 5 dpf – 6 dpf). These two developmental periods were chosen based on BBB formation in zebrafish. The longer exposure period (2 dpf – 6 dpf) coincides with brain angiogenesis and barrierogenesis while the 5 dpf – 6 dpf exposure occurred when the BBB is functional. Cardiovascular endpoints included cerebrovascular coverage, cerebrovascular vessel diameter, and heart rate. BBB permeability was assessed with 10 kda, 66.5 kda, and 2000 kda dyes to examine penetrance into the brain from the cerebrovasculature. In addition, survival and morphological deformities were assessed. None of the plastic exposures induced a significant amount of mortality up to 6 dpf. The 100 ppm exposure led to either a decrease in the number of larvae with inflated swimbladders following the 2dpf – 6dpf exposure or significantly smaller swimbladders after the 5 dpf – 6dpf exposure. Heart rate was significantly reduced in the 100 ppm PS NP exposure group regardless of exposure duration. Similarly, the 100 ppm PS NP exposure led to significantly decreased blood vessel area and vessel diameter in the brain. However, no changes in BBB permeability were observed. No correlation between BBB permeability and defects in the cerebrovascular system was observed. These results indicate that, in contrast to previous findings, even extremely high concentrations of plastic do not alter BBB function, but they do severely impact the cardiovascular system. [Supported by the US National Science Foundation (NSF) Graduate Research Fellowship Program and by a grant from WHOI Sea Grant (Award No. NA180AR4170104, project R/P-89).]

1.15.P-Mo-022 Modification of a Fast, Accessible Microplastics Screening Method and its Implementation on Cahaba River Water Samples

Josh Forakis, Hawaii Pacific University

Microplastics have emerged as a persistent anthropogenic pollutant, and little is known about their impact on humans and other organisms. Commonly, spectroscopic methods are employed to quantify microplastics in the environment, but these methods require time, expensive instruments, and expert knowledge. A current need in the field of plastic pollution research is a fast-screening method for environmental organizations to routinely estimate microplastics in nature. Nile Red is often discussed as a candidate to improve upon the imprecise visual detection method most employed by environmental organizations, but the Nile Red method itself has several challenges, including background fluorescence. To improve procedures, a novel water bath step (NR-B) was designed and assessed in a variety of experiments. While the standard dyeing method (NR-S) involves staining isolated particles directly on the filter, the NR-B method has isolated particles suspended in a small water bath (~30 ml) to maximize particle dye interaction. An optimal NR-B dyeing time was determined, compared to NR-S on various polymers (expanded polystyrene, polystyrene, nylon, and polypropylene), and applied to standing water samples from several locations along the Cahaba River to obtain microplastic particle counts from 0.5 mm to 5 mm. Results demonstrate that the NR-B method does reduce background fluorescence for all tested polymers except for expanded polystyrene and that this optimized Nile Red procedure is appropriate for screening environmental samples for microplastics. Recommendations for microplastics researchers using Nile Red in laboratory studies are also discussed.

1.15.P-Mo-023 Toxicity of Microplastics Explorer (ToMEx) 2.0

Leah Thornton Hampton¹, Dana Briggs Wylers¹, Bethanie Carney Almroth², Scott Coffin³, Win Cowger⁴, Darragh Doyle², Eden Hataley⁵, Sara Hutton⁶, Magdalena M. Mair⁷, Laura Monclus⁸, Emma Sharpe⁹, Samreen Siddiqui¹⁰ and Alvine Mehinto¹, (1)Southern California Coastal Water Research Project, (2)University of Gothenburg, (3)California State Water Resources Control Board, (4)Moore Institute for Plastic Pollution Research, (5)University of Toronto Scarborough, (6)GSI Environmental, Inc., (7)University of Bayreuth, (8)Norwegian University of Science & Technology, (9) Western Washington University, (10)California Department of Fish and Wildlife

Research on the toxicological effects of microplastics (MPs) is rapidly expanding as scientists, environmental managers, and policy makers seek to better understand the potential impacts of MPs on environmental and human health. The pace and intensity at which the field is developing presents a significant challenge in keeping up with the most recent advancements in MP toxicity research as well as identifying data that are appropriate for specific applications (e.g., hypothesis testing, risk assessment). To address this challenge, the Toxicity of Microplastics Explorer (ToMEx) was developed as an open-access database. The database is accompanied by an open-source Rshiny web application that effectively and efficiently catalogs, summarizes, and analyzes MP toxicity data for both ecological and human health. The first iteration of ToMEx (released in 2021) housed toxicity data from more than 200 peer-reviewed scientific studies and was initially used to develop preliminary health-based MP thresholds and identify critical future research needs. Many other scientists have used ToMEx for their own research applications, demonstrating its value amongst the MP community. Yet, publication rates have not slowed, highlighting the need to update ToMEx with the most recent data to maximize its utility. Thus, a four-part virtual workgroup series was hosted over the course of several months in 2023 to crowd-source the data mining and validation process required to create “ToMEx 2.0”. Participants were provided with training and guidance on how to mine data accurately and consistently. After mining data from at least two peer-reviewed studies from a pre-screened list, participants were randomly paired to swap data templates and validate each other’s work. More than 60 researchers from 14 different countries and across sectors submitted data from more than 160 studies, almost doubling the original database. In addition, a small pilot study to explore the potential use of artificial intelligence to facilitate data mining was also conducted. ToMEx 2.0 will be used to track research trends, improve thresholds for risk assessment, and advance the collective understanding of MP toxicity.

1.16.P-Tu-057 Establishing *Gladioferens pectinatus* as Regional Indicator Species for New Zealand

Andrew Barrick, Auburn University

Environmental risk assessments often implement standardized test species for the management of hazardous substances. These testing strategies often follow internationally accepted guidelines for characterizing the risk chemicals. However, some international countries have limited ability to implement their own testing strategies to address chemical contaminants present overseas and often rely on thresholds from overseas to establish environmental policy. New Zealand, as a pristine country, there are emerging concerns whether these reported thresholds are representative of how the country’s unique environments are likely to respond, should contamination occur. There is an inclination in New Zealand to use local test species to have stronger representation of how local environmental are likely to respond to anthropogenic perturbations. One potential candidate species for hazard assessments is the calanoid copepod, *Gladioferens pectinatus*, which is a species unique to the island and has a broad geographic range surrounding both the North and South Island, demonstrating its potential as an indicator species. The species is also euryhaline and is capable of surviving in broad ranges of salinities, promoting its flexibility in environmental hazard assessments. The purpose of this presentation is to highlight the research conducted in New Zealand to demonstrate the efficacy of *G. pectinatus* as a suitable alternative test species for hazard assessments. The presentation covers results for copper, emerging organic contaminants, and plastic additives. The presentation provides perspectives on research needed to further develop the species as a candidate for commercial testing. Finally, the study also highlights

the development of the first completed transcriptome for *G. pectinatus* to develop a mechanistic approach for hazard assessments and highlights the use of multigenerational testing for future ecotoxicity testing strategies in New Zealand.

1.16.P-Tu-058 Mercury Quantification in Freshwater Sediments from an Agricultural Population in Northern Colombia Using *Caenorhabditis elegans* as a Toxicity Model Organism.

Belkis Palacio, Maria C Garcia and Barbara J Arroyo, Universidad de Cartagena

Sediments act as reservoirs for chemicals that can adversely affect aquatic life. Sediments provide protective habitat for juvenile species, which is vital for reproduction of many species. Contaminants can bioaccumulate within aquatic animals and bioconcentrate at higher trophic levels posing a risk to human consumers. Mercury (Hg) is a ubiquitously distributed heavy metal that produces toxic effects on human health. The most important source of this exposure is fish consumption. The objective of this study was to use the nematode *Caenorhabditis elegans* as a biological model to evaluate the toxicity of the surface sediments of a major river system in an agricultural population in northern Colombia. Total mercury (Hg-T) concentrations were determined at four stations in the Marialabaja swamp by Atomic Absorption Spectrometry, using method 7473 US EPA SW-846. Hg-T concentrations (microg g(-1)) ranged from 0.117 ± 0.001 to 0.173 ± 0.001 in the sediments. All sediment samples had levels below the US-EPA value of 0.2 microg g(-1). The toxicity of the sediment extracts was evaluated in the nematode by exposing it to different concentrations of aqueous extracts for 24 hours. The endpoints were lethality and locomotion in wild-type Bristol N2 strains and oxidative stress expression in *sod1* mutant strains. K medium was used as a control. All procedures were performed in triplicate. No lethal effects on the nematodes were evident. There were significant differences with respect to the control in the movements of the worms. Exposure to the extracts produced overexpression of oxidative stress genes in *C. elegans*. Mercury has deleterious effects on organisms by altering the function and structure of their proteins or by generating ROS, mainly affecting the central nervous system. Our study induced oxidative stress and alterations in movements, which could be related to the affectation of the neurological system of the nematodes. *C. elegans* can be an useful tool in evaluating the effects of toxins on nervous system, furthering our understanding of the molecular mechanisms underlying toxicity.

1.16.P-Tu-059 Assessing Invertebrate Community Level Resistance to Insecticides in the San Francisco Bay Delta

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The invertebrate community within the San Francisco Bay Delta faces significant stressors, particularly from storm-driven contaminants including phenylpyrazole and pyrethroid insecticides. These stressors have significant impacts on the food web through shifts in prey items and invertebrate community structures. For example, there have been recorded instances of invertebrates evolving resistance to insecticides, particularly the non-target crustacean *Hyalella azteca*, which suggests ecological impairment and contributes to trophic transfer of high insecticide concentrations to fish. However, the extent of adaptive resistance across invertebrate communities is not known. To better understand the extent of adaptive resistance in the San Francisco Bay Delta, common invertebrates were collected for evaluation of resistance in two genes: resistance to dieldrin (RDL) and voltage-gated sodium channel (VGSC). These genes are common targets of phenylpyrazole and pyrethroid insecticides and mutations in these genes can confer resistance to these insecticides. The collected invertebrates were divided by their orders, including Trichoptera, Plecoptera, Diptera, Amphipoda, Cladocera, Copepoda, Ephemeroptera, and Odonata. For each order and gene, degenerate primers were made and tested for their validity on a small number of samples from each order. Most of the degenerate primers were able to produce the expected product size for their appropriate gene and order. Degenerate primers for RDL successfully amplified gene products for Trichoptera, Plecoptera, Diptera, Cladocera, Ephemeroptera and

Odonata and were identified correctly via sequencing. Degenerate primers for VGSC successfully amplified gene products for Trichoptera, Diptera, Ephemeroptera and Odonata and were identified correctly via sequencing. In our on-going work, we are determining if any of the invertebrates collected from different regions of the San Francisco Bay Delta have developed resistance to phenylpyrazole or pyrethroid insecticides by investigating target site mutations in RDL and VGSC. The goal of this project is to develop rapid molecular assays for identifying the presence of resistance mutations within freshwater invertebrate communities.

1.17.P-We General: Environmental Toxicology and Stress Response

1.17.P-We-011 Assessment of Reprotoxic Potential of Bisphenol Analogs in Male *Caenorhabditis elegans* *Katelyn D Waligora¹, Cole M Higley, Isabella Burke, Jessica R Clore, Shannon C Timmons and Aleksandra Kuzmanov, Lawrence Technological University*

Research shows that even low levels of bisphenol A (BPA), a widely-used plasticizer, can lead to meiotic errors resulting in poor reproductive cell quality and infertility. In recent decades, numerous reports of human health concerns have prompted the search for BPA alternatives. However, evidence suggests that the analogs currently used to replace BPA, such as bisphenol S (BPS), pose similar health risks. To gain insight into the influence of bisphenols on spermatogenesis, we have assessed three structurally diverse bisphenol analogs - BPA, BPS and tetramethyl bisphenol F (TMBPF) using *C. elegans*-based assays. The assays evaluate quality of sperm by assessing a total spermatid count, spermatid size and activation as well as *in vivo* oocyte fertilization. L4-stage male larvae were treated with 0.5 mM concentrations of the ethanol-dissolved bisphenol analogs for 48 hours in liquid medium. The chosen exposure concentration was previously demonstrated to approximate human internal BPA and BPS levels. Our preliminary results showed that exposure to BPA, BPS and TMBPF reduced spermatid size without significantly affecting spermatid activation. As we continue to assess other spermatogenesis parameters including a total sperm count, timing of activation and *in vivo* oocyte fertilization, we aim to explore how structural properties of bisphenols affect male reproductive health.

1.17.P-We-012 Development, Behavior, and Gene Expression Patterns of Two Zebrafish Strains Exposed to Two Fractions of Fine Particulate Matter (PM_{2.5})

Shayla Victoria, Connor Necaie and Courtney Roper, University of Mississippi

Fine particulate matter (PM_{2.5}) is the solid and liquid portion of air pollution that is 2.5 μm or smaller in aerodynamic diameter. Exposure to PM_{2.5} has been associated with various human health impacts, with most research focus being on conditions of the cardiopulmonary systems. The predicted underlying mechanism of action for PM_{2.5} toxicity is through the production of reactive oxygen species, inducing oxidative stress, thus affecting a range of tissues. However, little is known about the effects of PM_{2.5} organ systems outside of the cardiopulmonary systems, but recent epidemiological and rodent studies have highlighted the potential associations between PM_{2.5} exposure and reproductive impacts. Therefore, we aimed to identify changes to development, behavior and gene expression of zebrafish (*Danio rerio*) in response to PM_{2.5} based on wild-type strain and the fraction of PM_{2.5} isolated. Zebrafish embryos (6 hpf) from two wild-type strains (AB and 5D) were exposed to 12.5, 25, 50, 100, and 200 μg/mL whole particle and soluble fractions of fine particulate standard reference material (n=33/group). Health, morphology, and the behavioral endpoint, larval photomotor response (LPR), were measured. Morphological changes were strain-dependent, but not fraction-dependent, while changes to the LPR varied by concentration and by zebrafish strain. At the lowest exposure concentration of 12.5 μg/mL, swim distance differed significantly between AB and 5D strains (P=0.020, two-way ANOVA Holm-Sidak). To link the observed whole-organism impacts to molecular events, gene expression analysis by RT-qPCR is currently in progress to compare expression of various genes involved in reproductive function (vitellogenin) and oxidative stress responses (superoxide dismutase). Overall, this work contributes to filling knowledge gaps about the health impacts of PM_{2.5} exposure by examining less-studied organ systems while also

addressing potential limitations when comparing across zebrafish research studies that use varying wildtype strains and PM_{2.5} fractions.

1.17.P-We-013 Ecological Risk Assessment of Polychlorinated Biphenyls (PCBs) Levels in Water and Three Fish Species in Lagos lagoon, Lagos Nigeria

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Polychlorinated Biphenyls (PCBs) are highly carcinogenic, persistent, organic chemical compound that is becoming an emerging issue for aquatic health. Humans can contact PCBs from contaminated fishes. The objective of the study was to assess PCBs in selected and commonly consumed fish species in Nigeria waters. Environmental study of Polychlorinated Biphenyls (PCBs) in water and in three fish species (*Chrysichthys nigrodigitatus*, *Cynoglossus senegalensis* and *Pseudolithus elongatus*) were evaluated from Iddo, commodore channel and Takwa bay within Lagos Lagoon, Nigeria. Gas Chromatography coupled with Electron Capture Detector (GC-ECD) was used to detect PCBs levels in the samples. The results showed that the mean concentration of PCBs detected in the water across the stations were 0.51 ± 0.18 mg/l (Iddo), 1.39 ± 0.48 mg/l (commodore channel) and 2.39 ± 2.29 mg/l (Takwa bay) and were not significantly ($P > 0.05$) different. The values were above USEPA maximum limit for water (0.0005 mg/L). The highest mean concentrations of PCBs detected in *C. senegalensis*, *C. nigrodigitatus* and *P. elongatus* were 8.97 ± 1.02 , 12.50 ± 2.48 and 16.28 ± 5.05 mg/kg wet weight respectively and were not significantly ($P > 0.05$) different among the samples but were above the Environmental Canada, 2000 six indicator congener's regulatory body's permissible limit of 0.075 mg/kg wet weight) and WHO recommended maximum limit for fish (0.2 mg/kg). Hazard Quotient (HQ) for non-carcinogenic effects of dioxin-in the fishes is probably a risk factor and a high Hazard Quotient (HQ) for non-carcinogenic effects of dioxin-like PCBs for human especially in children consumption PCBs contaminated fishes. Therefore, regulatory bodies should enforce effective monitoring and regulations in the use and disposal of products that contain PCBs to ensure a safe and healthy environment.

1.17.P-We-014 U.S. National Recommended Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) Ambient Water Quality Criteria for Aquatic Life

James Justice, *Amanda Jarvis*, *Brian Schnitker*, *Mike Elias* and *Kathryn Gallagher*, U.S. Environmental Protection Agency

Perfluorooctanoic Acid (PFOA) and Perfluorooctane sulfonate (PFOS) are part of the broader group of Per- and Polyfluoroalkyl Substances (PFAS). Given their unique physiochemical properties, PFOA and PFOS have been used in a wide range of industrial and consumer products. Following use and disposal of these products, PFOA and PFOS may be released in the environment where they are highly persistent, being detected in water and organismal tissues within aquatic ecosystems. In accordance with the U.S. Environmental Protection Agency's PFAS Strategic Road Map, the Agency developed national recommended PFOA and PFOS Aquatic Life Ambient Water Quality Criteria. The goal of this presentation is to provide an overview of the PFOA and PFOS freshwater criteria and estuarine/marine benchmarks, describe the toxicity studies used to derive the PFOA and PFOS criteria and benchmarks, and summarize the scientific comments received from the public. EPA's national recommended PFOA and PFOS criteria reflect the maximum concentrations, with associated frequency and duration specifications, that support protection of aquatic life from acute and chronic effects associated with PFOA and PFOS in freshwaters. States and authorized tribes can adopt EPA's national recommended PFOA and PFOS Aquatic Life Ambient Water Quality Criteria into their water quality standards or can adopt other criteria that are scientifically defensible based on local or site-specific conditions. EPA's recommended criteria are not a regulation, nor do they impose legally binding requirements.

1.17.P-We-015 Evaluation of Aquatic Invertebrates as Vectors of Contaminants to Proximal Ecosystems

Allison DeLoache, Joslyn Kent, Casey Kennedy, Vince Hart, Chris Murray and Justin Anderson, Southeastern Louisiana University

Vertical transfer of toxicants within a food web is a topic of increasing concern when considering both bioaccumulation and magnification in higher level taxa. Identifying vectors for these contaminants is an important step towards more informed risk assessments and management strategies. Aquatic invertebrates are commonly used as indicators of water quality. As anthropogenic land use changes increase, aquatic and terrestrial pollution also increases. One popular insect that thrives during land use change as well as climate change, is the mosquito (Diptera: Culicidae). Because of its biphasic life cycle, and propensity for stagnant or low flow water, mosquito larvae are likely exposed to numerous toxicants prior to emergence as adults. Whether larvae or adult, mosquitoes are often the base of the food chain in ecosystems. We hypothesize that metamorphosing aquatic invertebrates act as vectors to carry toxicants from aquatic to terrestrial environments. In this study we examined whether 17 α -methyltestosterone (MT) and perfluoropentanoic acid (PFPeA) have any effect on larvae development, and explore if invertebrates raised in contaminated water will act as a vector to vertebrate species. We used *Aedes albopictus* as an insect model. Larvae were dosed (with MT or PFPeA) within 24 hours of hatching, and incubated for approximately 14 days in their respective treatment groups. As pupae developed, they were removed from the colony and placed in a separate cup to successfully emerge as adults. Adult mosquitoes were frozen and stored. To evaluate the vector potential of these mosquitoes into vertebrates, we used green anoles (*Anolis carolinensis*) as a predator in our system. After capture, anoles were assigned a treatment group, fasted for 5 days, then weighted and fed 4 adult mosquitoes per gram of anole body weight. MT treatment group anoles were fed for a total of 2 days before termination. PFPeA treatment group anoles were fed for a total of 5 days before termination. Blood was collected from each anole and standard extraction protocols were used on blood and adult mosquitos. Quantification of transferred toxicant concentration was performed using HPLC MS/MS in tandem. The goal of this study was to develop a better understanding of the potential mechanisms for toxicant movement using common prey items and vertebrate insectivores. A detailed presentation of the results will be provided.

1.17.P-We-016 Farming Antimicrobial Resistance: The Off-Target Effects of Fungicide Exposure on Plant Surface Bacteria

Natalie Wieber, Paul Koch and Jeri Barak, University of Wisconsin, Madison

Increasing antimicrobial resistance (AMR) poses a direct threat to human health, but the impact of routine agrochemical usage on the development of resistant bacteria on plants remains unclear. We assessed the effect that repeated applications of common fungicides had on plant surface bacterial community composition and their resistome to better understand the off-target effects of agrochemicals. The study was conducted on creeping bent grass in Madison, WI during the summer of 2022. Leaf material was collected prior to fungicide application, as well as 4 hours, 24 hours, 96 hours, and one week post application. HT-qPCR and 16s sequencing results were used to determine abundance of genes known to confer antimicrobial resistance and bacterial community composition respectively. This study will increase understanding of the impact fungicides have on plant surface bacteria and may lead to novel AMR management strategies.

1.17.P-We-017 Homologous Recombination Repair Protect Whale Cells to Avoid Cr(VI)-Induced Chromosome Instability

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Particulate hexavalent chromium [Cr(VI)] is a well-established human lung carcinogen, but the mechanism for Cr(VI)-induced cancer is uncertain. Chromosome instability (CIN) is a hallmark of lung cancer and is considered a major factor in Cr(VI)-induced lung cancer. Structural chromosome instability can result from unrepaired DNA double strand breaks. Homologous recombination (HR) repair protects against these breaks. In

human lung cells, we found Cr(VI) induces DNA double strand breaks while simultaneously inhibiting DNA double strand break repair, resulting in chromosome instability. Whales face long-term exposure to Cr(VI) and accumulate Cr in their tissues, but appear to have a low incidence of cancer. Thus, to further explore the mechanism of Cr(VI)-induced lung cancer, we tested the hypothesis that whales are resistant to Cr(VI)-induced chromosome instability. We measured the ability of Cr(VI) to induce DNA double strand breaks, HR repair, and chromosome damage in whale lung cells. We discovered Cr(VI) induces DNA strand breaks in whale cells, but whale cells avoid repair inhibition and maintain their HR repair response. Consequently, the amount of chromosomal damage was greatly reduced with no apparent chromosome instability. Next, we compared Cr(VI)-induced DNA double strand breaks, HR repair inhibition, chromosomal instability, and cytotoxicity in lung cells of whale, human and rat. We added some rat lung cell comparisons to clarify whether differences found between human lung cells and whale lung cells reflect whale specific outcomes. We found Cr(VI)-induced similar DNA double strand breaks levels in all three species' cell lines and only whale lung cells escape Cr(VI)-induced repair inhibition. Subsequently and consequently, there was no chromosome instability in the whale cell and less cell death, while the human lung cells incurred Cr(VI)-induced chromosome instability and increased cell death. We also found by comparing key select outcomes in rat cells, that the whale outcomes were likely specific to whale cells, as outcomes in rat cells were similar to those in human cells. The work was supported by the National Institute of Environmental Health Sciences [ES016893 to J.P.W].

1.17.P-We-018 Characterization of Thyroid Disrupting Chemicals (TDCs) by Modulation of Hepatic Metabolism: Adverse Outcome Pathway (AOP) Approach

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Many environmental chemicals have the potential to disrupt thyroid hormone (TH) homeostasis, leading to various adverse health outcomes. An increasing number of adverse outcome pathways (AOPs) have been proposed to explain the disruption of THs, encompassing synthesis, metabolism, and transport. However, there is a lack of comprehensive understanding on the related key events, particularly regarding hepatic metabolisms of THs. In this study, we chose ten potential thyroid disrupting chemicals (i.e., *p,p'*-DDE, PFOS, DEHP, DINCH, BPA, BP-3, EHMC, AHTN, HHCb, and MK), and measured the responses of several key events (KEs) employing both *in vitro* and *in vivo* models. HepG2 (human liver), McA-RH7777 (mouse liver), and ZFL (zebrafish liver) cells; and larval and adult zebrafish were also used. Exposure to PFOS, BPA and BP-3 significantly up-regulated *PPARA* and *UGT1A1* genes in HepG2 cells. In McA-RH7777 cells, only EHMC significantly down-regulated *Ugt1a1*, *Sult1b1* and *Ppara* genes. In ZFL cells, exposure to BPA, *p,p'*-DDE, DEHP, or BP-3 induced *ugt1ab* gene. In larval zebrafish, BP-3, EHMC, HHCb, and MK demonstrated a significant decrease of TH levels and locomotor activities, accompanied by transcriptional changes of several genes. However, the direction of regulatory change varied among chemicals, indicating different modes of action affecting hepatic regulations. In adult male zebrafish, exposure to BP-3 and EHMC activated hepatic xenobiotic nuclear receptors (*pxr*, *ahr2*) and their downstream KEs (*cyp1a*, *cyp2* and *cyp3a65*, *ugt1ab*, and *sult1st5*), suggesting that the reduction of THs might be attributed to enhanced THs excretion by hepatic metabolism. Overall, our results demonstrate that chemicals can be classified by the pathway hepatic metabolism of THs is disrupted. Furthermore, we found that an AOP-based strategy, combining *in vitro* and *in vivo* approaches, is useful to screen and prioritize thyroid disrupting chemicals.

1.17.P-We-020 Particulate Hexavalent Chromium Induces DNA Damage Response Failure in Human Cells but Not in Alligator Cells

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Hexavalent chromium [Cr(VI)] is a metal known to cause lung cancer in humans. While lung cancer is primarily associated with smoking, many deaths are due to other factors, still making lung cancer in non-smokers one of the top 10 deadliest cancers worldwide. Cr(VI) is an environmental pollutant, impacting aquatic ecosystems. Alligators skin samples show high levels of Cr(VI) accumulation, but alligators have few reports of cancer. Cr(VI) has been shown to induce chromosome instability (CIN), but mechanism for how they induce CIN are poorly understood. We focused on Cr(VI) as a representative metal as it is a known human lung carcinogen that induces DNA double strand breaks while simultaneously inhibiting the repair of those breaks resulting in CIN, an early event of lung cancer. To develop a better understanding of how Cr(VI) causes carcinogenesis in humans, we investigated the high-fidelity DNA damage repair, homologous recombination (HR) response in human lung cells and compared this to the response in alligator lung cells. Cr(VI) targets RAD51, a key effector protein within the HR pathway, and prevents its loading onto a nucleoprotein filament. HR is regulated by RAD51 loaders (RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3). The effect of Cr(VI) on these loaders are poorly understood and largely uninvestigated. Thus, we hypothesize that Cr(VI) disrupts RAD51 and its loaders, altering RAD51 filament-loading, which inhibits HR repair in human lung cells. We measured the effect of Cr(VI) on RAD51 and RAD51D as representative DNA damage response proteins. Human and Alligator lung cells were exposed to acute (24 h) and prolonged (120 h) Cr(VI) concentrations of 0-0.3 ug/cm². Using immunofluorescent foci formation for DNA repair, western blot for protein levels, and QRTPCR for mRNA levels, we found alligator lung cells are resistant to Cr(VI)-inhibited HR repair. Completion of this study will identify key mechanisms for how hexavalent chromium causes CIN. Supported by NIEHS grants R01ES016893 and R35ES032876 (J.P.W.) and T32ES011564 (A.W. and J.P.W.).

1.17.P-We-022 Acute Beta-N-methylamino-L-alanine (BMAA) Effects on Adult Zebrafish Brain, Locomotion, and Recoverability

Dani J Hamilton and Sherri A Emer, Florida Gulf Coast University

Beta-N-methylamino-L-alanine, or BMAA, is a neurotoxin produced by cyanobacteria. BMAA resulting from blue-green algae blooms may negatively affect aquatic and terrestrial life, including humans. Previous studies of BMAA effects in fish have focused on embryonic development, and have failed to address impacts in adulthood. Results from our preliminary study suggested that BMAA has potential negative effects on the central nervous system and locomotion within adult zebrafish. Specifically, we observed in BMAA-exposed fish significant increases in an apoptosis marker in the brain and decreased swimming compared to control fish. Apoptosis labeling most often occurred in the telencephalon, optic tract, primary motor cortex, diencephalon, and other areas functioning in movement, integration, and metabolism. Our current goal was to validate those data and test the hypothesis that fish recover following acute exposure to BMAA. Fish were exposed for 30 days to low and high concentrations of BMAA that were based on levels reported locally, from Sarasota Bay. Fish were subsequently allowed to recover in untreated water for 15 or 72 days. Throughout BMAA treatment and return to normal water, swimming behavior was recorded weekly for future locomotion analysis. Following exposure to normal water, brains were immunolabeled with the apoptosis marker caspase and imaged with fluorescence microscopy. Preliminary analysis suggests that apoptosis was maintained in the brains of the fish even following long term removal from BMAA. While zebrafish have been shown to regenerate some neurons after injury, these results raise new questions regarding recoverability from acute neurotoxin exposure, particularly in adults. These results have important implications to the relationship of neurodegenerative conditions and environmental toxins, and possible life history stage-dependent consequences that can impact the overall health of nonhuman animals and humans alike.

1.17.P-We-023 Linking Insecticide Body Residue With Thermal Performance and Behavioral Impairment in Juvenile Chinook Salmon

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Insecticides are used globally in agricultural, industrial, and household settings and are detected in surface waters worldwide. The California Delta is no exception, where contamination has resulted in it being listed as impaired under the Clean Water Act. Insecticides distributed throughout the system have been linked to a wide spectrum of adverse biological effects ranging from sublethal impairments to lethality. Pesticide residues commonly bioaccumulate in fish, but the potential effects of that body burden remain largely unknown. To that effect, our global objective is to develop a response spectrum framework that associates body residue with adverse biological effects. As part of this broader project, here we present two sub-lethal endpoints: thermal tolerance and behavioral alterations in juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) exposed to bifenthrin. We hypothesized that sub-lethal levels of bioaccumulation are high enough to affect thermal performance and organismal behavioral, and thus potentially migratory success. To test this hypothesis juvenile Chinook Salmon were exposed for 10 days to four concentrations of bifenthrin (125, 500, 1000 and 2000 ng/L) and a control. After exposure, behavioral assays were conducted using video-tracking software, from which total distance moved, thigmotaxis, and social behavior were evaluated. To evaluate thermal performance and upper thermal limits we investigated the critical thermal maxima (CT_{max}). Time to loss of equilibrium for the 500 ng/L condition was different from the control and the 125 ng/L condition (p<0.05). We will present this, along with behavioral data across conditions, along with body burden analysis and water concentration data. This information will be used to interpret the measured sublethal effects to better understand how insecticide residue from field-caught Chinook Salmon can be used to monitor the impact of contaminants on their outmigration success.

1.17.P-We-024 Expression of CDC2 and POLD 1 Genes and Oxidative Stress Biomarkers in Cambray Onion (*Allium fistulosum*) Roots Exposed to Chloroform, Chlorpyrifos, and Lindane

Eunice Danilú Couh Puga, Maria Concepción Gómez Maldonado and Gabriela Rodriguez-Fuentes, National Autonomous University of Mexico

The presence of pollutants in groundwater has caused global concerns about the toxic effects and adverse consequences to the environment. As an economic and representative proposal to assess the negative effects, the use of the *Allium* sp. bioassay stands out. This work aimed to assess gene expression, oxidative stress biomarkers and its possible relationship with concentration of three commonly found pollutants in Yucatan groundwater. In this context, Cambray onion (*A. fistulosum*) roots were pre-cultured in distilled water during 48 h, and then exposed to different concentrations of Chlorpyrifos (0.1, 1 and 10 µg/L), Lindane (100, 300 and 500 µg/L), Chloroform (50, 100 and 200 µg/L) for 24h. Preliminary findings showed that different concentrations of pollutants had significant effects on the oxidative stress biomarkers, moreover, cell division genes, *CDC2* and *POLD 1* presented significant differences between treatments. Thus, the effects on cellular division of the pollutants in the roots of *A. fistulosum* are probably related to the generation of oxidative stress. The results of the present study clearly indicate that bioassays with sensitive plants such as *A. fistulosum* are valuable and complementary tools to evaluate the environmental impact of pollutants.

1.17.P-We-026 PFAS Bioaccumulation, Depuration, and Associated Energetic Costs in the Eastern Oyster, *Crassostrea virginica*

Kayla Taylor Boyd, Vanisree Mulabagal, Joel Hayworth and James Stoeckel, Auburn University

Per- and poly-fluorinated substances (PFAS) are a class of man-made chemicals that are widespread and persistent in the environment, including Mobile Bay, AL. Impacts of PFAS on the health of oysters on natural reefs, as well as off-bottom cages are an emerging concern to conservationists and oyster farmers alike. Oysters

in estuaries may be chronically exposed to contaminants such as PFAS, which can bioaccumulate within oyster tissue. Depuration of contaminants has been shown to be energetically expensive in some bivalves and there is concern that this energetic cost may have a negative effect on organismal health. To address these concerns, eastern oysters (*Crassostrea virginica*) were exposed to an ecologically relevant mixture of PFAS (PFPeA, PFHxA, PFBS, PFOA, and PFOS) at a nominal concentration of 2 µg/L per compound (10 µg/L combined) for 28 days and then transferred to clean water for 5 days. Concentration of PFAS in soft tissues was quantified in a subset of animals (Day 2, 4, 7, 10, 14, 20, and 28 of exposure and day 1, 2, and 5 of depuration) using ultra high-performance liquid chromatography-MS/MS to monitor bioaccumulation and depuration. Respiration rates were measured on day 10 of exposure and 24 hours following transfer to clean water to assess energetic costs associated with exposure and depuration. There was no significant difference in respiration rate between treatment and control oysters during exposure or during depuration. Oysters exhibited different BAF values for each PFAS compound during exposure but depurated PFAS to below detection limits within 24 h of transfer to clean water. We found no evidence that exposure to ecologically relevant mixtures of PFAS at concentrations (i.e. 10 µg/L) higher than we measured in ambient Mobile Bay waters are likely to adversely affect the energetic health of wild or farmed oysters. We did find evidence that oysters can quickly depurate PFAS mixtures upon transfer to clean water – a characteristic that may be useful when addressing food safety concerns.

1.17.P-We-027 Using A Toxic Aging Coin to Address Concerns for the Global Aging Crisis: Heads for Age Differences, Tails for Accelerated Aging

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We are currently facing an aging crisis, as 20% of the U.S. population will be classified as geriatric (65+) by 2030. Growing geriatric populations will challenge healthcare systems with increased prevalence of age-related diseases (e.g., neurodegenerative disease). Compounding this crisis, environmental pollution contributes to many age-related diseases and may impact geriatric health distinctly from younger individuals. We tackle this two-sided challenge with a “toxic aging coin” approach. One side examines how age impacts toxicity; the other side examines how chemicals accelerate aging. Cr(VI) is a widespread environmental pollutant that induces neurotoxicity. There are currently no environmental regulations considering Cr(VI) neurotoxicity and few considerations for geriatrics, adding to the significance of this work. We hypothesize Cr(VI) will induce different neurotoxic effects in rats at different life stages. We exposed rats from three age groups (3-, 7-, and 18-months old) to Cr(VI) in drinking water at 0.05 and 0.1 mg/L for 90 days. We assessed neurobehaviors using elevated plus maze, Y-Maze, open field assay, and grip strength assay. We assessed brain Cr levels using inductively coupled plasma mass spectrometry and neurodegeneration using silver stain. Our data suggest Cr accumulated in the male and female hippocampus. Cr(VI) increased anxiety in young male rats exposed to both concentrations of Cr(VI) and middle-aged male rats exposed to 0.1 mg/L Cr(VI) and impaired spatial memory in geriatric male rats. Cr(VI) increased anxiety in young and geriatric female rats after exposure to both concentrations and in middle-aged females exposed to 0.05 mg/L Cr(VI). Grip strength assay indicated concentration-associated frailty in young male and female rats but demonstrated a decrease in frailty in geriatric rats. Silver stain demonstrated Cr(VI)-induced neurodegeneration in the hippocampus, amygdala, and striatum of male and female rats; silver-stained neurons increased in frequency with age. In sum, our data suggest Cr(VI) induced neurotoxic effects that changed with life stage. Future work will assess the mechanism of Cr(VI) neurotoxicity. Support by R21ES033327 (JPWJr), R35ES032876 (JPWSr), NIEHS R35-ES028373 (MC), and Jewish Heritage Fund for Excellence Faculty Recruitment Grant Program at the University of Louisville, School of Medicine (JYW).

1.17.P-We-030 Examination of Acute Exposure Effects of Untreated and AOP-Treated OSPW Using a Human Immune Cell-Based Bioindicator System

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Mining of Alberta oil sands bitumen deposits produces large volumes of oil sands process affected water (OSPW), which is a dynamic mixture of organic and inorganic components and shown to have toxic effects. Therefore, an effective remediation procedure is required to remove the potential toxic components from the OSPW and remediated OSPW needs to be examined properly before releasing into the environment. In response to this concern, advanced oxidation processes (AOPs) including electro oxidation and solar oxidation are being investigated to treat OSPW which removes the major organic toxicants of OSPW such as naphthenic acids (NAs). We developed an *in vitro* immune cell-based bioassay system to examine the immunotoxic effects of OSPW before and after treatment procedure. In the present study, human macrophage-like cells (THP-1) were exposed to untreated and AOP-treated OSPW to evaluate their immunomodulatory effects through measuring the expression levels of immune genes, cell surface proteins, and cytokine secretion levels using quantitative(q)-PCR, flow cytometry and human cytokine secretion assay, respectively. Transcriptome analysis was performed on THP-1 cells to examine the exposure effects of untreated and AOP-treated OSPW. Results showed that untreated OSPW significantly induced the gene expression and secretion levels of inflammatory cytokines including interleukin (IL)-1 β , IL-6, IL-8 and tumor necrosis factor (TNF)- α . Furthermore, untreated OSPW significantly increased the gene expression and protein expression levels of two potential immune cell surface markers which includes CD40 and CD54. Our results indicate that untreated OSPW contains potent immunostimulatory compounds that can activate human macrophages. However, results showed that AOP treatments effectively abrogated the immunostimulatory effects of OSPW indicating that AOPs remove inflammatory components from untreated OSPW. Finally, transcriptome analysis demonstrated the robust inflammatory effects of untreated OSPW on human macrophages and supports that AOPs can effectively diminish the inflammatory effects. Overall, our study indicates that THP-1 cell based bioindicator system can be used to examine the immunotoxicity of OSPW suggesting it as a sensitive system for evaluating the effectiveness of OSPW remediation strategies.

1.17.P-We-031 Novel Microbe-Based Toxicity Assessment Tools for Examining Oil Sands Processed Waters

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Clark hot water separation is a petroleum extraction technique used in Alberta oil sands deposits to separate bitumen from oil sands. This technique uses hot water to extract the bitumen for refinement to produce a variety of petroleum products. In 2021, for every barrel of bitumen extracted by this process, approximately 3 barrels of water from the Athabasca River are used. After use in bitumen extraction, these oil sands processed waters (OSPW) are alkaline, moderately brackish waters that contain several inorganic and organic components that contribute to both acute and chronic toxicity. Due to the absence of provincial and federal regulation, OSPW is under a zero-discharge approach, resulting in the accumulation of large volumes of contaminated waters in need of remediation. As these water mixtures are complex, the tools used to assess their biological effects are equally varied and sensitive. Our lab uses rapid, cost effective, and high-throughput *in-vitro* cell-based bioassays as effective tools in monitoring environmental and wastewater quality. Microbial toxicity assays are one such standardized approach we use to screen waters for potential toxicants of concern. In clinical settings, Minimum Inhibitory/Eradication Concentration (MIC/MEC) assays are a reliable method to determine effective concentrations of antimicrobial compounds against indicator organisms. Through modification of traditional MIC/MEC approaches, we have been able to evaluate viability of *Escherichia coli* and *Staphylococcus epidermidis* lab strains following exposure to OSPW. After 20-hour exposures to OSPW, a Log reduction of 1.13 ± 0.19 Log₁₀CFU/Well (92.7% kill) and a 2.00 ± 0.06 Log₁₀CFU/Well (99.0% kill) was observed for *E. coli*

and *S. epidermidis*, respectively. Notably, treatment of OSPW with approaches such as UV/LED peroxydisulfate (PDS) treatment, which reduces organic toxicants in these waters, partially abrogate the observed loss of viability with a Log reduction value of $0.08 \pm 0.14 \text{ Log}_{10}\text{CFU/Well}$ (18.6% kill). These robust approaches allow for longer exposure, less manipulation of source water, and direct assessment of cell viability when compared with industry standard microbial assays for water toxicity. This method is a proven effective and sensitive biological screen for contaminants in treated and untreated samples, though this novel *in vitro* microbe-based assessment method requires further standardization with regulatory *in-vivo* approaches.

1.17.P-We-032 Biomarkers Development to Detect Environmental Contaminant Related Stress in Sharks and Rays

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The world has witnessed rapid environmental changes, especially due to anthropic interference in aquatic ecosystems threatening marine and ecological diversity. These factors impact aquatic trophic chains and affect the supply of resources and the human quality of life. To monitor and evaluate these anthropic impacts, bioindicator species can be used. Elasmobranchs, including sharks and rays are a good model, as they are at a high trophic level and often incur pollutant biomagnification. Biomarkers represent changes in organismal functions and can be important indicators of pollutant induced stress. The goal of this work is to gain sequences and build qPCR primers for elasmobranch genes commonly used as biomarkers of pollutant impacts in osteichthyes species. Specifically, we aim to sequence and develop primers for elasmobranch glutathione S-transferase (GST), acetylcholinesterase (Ache) and several cytochrome P450s (Cyp1a, Cyp3a and Cyp19a). To develop versatile polymerase chain reaction (PCR) primers for variable species of interest, we gathered available elasmobranch sequences from NCBI and aligned gene sequences in ClutalOmega. Primers were developed in areas of high sequence similarity and PCR ran using total RNA extracted from skeletal muscle biopsies from thornback rays (*Raja clavata*) collected in Seal Beach, California. Preliminary results show high quality PCR amplification for GST, Cyp3A and Cyp19a in the thornback ray and these PCR amplicons are currently undergoing sequencing. Additional validation is needed for AChE and Cyp1a PCR primers. Once valid PCR primers are developed and sequences are obtained quantitative PCR primers can be applied to elasmobranch tissue samples collected from urban and rural locations globally.

1.17.V-019 Effects of Black Carbon, PM_{2.5} and Temperature on Daily Asthma Admissions in a City in Mississippi, USA

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Asthma is an environmental justice concern across the world with several air pollutants potentially being associated with asthma cases. Black carbon (BC) concentrations were measured in ambient fine particulate matter (PM_{2.5}) in Jackson, Mississippi over two years (January 1, 2014, to December 31, 2015). We investigated the effects of air pollutants (BC and PM_{2.5}) and temperature on daily hospital admissions for asthma. Positive associations between asthma hospitalizations and both BC and PM_{2.5} were observed using a Pearson's correlation analysis, while a negative correlation obtained with temperature. The results of applying the quasi-Poisson regression in the generalized additive model and the distributed lag non-linear model to explore the exposure-response between daily asthma admissions and potential confounders demonstrated that lower temperatures were linked to a higher risk of asthma hospital admissions. Exposure to BC at the 25th percentile was associated with asthma hospital admissions at a cumulative 14-day relative risk (RR) of 1.07 (95% CI: 0.83-1.38), whereas exposure to BC at the 99th percentile resulted in a RR of 1.73 (95% CI: 0.23-12.82). The effect of BC concentration on asthma hospitalizations was stronger compared to that of PM_{2.5} concentration. These findings emphasize that BC has negative effects on human health in an urban area.

1.18.P-Th Late Breaking Science: Environmental Toxicology and Stress Response

1.18.P-Th-165 Working in Groups: An Evaluation of Different Chemical Grouping Strategies When Generating Protective Thresholds in EnviroTox

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EnviroTox is a large, diverse dataset of aquatic toxicity assays developed by Middle Tennessee State University on behalf of the Health and Environmental Sciences Institute (HESI) to support ecological risk assessment. Leveraging assays conducted across trophic levels, the EnviroTox platform can be used to predict ecosystem protective toxicity thresholds, chemical toxicity distribution (CTD) and Ecological Threshold of Toxicological Concern (EcoTTC) for a group of related or similarly acting chemicals. Application and use of the tool could be enhanced by the knowledge of appropriate grouping methods. This poster will analyze toxicity thresholds derived using two grouping strategies: 1) an effect endpoint grouping method utilizing known Classification, Labelling and Packaging and Globally Harmonized System (CLP/GHS) regulatory categories and 2) a chemical structure-based approach applied using ECOSARs (Ecological Structure - Activity Relationships). This will lead to a discussion of the key considerations for relevant groupings such as the representativeness of chronic and acute endpoints within the EnviroTox database, the importance of chemical group size, and the necessity of information such as mode of action when identifying thresholds for chemical groups. Finally, based on the opportunities and limitations identified when selecting groups in EnviroTox, potential applications of generated thresholds are proposed.

1.18.P-Th-166 Wetland Management Activities: Sustainable Tshirela Wetland Systems for Communal Beneficial Purposes

Mzimkhulu Monapathi, North-West University

Wetlands provide a range of environmental, cultural, and economic values. However, to some extent, they continue to be degraded and destroyed at an alarming rate. Wetlands in Tshirela, Vaal region, Gauteng Province in South Africa are damaged by communities via illegal dumping, human settlement, uncontrolled cattle farming, and spillage of chemicals from industry. This is worrisome as some of the goods and services associated with these systems are lost. A one-day World Café workshop was held in Tshirela for Itereleng based community organisation, Tshirela community members and academic researchers. The aim of the workshop was to identify activities that could sustain the wetland in Tshirela, and other wetland systems in general, for communal beneficial purposes. The present review reports on the findings from the workshop. These are supported by previously published work that address activities that could be implemented in the management of wetlands. Findings from the workshop included implementation of education and awareness campaigns, cleaning activities on the wetland systems, legal interventions, recreational activities, agricultural activities, involvement from Non-Governmental Organisations, establishment of forums, intervention from water culture religion, arts and culture, construction of barricades and enforcing waste management and recycling. Stakeholders from the government, private sector, academia, and the community have a huge responsibility to learn and teach about the importance and values of wetlands to protect and conserve these valuable systems.

1.18.P-Th-167 Harnessing Nature's Nanoplastic Bioremediators: Developing Dual-Purpose Solutions for Sustainable Agriculture and Environmental Cleanup

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Nanoplastic pollution has emerged as a global environmental crisis, necessitating innovative and eco-friendly solutions. We propose a pioneering approach 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 plant growth-promoting *Bacillus* species, *B. inaquosorum* and

B. velezensis, investigating their adaptability to nanoplastic-rich environments and plastic degradation capabilities. We previously showed that *B. inaquosorum* (F5) and *B. velezensis* (QST713) can utilize polystyrene nanoplastics. Now, we are aiming to ramp up their polystyrene metabolism. Through experimental evolution, we exposed replicate evolutionary lines of F5 and QST713 to Luria broth with polystyrene nanoplastics over five transfers. Intriguing light-cream colony morphologies emerged, distinct from normal morphotypes. Notably, after five transfers, the population density substantially increased in nanoplastics, indicating adaptation. These findings suggest the potential of *B. inaquosorum* (F5) and *B. velezensis* (QST713) to adapt to and degrade nanoplastics. Future work will isolate distinct morphotypes and assess their degradation abilities. Our study reveals the prospect of using nanoplastic bioremediators for environmental cleanup and crop growth promotion. Understanding the plastic metabolism of *B. inaquosorum* and *B. velezensis* will inform sustainable agriculture strategies and cleaner environments.

1.18.P-Th-168 Aroclor vs. Total PCB Concentration: Accuracy vs. Precision

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Polychlorinated biphenyls (PCBs) are widely known synthetic environmental contaminants strongly linked with negative impacts on environmental and human health. Despite toxicity often being associated with individual congeners, PCBs were exclusively manufactured as commercial mixtures (i.e., Aroclors) containing multiple congeners. Many exposure modules utilize mixtures such as Aroclors to better emulate mixtures that were historically released. The difficulty in quantifying Aroclors is the inconsistency in congener profile. Aroclors are categorized based on proportional weight of chlorine, not proportion of congeners. This results in batches of Aroclors, such as Aroclor 1254, with the same weight ratio but different congener profiles. In addition, environmental samples often consist of a mixture of PCB congeners unrelated to specific Aroclor mixtures. Understanding these discrepancies, organizations such as the EPA and other private analytical chemistry companies have developed methods to quantify Aroclors and total PCBs that rely on only quantifying a small suite of specific congeners. Using extensive chemistry data from total PCB congener analysis, we show that these methodologies have three major issues: 1.) So long as the congeners needed to calculate the Aroclor mixture are present, the concentration of that Aroclor can be calculated (The concentration of Aroclor 1242 can be calculated from an Aroclor 1254 stock), 2.) The calculated concentration of Aroclor from a sample does not fully account for all PCBs present in that sample and the ratio of total Aroclor to total PCB concentrations can change depending on the overall concentration of PCBs, 3.) Congener profiles from environmental samples vary dramatically and utilizing a small number of congeners to calculate total PCBs does not accurately represent all samples. Here we suggest reporting total PCBs as a more accurate measure of PCB concentrations in lab exposures and environmental samples. When conducting lab exposures, reporting the specific Aroclor used can provide relevant information indicating the general profile of congeners. We also show that total PCBs can be calculated using a few congeners rather than measuring and summing all 209 congeners as a cost saving measure.

1.18.P-Th-169 Ziram Cytotoxicity in Fish Gill Cell Lines for Nuisance Species Pesticide Development

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Pesticides are an important tool used by fishery managers to manage populations of undesirable and invasive fishes. Grass carp (*Ctenopharyngodon idella*) negatively impact aquatic ecosystems had reproducing populations in the Great Lakes. Ziram, a dimethyl-dithiocarbamate containing zinc ($\log K_{ow}$: 1.23), currently registered as a fungicide with the U.S. Environmental Protection Agency and may have promise as a pesticide to control undesirable fishes, like grass carp. Traditional *in vivo* screening is labor intensive and requires large numbers of animals. The use of *in vitro* methods to predict *in vivo* toxicity could help prioritize candidate pesticides and reduce the use of animals. In this study, according to the Organization for Economic Co-

operation and Development Test Guideline No. 249: Fish Cell Line Acute Toxicity, gill cells from rainbow trout (*Onchorynkuss mykiss*; RTGill-W1) and newly developed grass carp gill cell lines were exposed to ziram (0.01 – 1250 µg/L) for 24-hours to characterize fish cytotoxicity (EC₅₀), with three endpoints (metabolic activity, lysosome membrane integrity, and cell membrane integrity), to evaluate an *in vivo* approach. 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 are less than 2-fold. This presentation will discuss the screening of pesticide candidates through *in vitro* fish cell lines and ziram cytotoxicity in target (nuisance) and non-target fish and whether the approach predicts whole organism toxicity for prospective development of a nuisance fish pesticide.

1.18.P-Th-170 Cell-Based Assays are Sensitive Tools for Quantifying the Cumulative Bioactivities of Endocrine-Disrupting and Polyaromatic Hydrocarbon Pollutants in Environmental Water Sources

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Current protocols for water safety evaluation primarily focus on targeted chemical analysis, but this approach does not alert us to the presence of hazardous bioactivities from non-target chemical contaminants. Importantly, the cumulative bioactivities from low concentrations of numerous mixed chemical pollutants cannot be predicted using targeted analytical approaches. As a complement to analytical chemistry approaches, mammalian cell-based assays have been developed to provide a sensitive assessment of potential adverse effects from chemical contaminants in water such as endocrine-disruption (ED). Herein we demonstrate the utility of new cell-based luciferase reporter assays encompassing chemical-sensing receptors that are directly relevant to environmental biomonitoring applications. These include the polycyclic aromatic hydrocarbon (PAH) sensing Aryl Hydrocarbon Receptor (AhR), and the ED targets Androgen Receptor (AR), Estrogen Receptor (ER), Glucocorticoid Receptor (GR), and the Mineralocorticoid Receptor (MR). Environmentally relevant reference ligands for each receptor were tested in the assays, evaluated for sensitivity, and compared to current or suggested monitoring trigger levels (MTLs). Surface water samples collected from nine sites in the Spruce Creek watershed in Central Pennsylvania were tested in these assays. The assay data were compared to targeted chemical analyses of the sites to determine if the assays were able to detect biological activities that the chemical data would not have predicted. The results demonstrate that the limit of detection (LOD) for each assay is consistent with proposed MTLs presented in the literature. All samples exhibited detectable agonist activity for AhR, AR, ER, and MR, but minimal to no activity for GR. The comparison between bioassay and chemical analysis data demonstrated that using both methods together provide a better representation of the quality of the water samples, and that chemical analysis alone cannot predict the accumulated bioactivities revealed by the bioassays. These results demonstrate the utility and sensitivity of function-based assays for generating comprehensive water quality assessments. Bioassays provide reliable, actionable data that inform the decision-making process as to the need and extent of remediation required for a given water source.

1.18.P-Th-171 Testing with Saline Water: Refining Laboratory Techniques for *Acartia tonsa*

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Acartia tonsa is a calanoid copepod found in most estuaries throughout the world. In 2021, the Metal and Diamond Mining Effluent Regulations in Canada were amended to require the use of Environment and Climate Change Canada's newly published reference method STB 1/RM/60 (ECCC, 2019) for determining acute lethality using *A. tonsa*. The method is required for mines discharging final effluent into marine waters where salinity is > 4 g/kg. The 48-h tests are conducted using eggs ≤24 hours old that are acquired from adults which can vary in age class, provided they achieve at least 80% survival in a culture health test. Similar to the culture health test, a toxicity test using *A. tonsa* is considered invalid if there is 20% or more control mortality, where mortality is defined as unhatched eggs, immobile (no movement after 30 second observation) or missing

organisms. In carrying out tests with this relatively new regulatory test method, we investigated further optimization of both culture and test conditions. The objectives of this study were to determine if, for tests with 'clean' artificial seawater: 1) The **age of eggs** used to initiate the tests impacts % survival. 2) The **age of the adults** used to obtain eggs for testing impacts % survival. 3) The **salinity of the water** (10 vs. 30 g/kg) impacts test outcomes specific to egg and adult age, and. 4) The extent to which any of these parameters also impacted overall health. The repeated 48 h culture health tests with *A. tonsa* conducted in our study (with clean artificial seawater) have led to a better understanding of factors that impact test outcomes. Namely, eggs collected from older adults (21-28 days old) resulted in reduced survival and overall health relative to the younger age classes evaluated. Similarly, the use of eggs ranging in age from 0-24 h to initiate the culture health tests had reduced survival relative to eggs selected from a narrower age-bracket of 0-19 h old. The findings specific to adult and egg age were consistent for the 10 and 30 g/kg salinity. Restricting the age of the adults used to generate eggs for testing, and also narrowing the age range of eggs used in tests, will potentially help to reduce the extent of variability observed in test outcomes. It may also reduce the number of tests that need to be reset because survival was < 80%. This work has direct implications for regulatory testing specific to industrial discharge of final effluent in saline waters.

1.18.P-Th-172 Metabolism of Per- and Polyfluoroalkyl Substances (PFAS) in Rainbow Trout (*Oncorhynchus mykiss*) Using an Ex Vivo Model

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Per- and Polyfluoroalkyl Substances (PFAS) are a chemical class defined by their carbon-chains bonded with fluorine. PFAS, due to their persistence in the environment and toxicological impacts, are considered to be contaminants of emerging concern (CEC). A critical aspect of assessing exposure and effect characterizations is understanding xenobiotic metabolic capabilities. Limited availability of fish PFAS metabolic pathways contribute to inherent uncertainties in ecological risk assessments. A systematic process utilizing MetaPath (Metabolic Pathways knowledgebase) designed for the capture of PFAS biotransformation data from the open literature was developed. The resulting database provided a means to assess for deficiencies regarding available fish metabolic maps as well as potential for species extrapolation. This analysis provided insight into chemical selection and experimental design to fill the data gaps. The *ex vivo* Rainbow Trout (*Oncorhynchus mykiss*) Liver Slice model has been shown to be an effective tool to study the metabolic fate of a chemical within liver slices featuring a full complement of Phase I and Phase II enzymes. The advantages of *ex vivo* modeling include reduced number of live fish needed for testing and maintenance of elevated level of biological matrix complexity. Using slices allows for testing of several different chemicals and time points from one fish liver. In this study, individual liver slices were exposed to PFAS for up to 72 hours at 11° C in 12 well culture plates. Both incubation media and slice samples taken at various time points were analyzed for the loss of parent chemical concentration. For the analysis of chemicals, a method was developed that allows analysis of multiple classes of PFAS using a modified SPE approach combined with a universal LCMS method for volatile and nonvolatile chemicals including perfluoroalkylcarboxylic acids and fluorotelomer alcohols. The fluorinated acids as well as 4:3 Fluorotelomer carboxylic acid, demonstrated no loss of parent chemical thus indicating a lack of metabolism. The 6:2 and 8:2 Fluorotelomer alcohols show the potential for metabolism as indicated by loss of parent chemical. Results from this assay and the future metabolite identification will contribute to the MetaPath database of PFAS metabolic pathways and will give further context to bioaccumulation, toxicity, and kinetics research. *The contents of this abstract neither constitute nor necessarily reflect US EPA policy.*

1.18.P-Th-173 Development of a Probabilistic Loading 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 (R_{max}), a release rate into water (k), and time (t) spent in the water. Distributions of skin loads are combined with three matched sets of R_{max} and k values combined with 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 short-lived plume concentrations and predict UVF concentrations in larger recreational areas.

1.18.P-Th-174 Improving Sensitivity and Specificity of Larval Zebrafish Behavior Assays through Identification of Negative Control Chemicals

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Identifying reliable positive and negative control compounds is integral to determining the sensitivity and specificity of chemical assays. Our laboratory screens chemicals for developmental neurotoxicity potential using assays that assess larval zebrafish locomotor behavior in response to visual stimuli. For such assays, there are established positive controls; however, well-characterized negative controls are lacking. Martin and coworkers (PMID: 35908584) conducted a literature review that identified nine chemicals that appeared to have no evidence of developmental neurotoxicity in mammals. To test these candidate negative controls in the zebrafish behavioral assay, we first performed a range-finding study to assess lethality and teratogenic potential of each chemical ($\leq 100\mu\text{M}$). Concentrations that caused lethality or malformations were excluded from further testing. Then, developing larvae were exposed to each chemical, and the locomotor activity profile was assessed at 6 days post fertilization using a light/dark transition assay. Various aspects of this locomotor profile (e.g., average speed, habituation, maximum and minimum activity, aspects of the startle response, range of activity, and area under the curve [AUC]) were analyzed to construct a benchmark concentration for each endpoint if there was a significant concentration-related change in that endpoint. The positive control compound, fluoxetine, showed a significant concentration-related change for five of thirteen endpoints: average speed in the light or dark, total average speed, range of activity in the light, and the dark AUC:light AUC ratio. Developmental exposure to eight of the candidate negative controls (i.e., L-ascorbic acid, D-mannitol, saccharin, sodium benzoate, metformin hydrochloride, ibuprofen, glycerol, or omeprazole) did not affect any endpoint; however, selegiline hydrochloride showed a significant change for average speed in the light, and the dark AUC:light AUC ratio. Therefore, many, but not all, of these chemicals may be appropriate negative controls for the zebrafish larval light/dark transition assay. Standardizing a set of negative control chemicals is

essential for researchers to properly evaluate the behavioral results obtained from unknown chemicals and will assist with assessing new chemical screening methods. *This abstract does not reflect the official policy of the US EPA.*

1.18.P-Th-175 Using Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) to Evaluate Cross-Taxa Species Susceptibilities to Antimycin-A

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Antimycin-A (ANT-A) formulations were previously used as effective liquid pesticide to control nuisance fish species. Historically, the development of new chemical controls required testing on numerous animals; however, new approach methodologies (NAMs) have been developed in order to reduce such testing in the support of prioritization assessments and ecological risk assessment. In this investigation, the U.S. Environmental Protection Agency's Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool was applied to measure amino acid sequence conservation at the toxicological target binding site of ANT-A in cytochrome b of the electron transport chain occurring across taxonomic groups. Antimycin-A is a mitochondrial inhibitor that interacts with cytochrome bc1 on the electron transport chain (Qi subunit of complex III) and the ANT-A binding site is evolutionarily conserved. Differences in the amino acid sequence of subunit Qi at the ANT-A binding site could help explain species susceptibility to the pesticide. The cytochrome b primary amino acid sequence for Silver Carp (*Hypophthalmichthys molitrix*; accession number: AAC98756.1), was queried in SeqAPASS Levels 1 and 2 to determine orthologs to Silver Carp cytochrome b protein and the Qi binding domain (cd00284). Using chicken (*Gallus gallus*) crystallography data on the cytochrome b protein and binding site motifs, identified by Baudoin et al. (2021), the Level 3 query results identified one amino acid residue difference among bony fish (Actinopteri) and wildlife (e.g., Mammalia, Aves). Toxicity data indicates that Paddlefish are one of the most sensitive fish species to ANT-A and the results from the SeqAPASS queries indicated two amino acid residues differed in Paddlefish compared to less sensitive species (e.g., Silver Carp). Otherwise, several amino acid residues characterized to interact with Antimycin-A binding are fully conserved across multiple taxonomic groups. Whether other amino acid residues measured for the chicken chemical binding site (~14) are also positively associated with species sensitivity will be discussed. Overall, based on the available data, SeqAPASS shows promise to help characterize species susceptibility to Antimycin-A. *This abstract neither constitutes nor necessarily reflects U.S. Environmental Protection Agency policy.*

1.18.P-Th-176 In Vivo and In Silico Assessments of Anti-Androgenic and Anti-Estrogenic Potentials of BPA and its Analogs Using Zebrafish

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BPA has been widely used in plastic products such as food containers and thermal receipt paper. The anti-androgenic and anti-estrogenic effects of BPA have been reported. However, these evaluations for BPA analogs are still insufficient. The present study aimed to measure the anti-androgenic and anti-estrogenic potentials of BPA and its analogs using *in vivo* and *in silico* approaches in zebrafish. Zebrafish embryos were exposed to androgen receptor (AR) agonist 17 α -methyltestosterone (TES) and estrogen receptor (ER) agonist 17 β -estradiol (E2) alone or in combination with BPA and each of its analogs. After 24 h of exposure, all samples were collected to measure the mRNA expression levels of sulfotransferase 2st3 (*sult2st3*) and cytochrome P450 19A1b (*CYP19A1b*). To better understand the ligand binding affinity, we analyzed the binding mode of bisphenols to the zebrafish AR and ER subtypes (ER α , ER β 1, and ER β 2). BPAF, BPE, BPA, BPF, and BPB inhibited the expression of TES-induced *sult2st3* with IC₅₀ values of 0.53, 3.7, 4.7, 12, and 87 μ M, respectively. BP C2, 2,2'-BPF, and Bis-MP showed an inhibitory effect on TES-induced *sult2st3* at the highest tested

concentrations. However, neither BPZ nor BPS exhibited inhibitory effects on TES-induced *sult2st3*. In AR ligand binding domain, BPA formed a hydrogen bond with Met695, while most of BPA analogs hydrogen-bonded with Asn655 and/or Gln661, indicating that these amino acid residues are essential for exhibiting anti-androgenic activity of bisphenols. The interaction energy of these bisphenols is much lower than that of negative control chemicals, indicating their stable binding to AR than negative chemicals. Furthermore, BPAF, BPA, BPF, BPB, BP C2, and Bis-MP showed inhibitory effects on E2-induced *CYP19A1b* expression, indicating their anti-estrogenic effects, although these effects did not follow the concentration-response relationship. The binding characteristics of BPA and its analogs toward ER subtypes differed between their antagonistic and agonistic modes, with ER β 2 exhibiting completely distinct binding modes, suggesting the potential role of ER β 2 in distinguishing agonistic and antagonistic activities of bisphenols. In summary, the majority of tested bisphenols exhibited both anti-androgenic and anti-estrogenic effects, with distinct orders of potential possibly due to differing receptor selectivity of each bisphenol in their antagonistic binding modes.

1.18.P-Th-177 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 a necessary 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.

1.18.P-Th-178 Toxicological Implications of Differential Weathering in Oil Residues

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A consequence of heavy fuel oil spills in coastal environments is the deposition of oil residues on rock and debris which may persist for years. These oil residues naturally weather, changing their physical properties and chemical composition. Photodegradation and other weathering processes are focused on the surface of an oil and can lead to the formation of a distinct skin that physically shields the bulk oil beneath. Visual inspection shows this surface layer has distinct color and textural changes while the material directly underneath appears shiny and unweathered. These physical changes hint at differential chemical changes between the layers which could have toxicological implications. Oil residues sampled following a spill are generally collected and analyzed in bulk, a methodology that may result in overestimating environmental exposure to toxic oil

components. Here, we examine if sampling laboratory-weathered oil with a novel approach, using sliced vertical sections, would show chemical and toxicological differences not evident with traditional sampling methods. Our results reveal that surface layers of oil residues show more extensive weathering than deeper layers, with more significant loss of lower molecular weight components and more oxidation than the bulk oil. Because oxidation is linked to increased compound polarity and higher toxicity, we examined the relative toxicity of these different oil layers. Here we present the results of IR spectroscopy exploring sample oxidation and bacterial luminescence toxicity testing for vertical sections of laboratory-weathered oil samples.

1.18.P-Th-179 Particulate Hexavalent Chromium Targets Genes in Lung Cancer and DNA Repair Pathways

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Hexavalent chromium [Cr(VI)] is a well-established human lung carcinogen with widespread environmental and occupational exposure. Cr(VI) causes DNA double-strand breaks and loss of DNA break repair, leads to chromosome instability, which is a driving mechanism in Cr(VI) carcinogenesis. However, the ability of particulate Cr(VI) to inhibit DNA break repair across repair pathways is poorly understood. Our previous data show Cr(VI) alters the expression of mRNAs related to DNA double-strand break repair; however, these studies focused on individual genes and not the broader impact on repair genes across the genome. Thus, the aim of this study was to characterize the global transcriptional changes in mRNA expression and specifically consider DNA repair pathways in human lung cells after acute (24 h) and prolonged (>72 h) particulate Cr(VI) exposure. Cells were exposed to various concentrations (0.1, 0.2 and 0.3 $\mu\text{g}/\text{cm}^2$) of zinc chromate, a representative particulate chromate compound. Cells were harvested, and RNA was extracted, followed by a library preparation step and next generation sequencing. Our results show exposure to particulate Cr(VI) induced a time- and concentration-dependent increase in the total number of upregulated and downregulated genes. Using software MetaCore, we compared our list of Cr(VI) targeted genes with disease databases and found Cr(VI)-induced differentially expressed genes were involved in lung cancer. Pathway analyses revealed differentially expressed genes were involved in key DNA repair pathways and DNA maintenance. Thus, we generated heatmaps for genes involved in 8 different DNA repair pathways: 1) homologous recombination repair, 2) non homologous end joining, 3) microhomology-directed end-joining, 4) single strand annealing, 5) mismatch repair, 6) base excision repair, 7) nucleotide excision repair and 8) crosslink repair. Our data show global downregulation of genes involved in all eight DNA repair pathways compared to untreated controls indicating particulate Cr(VI) modulates all of them. Altogether, our data show Cr(VI) exposure in human lung cells leads to differentially expressed genes involved in lung cancer and induces a global downregulation of genes involved in high fidelity DNA repair pathways, which can lead to chromosome instability and cancer. This work was supported by NIEHS grants R01ES016893, R35ES032876 (JPW), and T32ES011564 (RMS and J.P.W.).

1.18.P-Th-180 Perfluorobutanoic Acid (PFBA) Modulates Molting and Development-Regulating Genes in the Fall Armyworm

Eguono Wayne Omagamre and *Joseph Pitula*, University of Maryland, Eastern Shore

Emerging studies indicate that per- and polyfluoroalkyl substances (PFAS) pose environmental concerns. A prior investigation from our lab showed that a low exposure dose of perfluorobutanoic acid (PFBA), a four-carbon PFAS, stimulated the size and time to larval-pupal transition of *Spodoptera exigua* (beet armyworm), highlighting potential implications for pest management due to the ready accumulation of this PFAS in the aerial parts of plants. In this study, we explored the molecular mechanisms underlying this phenomenon, employing *Spodoptera frugiperda* (fall armyworm) as a model organism. Third-instar larvae were exposed to an artificial diet spiked with 1 $\mu\text{g}/\text{g}$ PFBA, while a control group received a distilled water-infused artificial diet. Larval weight measurements were taken 24 hours post-exposure, after which whole-body total RNA was extracted from the larvae. Isolation and sequencing of mRNA were subsequently carried out. Differential

expression analysis of the obtained gene counts was performed after mapping the short read sequences to the fall armyworm genome. Remarkably, larval sizes indicated that the exposed group had a 45% weight gain over the controls at day 1 post-exposure. Among the 19,865 genes with over 100 gene counts in both groups, 1121 genes exhibited differential expression, with 36% upregulated and 64% downregulated in the exposed group. Notably, genes encoding ecdysone oxidase and ecdysone-like isoform proteins were downregulated up to 13-fold compared to controls. Similarly, several isoforms of juvenile hormone epoxide hydrolase and juvenile hormone esterase genes were all downregulated, with fold decreases ranging from 2 to 9 folds. Additionally, three circadian clock-controlled daywake proteins gene isoforms were downregulated up to 5-fold compared with the controls. Furthermore, genes encoding 10 distinct CYP450 isoforms, catalase, superoxide dismutase (Cu-Zn), NADP-cytochrome P450 reductase, and a cytoglobin-1 isoform were all downregulated, suggesting an impact on the immune response at low PFBA exposure. Finally, 15 genes coding for different larval cuticle proteins were downregulated 4 to 11-fold. Collectively, our findings suggest that low-dose PFBA modulated molting and development-regulating genes in the fall armyworm, possibly resulting in the observed weight stimulation.

1.18.P-Th-181 Initial Characterization of a Fathead Minnow Liver Cell Line Using RNA-Seq

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Fish represent a large portion of the animals used for vertebrate testing, and thus, continued development and validation of new approach methodologies (NAMs) applicable to aquatic species is critical to reducing reliance on animal testing. In contrast to the use of cells in mammalian hazard evaluation, the implementation of fish cell lines in ecological risk assessment is a relatively unexplored area of research. There are myriad cell lines derived from multiple tissues and species of fish available through commercial vendors and academic researchers. While some have been used in toxicology studies, comparatively little work has been done to assess how well fish cell lines reflect *in vivo* effect. We proposed to narrow this knowledge gap by characterizing FHML2-6, a cell line derived from fathead minnow liver by colleagues at the University of Fraser Valley, towards determining the feasibility of using these cells in high-throughput and high content assays. First, we examined expression of three hepatocyte-specific marker genes (retinol-binding protein 4, transferrin A, transthyretin) using PCR. Next, we investigated the biological responsiveness of FHML2-6 cells by treatment with prototypical inducers of pathways important in toxicology—cadmium chloride, copper sulfate, dexamethasone, ethinylestradiol, gemfibrozil, and TCDD. Following 24 hour exposure, RNA was isolated and transcriptomic analysis (RNA-Seq) was performed to get a more complete picture of baseline expression and pathway activation in FHML2-6 cells. A wealth of *in vivo* toxicology data from fathead minnows are available for the compounds tested, which provide an important reference/comparison dataset with which to compare. Our results suggest that FHML2-6 cells do express certain characteristics of epithelial hepatocytes, but also those of non-parenchymal cells. Furthermore, inducibility of some toxicologically-relevant pathways (e.g., estrogen receptor, PPARα, PXR) appears to be inconsistent with *in vivo* responsiveness. Continued analysis is needed to confirm these findings at the transcriptomic and protein levels. Data generated through this research will provide greater clarity as to the utility of fish-based cell lines and aquatic NAMs in next generation ecotoxicology study. *This abstract neither constitutes nor necessarily reflects USEPA policy.*

1.18.P-Th-182 Application of a Probabilistic UV Filter Loading Model to Estimate Water Column Concentrations in Swimmers' Plumes and at a Recreational Beach

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Measured levels of UV filter actives (UVFs) in recreational waters vary widely due in part to the proximity of sample locations to swimmers. Use of measured levels in exposure and risk assessment can be complicated by this high variability and a lack of detail on spatial and temporal aspects of sampling. Additional understanding

of UVF concentrations can be obtained using mass transfer models that estimate UVF loading from swimmers wearing sunscreen (see accompanying poster). In this study, UVF loadings were used to estimate concentrations in plumes released into water in the immediate vicinity of swimmers. These concentrations, while ephemeral, are likely to be the highest that will occur or be measured in recreational waters on any occasion anywhere, and represent the highest short-term exposures, but are unlikely to be representative of longer-term exposures due to their rapid dissipation. A minimum plume volume was derived for five UVFs from the highest measured concentrations reported in the literature and distributions of the mass loads during a swim event that could result in these levels. This minimum volume was then combined with individual UVF loading distributions to generate distributions of maximum plume concentrations. Loading models also make it possible to estimate water column concentrations at a more macro level as beachgoers visit and leave a recreational site. These calculations are less generic and more complex involving information on loading and turnover rates, water volume and sunscreen products being used, all of which can be specific for a given water body and even a given day. Distributions of mass loads for oxybenzone, avobenzone, and octocrylene were combined with other information reported by Labille et al. (2020) at Prophete Beach. Loading rates were calculated from the number of swim events occurring on an hourly basis throughout the day, while turnover rates were based upon the dissipation of these UVFs after peak recreation time. The result of this modelling were distributions of UVF levels for which the median concentrations corresponded closely with measured concentrations at 4:00 PM on July 5, 2017. Given similar site-specific information, this approach can predict concentrations on different days and at other recreational waters helping to form the basis for more robust risk assessments.

1.18.P-Th-183 Macrophage Activation as a Screening Tool for Immunotoxicity: Many Environmental Contaminants Considered to be Potentially Immunotoxic May Not Target NF- κ B Pathways

Alyssa Whisel and Charles D. Rice, Clemson University

The field of immunotoxicology has demonstrated that several environmental contaminants target the immune system, though no standalone adverse outcome pathway (AOP) is predictive of the outcome. Our lab has employed the murine macrophage cell line RAW264.7 in screening assays to possibly predict immunotoxicity in other immune cell types, and to explore mechanism(s) of action. RAW264.7 cell are well characterized and a common *in vitro* model for understanding macrophage-related inflammation profiles in immunotoxicology and immunopharmacology. Herein we used lipopolysaccharide (LPS) to activate inducible nitric oxide synthase (iNOS) for screening common environmental contaminants for the ability to modulate iNOS activity as a biomarker of pro- or anti-inflammatory properties. This pathway to activation is driven primarily by the transcription factor NF- κ B. We then compared these responses to endogenous anti-inflammatory compounds such as short chain fatty acids (SCFA), bile acids (BA), and pharmaceutical derivatives of natural products. The SCFAs butyrate, propionate, and acetate suppressed iNOS activity at sub molar levels. Among synthetic phenolic antioxidants tested TBHQ was active at sub μ M levels. A derivative of natural indirubin (E804) was very potent at sub μ M levels, as was the case with dexamethasone, both being known to target NF- κ B. The biocide tributyltin (TBT) was predictably very potent at low μ M levels. However, the biocide propyzamide was ineffective at 50 μ M and lower. Six BAs were tested and shown to be active at 50 μ M and higher. Of keen interest to environmental toxicologists, the polyfluoroalkyl compounds PFOS, PFOA, GenX, and PFC-B had no effect on macrophage activation up to 40 μ M, as was the case with phthalates BPA and DEHP, up to 20 μ M and 50 μ M respectively. The flame retardant TPP was effective only at 50 μ M and higher. Chlorinated aryl hydrocarbons (HAHs) are known potent immunotoxic compounds, though neither PCB-126, PCB-104, or PCB-157 were effective at 25 μ M or lower. That same was true of the polyaromatic hydrocarbons (PAHs) 3-MC, BKF, and retene at 25 μ M or lower. Tire wear particle compounds of interest 6PPD and 6PPD-Q were also ineffective at modulating iNOS activity up to 20 μ M. Taken together, our data suggest that the effects of pollutants of interest on innate immune functions such as macrophage activation should be considered within the perspective of the comparative effects of known NF- κ B-targeting agents.

1.18.P-Th-184 Estimating The Mass of Per- and Polyfluoroalkyl Substances in Building Materials

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Water and air in urban environments are contaminated with neutral and ionic Per- and Polyfluoroalkyl Substances (PFAS), however sources of and contributions to this contamination are poorly known. We investigated exterior building materials as a source of PFAS release into the environment and quantified the mass of PFAS released from this source. Based on our testing of total Fluorine (F) and organic Fluorine using Particle Induced Gamma Ray Emission (PIGE), F-NMR, and Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS), PFAS can be found in building materials such as paints, sealants and textiles. Our weathering results showed that under the influence of UV, the surface chemistry of PFAS coatings on surfaces such as tile changes by increasing the abundance of mobile and potentially harmful short-chained PFAS. We estimated the mass of PFAS used in exterior surfaces before the building was subject to weathering. To calculate the mass of PFAS used in archetypal buildings, we obtained architectural drawings for several building types in the United States and Canada. We calculated the surface areas of all exterior materials that could contain or be coated in a material made with PFAS. To estimate the mass of PFAS in the building products we used the median total F concentrations for product samples with detectable total F ($\mu\text{mol F/g}$ wet weight) measured by PIGE. For example, we estimated that approximately 60% of a small single-family house could be coated with 6.4 g of PFAS on brick veneer and fiber cement panels. We will present results for several additional building archetypes to better understand the extent of use of PFAS in a city.

1.18.V Late Breaking Science: Environmental Toxicology and Stress Response

1.18.V-021 Assessing Differences in the Concentrations of Trace Metals from Different Tomato Varieties Harvested from Soils Treated with Municipal Waste Sludge

Olawole Aina, Joshua Oluwole Olowoyo and Nditsheni Matodzi, Sefako Makgatho Health Sciences University

The ever-increasing world population is putting extreme pressure on the already limited agricultural resources for food production. Different soil enhancers such as municipal sewage sludge are used in improving soil productivity thereby increasing food production. This study investigated the differences in the concentrations of heavy metals in four different tomato varieties cultivated on soil treated with municipal waste sludge in Pretoria, South Africa. Fruits were harvested at maturity and analysed for trace metals content using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The study showed that different varieties of tomatoes bioaccumulated different types of trace metals from the soil. Rodade tomato bioaccumulated the highest concentrations of Mn, Cr, Cu and Ni. Roma bioaccumulated the highest concentrations of Cd, Fe and Pb while Heinz bioaccumulated the highest concentrations of As and Zn. Cherry tomato on the other hand, recorded the lowest concentrations for most metals, Cd, Cr, Cu, Mn, Ni, Pb and Zn. Some of the values recorded for some heavy metals in the harvested tomatoes fruits were higher than the WHO recommended limit. The bioaccumulation index showed that the tomato fruits can bioaccumulate trace metals from the soil owing to the availability of the trace metals in soil. The tomato varieties used in the study, bioaccumulated the toxic trace metals in their fruits and values obtained for Zn, Pb, Cu, Cr, and Fe were higher than the acceptable limit set by the World Health Organisation (WHO) and the Agency for Toxic Substances and Disease Registry (ATSDR) (2008), care should be taken when these tomato varieties are either cultivated or harvested from polluted areas.

7.12.V Late Breaking Science: Policy, Management and Communication

7.12.V-027 Management for a Listed Species Using Contaminant Performance Metrics

Shawn Acuna, Metropolitan Water District of Southern California (MWD)

Water management and listed species management have significant hurdles in regard to making the best decision to either reduce impacts and/or promote recovery while still providing high quality water. Contaminant impacts can be one of those hurdles as there are numerous interactions that if not accounted for could lead to unrealistic predictions or spurious conclusions. Recent efforts to manage water in the San Francisco Estuary and the impacts of that management on the listed species Delta Smelt (*Hypomesus transpacificus*) have attempted to use an integrative approach to incorporate contaminant effects in decision making. The Structured Decision Making framework was used to evaluate different action alternatives. The effort was done to determine whether water project management decisions need to begin including contaminant impacts in their decision making regarding Delta Smelt. The effort identified significant gaps in data and updated ecosystem conceptual models regarding water operations, contaminants, and Delta Smelt that were used in making decision.

Track 2: Aquatic Toxicology, Ecology and Stress Response

2.01.P-Tu Advancing Aquatic Toxicity Test Methods: Developments in Culturing, Testing and Data Analysis of Toxicity Test Methods

2.01.P-Tu-061 Toxicity Identification and Evaluation (TIE) for Ammonia Contaminated Sediment and Metabolomics-Based Effect-Directed Assessment (EDA) Using *Glyptotendipes Tokunagai*

Rahul Singh, HUFs GLOBAL

Sediment TIE methods developed by United States Environmental Protection Agency (USEPA) provide a way to identify the presence of toxicants and measure their toxicity in freshwater sediments. In this work, we have validated the sediment TIE method using domestic organism to evaluate the sediment toxicity due to ammonia under varying concentrations. This study is a part of the project that attempts to develop the sediment TIE method manual for ammonia toxicity in South Korea. Using domestic organisms for sediment TIE are more likely to provide plausible results due to their representativeness to the domestic wildlife. We chose *Glyptotendipes Tokunagai* as the test organism that habitats across East Asian countries such as South Korea and Japan. We utilized the LC₅₀ metric to measure the toxicity of the sediment according to the USEPA TIE method. We performed a two-stage experiment to verify the presence of ammonia in a sediment that was contaminated by the addition of ammonia solution. In the first stage i.e., initial toxicity test, we added the test organisms in the sediment along with synthetic ammonia solution. At the end of initial toxicity test, we observed the death ratio of the organism, which was found to be approximately 0.47%, and the corresponding LC₅₀ ammonia concentration was 400 mg/L. These results verified the sufficient ammonia toxicity required for stage two experiments. In the second stage i.e., manipulations by zeolite addition, we added zeolite, which is an ammonia adsorbent, to the toxic sediment samples. Ammonia concentration in the sediment samples was manipulated by adding different amounts of zeolite to them. At the end of stage two, we observed that the death ratio of the test organisms was found to be inversely proportional to the amount of zeolite under the same ammonia concentration. Addition of zeolite not only reduced the death ratio but also brought the ammonia concentration down. Due to the direct influence of zeolite addition on ammonia concentration and the death ratio, we concluded that the toxicant present in the sediment is ammonia. Through this experiment, we verified that ammonia present in the sediment is toxic to the test organisms and validated the sediment TIE for ammonia in South Korea. In further analyses, we will analyze the body samples of *Glyptotendipes Tokunagai* for quantifying metabolome. The metabolic information will be used to design further development of metabolomics-applied effect-directed assessment.

2.01.P-Tu-062 Developing a Novel Dietary Toxicity Test with the Freshwater Amphipod, *Hyalella azteca*
*Joshua Fischer*¹, *Mark Fitzmaurice*², *Steven Reyna*¹, *Collin Preftakes*¹, *Michael Bradley*² and *Christopher Brown*¹, (1)*Bayer Crop Science*, (2)*Smithers*

Freshwater invertebrate testing is a critical component of comprehensive ecological risk assessments for various classes of pesticides. *Daphnia* sp. are commonly used test species for meeting freshwater invertebrate testing requirements employing a water column exposure. Some classes of pesticides, including insect-control proteins and biologicals, pose significant challenges in water column test designs due to their limited solubility. *Hyalella azteca* is a freshwater epibenthic amphipod that serves as a representative species for freshwater shredders and is a standard test organism in sediment toxicity tests due to its amenability for culture and sensitivity to a variety of chemical substances. Insect control proteins from genetically modified crops exhibit dietary modes of action and pose additional challenges in standard sediment toxicity test designs due to their high affinity binding to sediment components, thereby significantly hindering analytical confirmation of test concentrations. Herein, we describe the development of an agar-based diet formulation that supports the growth and development of *H. azteca* in a toxicity test design. Dietary consumption by amphipods was demonstrated by comparing body weight measurements of organisms exposed to diet tablets to those in no-food avoidance control and standard guideline feeding regime treatment groups. An insect control protein from genetically-modified maize was incorporated into the diet to test for effects on *H. azteca* in a 10-day static daily renewal test design. Protein stability in the diet was confirmed under test conditions, and additionally, a potential positive control toxicant was identified and characterized. This method serves as the basis for a novel test design to evaluate the potential effects of test substances with limited solubility or dietary modes of action on a freshwater invertebrate species.

2.01.P-Tu-063 The First 30 Years Of Sediment Bioaccumulation Testing with *Lumbriculus variegatus*: A Meta-Analysis

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To evaluate the aquatic risk of a contaminant an understanding of both exposure and effects are needed, and the use of bioaccumulation assays have proven to be an invaluable tool in linking the two. This is especially true for the assessment of sediment-associated contaminants as bioaccumulation assays take into consideration both ingestion and dermal adsorption exposure routes when assessing the risk to benthic invertebrates. Although sediment bioaccumulation assays started in the late 1980s, it was not until the early 2000s that the U.S. Environmental Protection Agency chose *Lumbriculus variegatus* as the model freshwater organism for assessing bioaccumulation in sediments. The following meta-analysis examined published papers on the topic of sediment bioaccumulation testing with *L. variegatus* from 1988 to 2020. Of the papers that met the criteria for inclusion (123 in total), a closer analysis of the types of chemicals evaluated as well as the methods explored by each paper over the years was taken. In this review, we explored the frequency of field and laboratory spiked sediment bioassays, the temporal trends for bioaccumulation testing of chemical classes (i.e., which chemicals were being commonly evaluated each year), the types of bioaccumulation assessments and kinetic models utilized, as well as a general overview comparing Kow to BSAF for this test species. Overall, the goal of this review, was to better understand not only the current data gaps and limitations of the current sediment bioaccumulation testing literature, but also understand the advances of sediment bioaccumulation testing since its initiation in late 1980s.

2.01.P-Tu-064 New OECD Test Guideline Proposal for Sediment-Water Amphipod Toxicity Test
*Kyoshiro Hiki*¹, *Haruna Watanabe*¹, *Hiroshi Yamamoto*¹ and *Pascal Pandard*², (1)*National Institute for Environmental Studies (NIES), Japan*, (2)*Institut National de l'Environnement Industriel et des Risques (INERIS), France*

Sediment-water system is complex and benthic organisms are exposed to contaminants via multiple exposure pathways. Therefore, sediment toxicity tests using species with different taxonomic groups, habitats and feeding

modes are desirable for regulatory chemical assessments of insoluble, adsorptive, or persistent chemicals. Currently, benthic test species adopted in OECD test guidelines (TGs) are limited to only two animals (*Chironomus* sp. [TG 218, 219, 233], *Lumbriculus variegatus* [TG 225]) and one plant *Myriophyllum spicatum* (TG 239). However, these limited species do not satisfy the lowest assessment factor requirements for deriving predicted no-effect concentration (PNEC) in certain regions and countries, potentially resulting in unrealistically low PNEC levels. To derive reliable sediment PNEC values, it is beneficial to standardize sediment toxicity tests using the freshwater amphipod *Hyalella azteca* or other freshwater amphipod species as an OECD TG, which has different habitat and feeding mode from the aforementioned species. To develop a new OECD TG for amphipod 10-day sub-chronic and 42-day chronic sediment toxicity test using *H. azteca*, we have harmonized existing test protocols from various sources (e.g., ASTM, USEPA, Environmental Climate Change Canada, and ISO) into a draft guideline. The proposed guideline is designed to assess the effects of sediment-associated chemicals, by exposing *H. azteca* to laboratory-contaminated (i.e., spiked) sediments and evaluating survival, growth, and reproduction. The draft guideline needs now to be validated by conducting an interlaboratory comparison, wherein multiple laboratories will perform sediment toxicity tests according to the proposed draft guideline to evaluate the robustness of the protocol and the reproducibility of the results. While several published papers have conducted such interlaboratory comparisons for 10-day sub-chronic toxicity tests using spiked-sediments, the interlaboratory validation of 42-day chronic toxicity tests has only been performed under control conditions (i.e., no test chemical added) and not using spiked-sediments. In this presentation, we will present the preliminary results of the validation comparisons for 42-day chronic toxicity tests using two hydrophobic organic chemicals (pyrene and bifenthrin).

2.01.P-Tu-065 Distributions of Oxygenated PAHs in Sediments of Japanese Coastal Areas and their Risk Evaluations with Toxicities in Fish Embryos

Seiichi Uno, Mirai Uchimura, Machi Kawano, Aya Maehara, Kazuki Imamura, Masatoshi Yamasaki and Kokushi Emiko, Kagoshima University

Oxygenated polycyclic aromatic hydrocarbons (oxyPAHs) are directly discharged into the atmosphere with exhaust gas from diesel engine automobiles and industries, and also generated through photo-oxidation and/or microbial metabolism of parent polycyclic aromatic hydrocarbons (PAHs). Because oxyPAHs have diversity of forming process, they distribute widely in environment, especially around urban area, and this is not exception in aquatic environment. Information for oxyPAHs including the distributions in the aquatic environment and their toxicities to aquatic organisms is limited yet. Therefore, risk assessments are difficult in real aquatic environment. We have been examining the toxicities of some of oxyPAHs to fish embryos, and they cause the malformation such as blue-sac syndrome on the fish juveniles after hatching. However, toxicities of some oxyPAHs to fish embryos are unknown, and should accumulate their toxicity information. This study investigated the distributions of oxyPAHs in sediments of the urban coastal area in Japan. Additionally, we examined the additional toxicities of oxyPAHs to Japanese medaka, *Oryzias latipes*, and Java medaka, *Oryzias javanicus* to perform the risk assessments in the real environment. Sediments were collected from the bay areas close to urban areas in Japan such as Tokyo and Osaka Bays. Eleven oxyPAHs in sediments were extracted by dichloromethane and measured by GC/MS or LC/MS/MS. As a result, the oxyPAHs were detected 53.9 to 1060 ng/g dry weight in Osaka Bay. We will show additional distributions in the sediments in the coastal areas, Japan. We newly observed for causing the malformations on juveniles hatched from the embryos exposed benzantrone and pyrene-4,5-dione. However, anthrone and 9,10-anthraquinone could not be caused any effects on the embryos. In this study, we will also show the abnormality of heartbeat and arrhythmia in the embryos. At last, we will try to evaluate the risks in field using the toxicities data.

2.01.P-Tu-066 Evaluation Method of Ecotoxicity for Biodegradable Plastics

Norihisa Tatarazako and Yukiyo Okazaki, Ehime University

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 their development is underway around the world. 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 degraded products are concerned because they are supposed to be decomposed in the environment. Therefore, the method of ecological assessment for biodegradable plastics and their degraded products are required to be developed as soon as possible. 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 resulted in a low concentration of derived degradation products by a large amount of water used. Those reliability and validity are questionable as sample preparation method. In addition, the tested marine organisms (fish, crustaceans, and algae) are not general since those organisms are not easy to obtain in all countries, and it is not suitable for evaluation of the plastics which is biodegraded at terrestrial. The aim of this study is to develop a method with emphasis on simpler and quicker manner for preparing test sample, and with highly versatile ecotoxicity test results for evaluating the environmental impact of biodegradable plastics.

2.01.P-Tu-067 Testing Effluent Samples from Canadian Pulp and Paper Mills Using the Standard *Ceramium tenuicorne* ISO Test Method and Canadian Add-On Procedure

Melanie Gallant, Cintia Glasner Regis, Anna Sobaszek, Vanessa Chanes and Lisa N. Taylor, Nautilus Environmental Company

As indicated in the *Pulp and Paper Effluent Regulations* under the Canadian *Fisheries Act*, sublethal toxicity testing for effluent discharged into a marine or estuarine environment must be conducted using standard methods for marine algae species. Previously, the macroalgae *Champia parvula* had been used, but this test species is no longer being offered by North American labs due to concerns regarding data quality and the lack of available new plant material. Therefore, Environment and Climate Change Canada (ECCC) is investigating the use of the red macroalgae *Ceramium tenuicorne*; the genus is native to Canadian coastal waters, and this species was chosen due to its relative ease to culture in the laboratory. The ISO 10710:2010 method for *C. tenuicorne* is a 7-day growth inhibition test. To develop this method for use in a Canadian context, the Method Development and Applications Unit (MDAU) of ECCC drafted an add-on procedure that provides supplementary methods and conditions specifically for Canadian laboratories. Nautilus Environmental Company Inc. has been working with ECCC to test the viability of this method and add-on procedure. Results to date have indicated that culturing and testing of *C. tenuicorne* is not only possible, but can also be done efficiently and effectively. The reliability of the add-on procedure was demonstrated through reference toxicant testing and the establishment of a warning chart. The most recent testing aimed to further refine the add-on procedure for ease of use in commercial laboratories. Specifically, reference toxicant tests were initiated with two distinct organism size classes to determine if the sensitivity of the test is impacted by selection of more practical size ranges. Furthermore, testing of effluents voluntarily submitted by Canadian pulp & paper facilities with a variety of processing types (e.g., mechanical, chemical, papermaking/recycling) has characterized the sensitivity of the *C. tenuicorne* growth inhibition test. These results continue to support the development of a sublethal toxicity test for effluent discharged into a Canadian marine or estuarine environment using the add-on procedure for *C. tenuicorne*.

2.01.T Advancing Aquatic Toxicity Test Methods: Developments in Culturing, Testing and Data Analysis of Toxicity Test Methods

2.01.T-01 Evaluation of Potential Alternatives in Marine Toxicity Testing: Comparing the Sensitivity and Feasibility of Three Alternatives

Dalton Scott Allen, Katie Solomons, Maddie Wiencek, Michaela Kelly and Marlo K Sellin Jeffries, Texas Christian University

Larval growth and survival (LGS) tests are a predominant method for evaluating the toxicity of effluents

discharged into marine environments. Though these test have historically proven effective in identifying toxic effluents, there is a need to identify alternative methods that enhance animal welfare. This presentation will provide an broad overview of a project aimed at assessing the suitability of three alternative test types – the inland silverside fish embryo test (FET), sheepshead minnow FET test or mysid shrimp test – as potential replacements for the inland silverside LGS and sheepshead minnow LGS tests. The sensitivity of these testing strategies was compared using a suite of chemicals with different modes of action (i.e., 3,4-dichloroaniline, Ni, phenanthrene, and a water accommodated fraction of crude oil). In general, the the mysid shrimp test was found to possess either equivalent or greater sensitivity than the other test types suggesting that it may be a viable alternative to the LGS tests. Though FET tests were found to be the least sensitive of all test types evaluated, their sensitivity was substantially improved through the inclusion of sublethal endpoints, particularly hatch success. Results highlighting these relationships will be presented and the potential limitations associated with each alternative test type will be discussed to provide a robust and holistic assessment of these test types.

2.01.T-02 Advancing In Vitro Fish Alternatives for Regulatory Effluent Toxicity Testing: Perspective on Optimization and Incorporation into Current WET Guidelines

Justin Scott^{1,2}, *Shannon Mortensen*¹ and *Matteo Minghetti*², (1)*Cove Environmental*, (2)*Oklahoma State University*

Fish toxicity testing methods have been vital in predicting toxicity of pollutants dissolved in water such as effluents and receiving streams. Advancements with in vitro techniques have continued to gain momentum for their potential for regulatory applications. In vitro alternatives allow identification of a toxicant's mode of action (MoA), high throughput approaches, reduced test duration and number of animals used. These considerations are important for the principles of the 3R's (Replacement, Reduction, Refinement) and the additional 3Rs (Reproducibility, Relevance, Regulatory). We have recently investigated the use of animal alternative methods using cell lines and fish embryos to predict acute toxicity of WET chemicals of interest and actual effluents in fish. Current incorporation of these New Approach Methodologies (NAMs) requires careful consideration based on protocol comparisons. Specifically, test parameters regarding exposure duration and conditions, species comparisons, quantitative approaches, and advantages and limitations are addressed for transition and incorporation within current Whole Effluent Toxicity (WET) testing guidelines. In our studies, RTgill-W1 cytotoxicity assays and fathead minnow (*Pimephales promelas*) Fish Embryo acute Toxicity (FET) tests were successful in predicting acute toxicity of a broad list of legacy pollutants, chemicals of emerging concern (CECs) and wastewater samples. Moreover, subcellular, and teratogenic endpoints identified specific WET toxicants of concern. Results from our studies provide insight into the current state of in vitro animal alternatives regarding the incorporation and use of RTgill-W1 cytotoxicity assays (ISO 21115;2019) and FET tests (OECD 236:2013) for WET testing.

2.01.T-03 Interlaboratory Studies of the *Ceriodaphnia dubia* Chronic Test Method: The California Experience II

Darrin J. Greenstein, *Alvine Mehinto* and *Kenneth Schiff*, *Southern California Coastal Water Research Project*

The *Ceriodaphnia dubia* survival and reproduction test is an established whole effluent toxicity test method commonly used in regulatory programs including those adopted by the State of California in 2020. Both regulators and stakeholders recognize that some laboratories may need to improve their implementation of the *C. dubia* method to reduce intra-laboratory variability and increase comparability amongst laboratories. This study aims to investigate factors that can lead to test variability and to improve confidence in assessments of toxicity among laboratories accredited by the state of California. To evaluate the causes of test variability and develop guidance to improve data comparability, two interlaboratory studies were conducted. Eleven laboratories tested 4 different split samples: two types of moderately hard dilution water (one using the salts recipe and the other using the mineral-water based recipe from the USEPA test manual); and two dilution series of sodium chloride (one provided and diluted in the mineral-water based recipe and the other made by the labs

in their own dilution water). In the first study, the laboratories were allowed to use their usual test methods, but required to collect additional data on brood board health and water quality that most labs did not routinely document. In the second study, select laboratory methods were constrained including use of neonates from a restricted age window of females, food holding time and density, and randomization of test containers. The laboratories were also provided guidance to evaluate brood board health qualitatively and quantitatively, and to better document split broods. Results will be presented for key endpoints, such as mean and coefficient of variation for number of neonates per female and point estimates of the dilutions series. Comparisons will be made between the two studies to determine if the method constraints reduced intra and inter-lab variability. Data will also be used to develop method-specific recommendations intended to reduce test variability.

2.01.T-04 Improving the Laboratory Proficiency Processes for Whole Effluent Toxicity (WET) Test Methods

Teresa J Norberg-King¹, Rami Naddy², Stephen L. Clark³, Ginger Briggs⁴, Elizabeth West⁵ and Peter De Lisle⁶, (1)Aquatic Ecotox Solutions, (2)TRE Environmental Strategies, (3)Pacific EcoRisk, (4)Bio-Analytical Laboratories, Inc., (5)Louisiana Department of Environmental Quality, (6)Coastal Bioanalysts

Proficiency Testing (PT) programs provide the same or similar sample to multiple laboratories and compare their results with the group, with the aim to help laboratories improve their performance and ensure the integrity of environmental knowledge. Accreditation programs depend on PT programs. In the US, a voluntary accreditation program that sets quality standards and criteria for environmental laboratories is TNI's National Environmental Laboratory Accreditation Program (NELAP). It provides a framework for laboratories to demonstrate their competence and ability to produce reliable data for environmental monitoring and regulatory compliance. TNI's Whole Effluent Toxicity (WET) Testing Expert Committee updates and maintains TNI WET test standards, provides technical assistance on WET issues, and develops tools to aid implementation. In the US, EPA's DMR-QA program is a PT program designed to evaluate the analytical ability of laboratories that perform chemical, microbiological, and whole effluent toxicity analyses as required in NPDES permits. Analyses should be conducted as specified in the NPDES permit, but the permits can specify different test conditions. In 40 CFR 136 Table IA, EPA lists 16 WET methods, but only 11 methods are included in DMR-QA studies, and no West Coast methods are included. While study instructions specify the dilution water only for freshwater tests, and while permit tests may be conducted at 20C, the test is performed at 25C. In 2022, EPA solicited public comments on the DMR-QA program, and the TNI Proficiency Testing Program Executive Committee submitted comments. TNI recommends that EPA standardize the test conditions for each DMR-QA WET test, and recommended specifying the number of replicates, number of organisms/replicates, number of test concentrations, dilution water characteristics, and age range of organisms. Also, TNI recommended EPA DMR-QA require only point estimate test results (i.e., LC50 and IC25), given that point estimates are the preferred endpoint in the EPA WET promulgated methods. Point estimates can be averaged, whereas NOECs must not be averaged, as the NOEC values can only be one of the discrete test concentrations of the dilution series, and the effect endpoint is independent of permits effect endpoints. We will elaborate on desirable characteristics in PT programs and other standards recommendations of the TNI WET workgroup. *This abstract does not necessarily represent the position or policy of TNI.*

2.01.T-05 Adapting Whole Effluent Toxicity Testing Methods to Highly Variable Effluents- Challenges Presented by Discharges to Receiving Waters

Silvia Zavala, U.S Environmental Protection Agency

Whole Effluent Toxicity (WET) tests are used in the National Pollution Discharge Elimination System (NPDES) Permits Program to assess compliance of effluents with water quality standards and permit limitations on toxicity. Several WET methods are approved for use in compliance determinations and can be found in the regulations under 40 CFR §136. The method manuals describe each test and lay out the requirements for test

design, set-up, sampling methodology, and data analysis. WET tests can many times be a challenging component of a wastewater discharge permit for the permittee and for the regulatory authority. Most of the time it's not the test itself that is a challenge; the tests are straightforward. What becomes challenging is fitting the discharger's unique set of circumstances and variables in their effluent or discharge scenarios, into the regulatory structure of the WET methods, while using the flexibilities built into the methods. Effluents may present challenges such as intermittent discharges, unscheduled and unexpected discharge needs, viscosity and physical constraints, saline conditions, and others. Challenging discharge scenarios may include remote sampling areas combined with a short holding time for WET tests, multiple outfall scenario for the same discharge, several different receiving water bodies for the same discharge, toxic receiving water being used in processes resulting in toxic effluents, and others. This presentation will focus on highlighting some of the challenges presented by unique effluents and discharge scenarios as observed by a permit writer, and will offer considerations taken throughout the permitting process to ensure the permittee is able to conduct the WET test and it yields reliable data.

2.02.P-We Assessing Contaminant Effects in Ecosystems with Multiple Stressors

2.02.P-We-033 Design Verifications and Improvements to the In-Situ Toxicity Identification Evaluation System

Austin Crane¹, G. Allen Burton Jr.¹, D. Bart Chadwick², Megan Roe¹ and Aiden Nicholson¹, (1)University of Michigan, (2)Coastal Monitoring Associates

Currently available methods for detecting stressors in aquatic ecosystems like the US EPA Toxicity Identification Evaluation (TIE) method, while useful, often lack ecological realism due to the temporal limits of sample collection and the presence of manipulation artifacts. The *in-situ* Toxicity Identification Evaluation (iTIE) technology was designed to enable users to detect aquatic stressors with a better representation of environmental conditions. In the current iTIE prototype, water is collected either from the water column or from the sediment pore spaces of a potentially contaminated aquatic ecosystem. The water is then gently aerated, differentially fractionated through an array of diagnostic resins, and exposed to test organisms on site. This study addressed two design needs: 1) a technology update to improve the system's ability to pump water up a vertical lift and through the system interior, while reducing the formation of disruptive oxygen bubbles; and 2) a need to verify the ability of the system's porewater sampler to minimize surface water drawdown. To address the first design need, an additional booster pump and an overflow system were connected and tested. Results indicate that the addition of these two components met their intended goal of improving water flow to and through the system. To address the second design need, the porewater sampler was inserted into basins containing water-saturated sediments of varying particle sizes. The overlying surface water in each basin was spiked with a fluorescent tracer dye. The sampler was operated, and the resulting pumped water was analyzed to determine concentrations of tracer dye as a proxy for surface water drawdown rates. Early results indicate minimal surface water drawdown of the system's current porewater sampler. This prototype will continue to be field-verified and iteratively improved, with the eventual goal of wider application in aquatic ecotoxicology detection and characterization.

2.02.P-We-034 Does the Individual Microbiome Reflect Health and Contaminant Exposure?

Mary Ann Ottinger¹, Shailaja Mani², Sonja Krüger³, Brent Coverdale³, Sandi Willows-Munro⁴ and Leigh Combrink⁵, (1)University of Houston, (2)Baylor College of Medicine, (3)Ezemvelo KwaZulu-Natal Wildlife, (4)University of KwaZulu-Natal, (5)University of Arizona

Microbiomes are being recognized as critical to the overall health of individual organisms. There is growing interest in understanding not only the role of microbiomes, but also their essential functions. We have undertaken studies in endangered African vultures to understand how their microbiomes may enable their resistance to potentially extremely pathogenic microbes that they ingest as part of their scavenger role in the

ecosystem. As part of this project, we summarize research in avian scavengers and theories for their apparent resistance to illness associated with anthrax and other such pathogens. Buccal, facial, and cloacal microbiome samples were collected from four species of vultures (16 birds in total) housed in a controlled environment in a captive center in which these individuals were placed due to their inability to survive in the wild. Samples were also collected from five individuals undergoing rehabilitation to recover from injuries and prior to release back into the wild. In addition, samples were collected from 14 field caught White-backed vultures that were either juvenile or adult, based on wing coloration. These novel data will be used to characterize the microbiomes of African vultures and provide useful baseline data for microbiome composition for different body sites of these threatened birds. These data will be overviewed in terms of dominant and present microbes, and the presence of potentially harmful species. Initial recommendations for the use of microbiome in tandem with other health measures, including the presence of potentially harmful microbes, will be discussed based on insights stemming from the data collected in our pilot study and how such understanding will influence conservation management.

2.02.P-We-035 Unraveling 40 Years of Selenium Exposure in Burbot Populations: A Mining Story in the Elk-Kootenai Watershed

Noëlie Molbert¹, James L Dunnigan², Frederick Feyrer³, Rachel Johnson⁴, Travis S. Schmidt⁵ and Jessica Brandt¹, (1)University of Connecticut, (2)Montana Fish Wildlife and Parks, (3)U.S. Geological Survey, (4)National Oceanic and Atmospheric Administration, (5)U.S. Geological Survey

In the transboundary Koochanusa Reservoir-Kootenai/ay River system (Montana, USA and British Columbia, Canada), chronic selenium (Se) inputs from coal mining activity in upstream British Columbia watersheds have become an international concern and are suspected to contribute to the observed Burbot (*Lota lota*) population collapse. To evaluate Se exposure and toxicity in wild fish populations, especially the impact on population decline, a reliable account of environmental exposure during an individual's lifetime is needed. While soft tissues are often preferred for risk assessments, they only reflect recent exposure due to depuration, metabolic transformation, and tissue redistributions. To address the shortcomings of exposure monitoring using soft tissues, we will use an innovative microchemistry approach on calcified fish structures to access complete chemical records across ontogeny from larvae to adult fish. In the present study, otoliths – calcified and metabolically inert ear stones – were collected from Lake Koochanusa adult Burbot (n = 39) whose reservoir residence over time was confirmed by examining strontium isotope ratios (⁸⁷Sr/⁸⁶Sr). Selenium concentrations in otoliths will be analyzed using synchrotron-based scanning X-ray fluorescence microscopy to (i) elucidate individual-level temporal patterns of exposure and (ii) reconstruct time series of selenium loadings in Burbot population between 1982-2021. These results will be compared with long-term records of water column Se concentrations to evaluate how Se exposure in Burbot population respond to increases in Se loading to the Reservoir. Finally, a subset of Burbot otoliths with paired-tissue samples (n = 14 for muscle and liver, n = 6 for gonad) collected in 2021 will be analyzed to estimate relationship in Se concentrations between otoliths and soft tissues. This will help establish a predictive model to infer historic Se concentrations in Burbot soft tissues, which will be compared to current U.S. EPA's Aquatic Life Criterion. This presentation will introduce the study design, research questions, and initial results, which will be critically discussed.

2.02.P-We-036 Selenium Flux from Aquatic to Terrestrial Food Webs in the Upper Clark Fork River, Montana

Chloe Zampetti¹, Ashley M Bussell², Travis S. Schmidt², Bridger M Creel³, Ben Colman³, Johanna Kraus² and Jessica Brandt¹, (1)University of Connecticut, (2)U.S. Geological Survey, (3)University of Montana

Stream ecosystems are connected through a complex network of interactions, with emergent aquatic insects transferring organic matter and nutrients from aquatic to riparian food webs during their life histories. In polluted systems, these aquatic-to-terrestrial linkages also serve as pathways for contaminant exposure to riparian predators such as spiders and birds. The Clark Fork River (MT, USA) is heavily contaminated by metals from legacy and current mining and has had Superfund designation since 1986. The U.S. Geological

Survey has monitored this system for arsenic, cadmium, copper, lead, and zinc for over 30 years. Recent research shows that selenium levels are also elevated in this system, raising toxicity concerns where concentrations are greater than protective thresholds. However, selenium has not been included in long-term monitoring efforts and therefore represents a relatively under-studied contaminant. The current study serves to address important data gaps about selenium contamination and food web pathways in the Clark Fork system. In 2023, we will sample sediment, surface water, larval and emergent insects, and riparian spiders at six sites along an established selenium gradient along the Upper Clark Fork to better understand how selenium moves through the aquatic and terrestrial food webs and the potential risk of toxicity to *Arachnivorans riparian* birds. This presentation will provide an overview of the project scope and research questions, including initial results from the 2023 sampling season. We predict that selenium concentrations in biota will be highest at sampling sites nearest to the Beal Mountain Mine. Selenium concentrations will be compared to established and proposed toxicity thresholds to better understand potential risks for aquatic and terrestrial animals. We will also calculate insect-mediated selenium flux, and we hypothesize an inverse relationship between selenium concentrations and insect flux rates due to the potential for selenium exposure to inhibit emergence. The primary aims of this research are to understand selenium bioaccumulation along aquatic-terrestrial trophic pathways and risks for riparian predators exposed to multiple metals and stressors in the Clark Fork system. This will inform the need for ongoing monitoring and restoration.

2.02.P-We-037 Development of a Method to Monitor an Ecological Succession Remedy for Former Treatment Ponds at a Brownfield in Ohio

*Kathleen K Shearer*¹, *Hugh F Crowell*², *Valerie L Gill*¹, *Ian J Reider*¹ and *Edward J Pfau*¹, (1)*Verdantas LLC*, (2)*Hull & Associates, LLC*

A method was developed to monitor an ecological succession remedy for 16 man-made former industrial treatment ponds known to have been impacted by multiple stressors, as part of a novel remedy for a brownfield property proceeding through clean-up and redevelopment within Ohio EPA's Voluntary Action Program (VAP). The ecological succession monitoring method was developed because relevant state and federal programs lacked guidance for monitoring lentic (still) systems. A unique ecological monitoring plan was developed for the property, in part using documents recommended by Ohio EPA, including a pond survey guide from the United Kingdom that provided a general methodology for the assessment of lotic (flowing) systems and ponds. Using a combination of qualitative, semi-quantitative and quantitative parameters, the plan included procedures for initial assessment of the environmental quality of the 16 ponds to establish baseline field conditions, followed by periodic monitoring of primary ecological succession in a subset of seven representative ponds. Qualitative parameters included pond appearance, narrative description, plant community mapping, and documentation of substrate types. Semi-quantitative parameters included habitat quality metrics and pollution sensitivity metrics. Quantitative parameters included data evaluation including statistical analysis and predictable replicability. Six performance criteria were developed for periodic monitoring of primary ecological succession including: quality of the wetland fringe and riparian zone plant communities, aquatic macroinvertebrate pollution intolerance, food web complexity, pond cover, pond shoreline morphology, and aquatic vegetation quality. Baseline field conditions at the 16 ponds were documented in 2019. Statistical evaluations were conducted on ecological indices and surface water and sediment quality sampling results to determine significant differences in ecological quality based on history of construction (non-native berm substrates vs. natural berms) and historic use (dredged vs. never dredged). The statistical evaluations guided the selection of a subset of seven ponds for monitoring planned over a five-year period (2020-2025). Ohio EPA approved this novel ecological succession remedy as an effective alternative to the traditional dig-and-haul remedy often used at brownfields, which may result in additional impacts to aquatic habitats.

2.02.P-We-038 In The Context of Estuaries: Impacts of Hypersalinity, Temperature, and Per- and Polyfluoroalkyl Substances (PFAS) on Early Life Stage Red Drum

Kathleen Roark, Kerri Lynn Ackerly and Kristin Nielsen, University of Texas at Austin

Estuaries are important sites for many biological and ecological processes, including acting as a nursery habitat for larval and juvenile red drum (*Sciaenops ocellatus*), a commercially and ecologically important species in the Gulf of Mexico. Freshwater inflow from terrestrial sources and tidal inflow from the saline ocean cause natural salinity fluctuations and gradients. These factors, along with evaporation, are also likely to cause changes in temperature. Human activity within watersheds can also disrupt flow regimes, alter freshwater input, and result in contamination from anthropogenic activity. Temperatures and salinities outside of tolerated ranges are known to significantly impact development in fishes, but experimental data is limited in regard to red drum. There are also gaps in the data in regard to per- and polyfluoroalkyl substances (PFAS), a large class of manmade organofluorine compounds that are used in a variety of industrial and commercial applications. Due to the broad range of applications for per- and polyfluoroalkyl substances, these chemicals are found in a wide range of products and are thus in constant production, contributing to their continuous release into the environment via point and nonpoint sources. These compounds are highly mobile, persistent, and ubiquitously detected in sediment, soil, surface waters, wildlife, and humans. Estuarine habitats along the Gulf of Mexico are particularly vulnerable to PFAS contamination from a variety of sources, yet toxicological studies examining the effects of PFAS on aquatic biota overwhelmingly utilize freshwater models. Fewer studies examine how additional relevant stressors, such as temperature and salinity, may exacerbate the effects of PFAS on larval fish in terms of growth and development. Early life stage red drum were selected as an estuarine model for the present study, due to their abundance in the Gulf of Mexico, which experiences fluctuations in temperature, salinity, and is likely contaminated by PFAS. Experimental endpoints included survival and morphological parameters. Results of the present study address important gaps in our knowledge regarding the potential ecological risks associated with PFAS contamination, hypersalinity, rising temperatures in estuarine systems, and how interaction effects may impact early life stage red drum.

2.02.P-We-040 Investigation of Multiple Stressors on the Health and Survival of Juvenile Puget Sound Chinook Salmon

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

Extensive efforts are being conducted to improve the survival of juvenile Chinook salmon in the Duwamish River and surrounding watersheds in the Pacific Northwest. Increasingly, the focus has been on the health and survival of juvenile salmon preparing to migrate into the Puget Sound. To undergo that migration salmon must successfully complete the stressful process of smolt. However, juvenile salmon undergoing smolt are not faced with a singular stressor, but rather multiple stressors including exposure to legacy (e.g., PCBs), emerging contaminants of concern, and altered prey availability. However, toxicological effect data specific to 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, the current work is the first of three projects that will assess how restricted and contaminated feed impacts the health and survival of juvenile Chinook. Using an Aroclor 1254 spiked diet of commercially available chironomids at an environmentally relevant concentration of 200 ng/g the health and survival of juvenile Chinook was assessed over a 30-day exposure period. Initially results indicate that food availability is a likely contributing factor in increased mortality rates for juveniles. Analysis determining tissue accumulation, lipid content, and the activity of oxidative stress pathways (catalase, superoxide dismutase, and glutathione peroxidase) in the liver will further deduce the roles food restriction and contamination have on Chinook salmon.

2.02.P-We-041 Characterizing Metal Mixture Profiles in Japanese Water Environments and Illustrating Ecological Risk through Commonly used Approaches for Mixture Assessment

Wataru Naito, Yuichi Iwasaki, Hiroyuki Mano and Masashi Kamo, National Institute of Advanced Industrial Science and Technology (AIST), Japan

Ecological risk assessment and management of chemical substances has been conducted mostly on a single substance basis. In recent years, with the increasing use of small quantities and variety of chemical substances including metals, it is clear that humans and organisms are exposed to multiple chemical substances at low concentrations at the same time. Although hazard assessment studies on mixture toxicity have been conducted extensively, there is little guidance or other information that contributes to practical mixture assessments considering realistic exposure profiles conducted by regulatory agencies. To contribute to the knowledge base for pragmatic mixture assessment, the aim of this study is to characterize metal mixtures present in Japanese water environments and illustrate their metal mixture assessment by applying common mixture assessment approaches such as concentration addition. This presentation will report on the results of re-analysis of existing toxicity test data to consider the validity of concentration addition, the findings of simultaneous exposure profiles of multiple metals based on monitoring data in the Japanese aquatic environment, and the preliminary results of metal mixture assessment based on the monitoring data. Future research issues identified from this mixture assessment exercise conducted on the basis of available data will also be discussed.

2.02.P-We-042 Uncovering the Links Between Anthropogenic Activities, Microbial Communities, and Greenhouse Gas Emissions in Wascana Creek, Canada

Milena Doreen Esser, Cameron Hoggart, Phillip Ankley, Helen Baulch, John Giesy, Markus Brinkmann and Markus Hecker, University of Saskatchewan

Microbial communities play a crucial role in the functioning of freshwater ecosystems, yet they are highly susceptible to the impacts of anthropogenic activities. Understanding their responses is essential for effective ecosystem management. This study employed next-generation sequencing methods to investigate the diversity and activity of microbial communities in Wascana Creek, Southern Saskatchewan, Canada, in relation to local anthropogenic influences. Wascana Creek was selected as the study area due to its identification as a highly impacted river system in previous research. The research integrated targeted chemical analysis and suspect screening approaches, as well as nutrient and greenhouse gas analysis, to identify and characterize specific pollutants present in the creek and gain in-depth insights into the study area. By combining these analytical techniques, we aimed to unravel the intricate interactions between anthropogenic activities and microbial communities, elucidating their responses and potential functional alterations. The investigation was conducted in 2022 and revealed a substantial anthropogenic influence on Wascana Creek. Upstream of the City of Regina, elevated phosphorus levels originating from agricultural practices induced changes in microbial community composition and activity. These conditions resulted in decreased dissolved oxygen concentrations, leading to fish kill events. Furthermore, significantly increased emissions of methane were measured, which is known as a potent greenhouse gas. Downstream of the City of Regina wastewater treatment plant, the introduction of wastewater effluents led to significant increases in anthropogenic pollutants and nitrogen, as well as significant shifts in microbial community composition. These alterations were accompanied by increased emissions of nitrous oxide (N₂O), another potent greenhouse gas. These findings emphasize the direct influence of anthropogenic activities on microbial communities. Understanding the intricate interactions between anthropogenic activities, microbial communities, and ecosystem functioning is crucial. By examining microbial responses to anthropogenic stressors, valuable insights were obtained into the mechanisms underlying ecosystem disruption.

2.02.P-We-043 Assessing Potential Environmental Impacts of Exhaust Gas Cleaning System (EGCS) Discharges from Cruise Line Vessels

William A. Stubblefield¹, Valerie Chatterley², Michelle C Knowlen³, Joy A Mcgrath⁴, Mary Ann Rempel-Hester³, Dayang Wang⁵ and Jay Word³, (1)Oregon State University, (2)Venveco, (3)EcoAnalysts, Inc., (4)GHD, (5)Exponent, Inc.

Emissions from the combustion of high sulfur heavy fuel oil (HFO) have been suggested to increase risks to human health and the environment. Beginning in 2020, the International Maritime Organization International Convention for the Prevention of Pollution from Ships (MARPOL) required vessels to comply with new sulfur air emission standards by: 1) using a very low sulfur fuel oil (VLSFO) or 0.1% sulfur marine gas oil (MGO), or 2) installing an “appropriate exhaust alternative method.” To comply with the new standards, most cruise ship operators installed Exhaust Gas Cleaning Systems (EGCS) on their vessels. The EGCS removes 98 percent of sulfur oxides from exhaust air emissions, as well as many pyrogenic and petrogenic PAHs and metals, by spraying ambient seawater into the engine exhaust prior to vessel release. EGCS can be operated in a “closed-loop, i.e., minimal overboard discharge, “open loop”, discharging EGCS washwater “buffered” with ambient seawater, or hybrid configuration that can be operated in open- or closed-loop mode. The goal of this study was to evaluate potential environmental risks associated with the operation of open-loop EGCS. A combination of analytical determinations (PAHs and metals), modeling of washwater toxicity and post-discharge environmental concentrations, and validation of model predictions using Whole Effluent Toxicity (WET) bioassay methods were employed. Differences in exhaust gas composition has been attributed to engine load, characteristics of the fuel used, and engine differences (e.g., engine make, size, etc.). Possible differences attributable to fuel type were controlled by limiting the study vessels to a single HFO source with a sulfur content of 2.0-3.5%. Engine energy demand could directly affect fuel consumption and washwater composition. Two engine loads were evaluated in this study, low load ($\leq 50\%$, represents “in port operations”) and high load ($\geq 70\%$ represents normal transit operations). A representative set of samples were collected from six ships operated under low and high engine loads. Environmental samples were collected from the seawater intake, EGCS waters, and overboard discharge for each scenario. Analytical determinations (PAH and metals) were conducted on all samples. Additionally, WET testing was performed for each overboard discharge sample to determine potential toxicity to the organisms in the receiving waters. Results of all determinations will be discussed.

2.02.P-We-044 Six-years of Surface Water Quality Measurements from Four Tributaries in the Arkansas Delta

Brittany Singleton¹, Jennifer Bouldin¹ and Amber L. Spence², (1)Arkansas State University, (2)Summit Environmental

Many of the watersheds in the Arkansas Delta have been identified as areas that need more monitoring. Four tributaries to the channel-altered Bayou DeView River were sampled weekly from October 2016 to September 2022. Water quality parameters measured included pH, conductivity, dissolved oxygen, turbidity, total suspended solids (TSS), total and dissolved nitrogen, phosphorus, and lead (Pb). The major land use in the area is cultivated row crops primarily rice and soybeans. Year-round, multi-year sampling provides insight into the range of measurements, because it encompasses a variety of flow events, agricultural land practices, and precipitation events. The four tributaries sampled were Flag Slough Ditch, Three mile Creek, Cow Lake Ditch, and May Branch Lateral. Flag Slough Ditch was sampled within 0.2 km of the river and is impacted by backwater. Three mile Creek is accessed from the same road as Flag Slough Ditch and is frequently not accessible because of backwater flooding. Turbidity, TSS, and TN showed similar mean rank values at Flag Slough Ditch and Three mile Creek. However, there was a statistically significant difference between mean rank Total P between the same sites. Cow Lake Ditch, which had the forested riparian buffer removed and was dredged in 2018 had the greatest mean rank of both turbidity and TSS. May Branch Lateral contains the largest percentage of woody wetlands (8%) among the four tributaries and had the lowest turbidity and TSS. The sampling location at May Branch Lateral is located the farthest away from the river.

2.02.T Assessing Contaminant Effects in Ecosystems with Multiple Stressors

2.02.T-01 Identification and Prioritization of Stressors at a Groundwater Upwelling Site

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Addressing groundwater-surface water exposure in the face of multiple stressors can be challenging and complex, often requiring multiple lines of evidence to properly establish stressor causality. This study focused on determining the relative importance of natural fluctuations in dissolved oxygen (DO) as one line of evidence in a weight-of-evidence study of the potential impacts of groundwater upwelling in a freshwater lake. Previous work revealed dramatic diurnal fluctuations in DO and elevated concentrations of contaminants of potential concern (COPCs), with particular emphasis on ammonia. The present study involved summer sampling at the groundwater upwelling site and a nearby reference site. Differentiation and quantification of the impairments associated with each stressor was achieved through diurnal monitoring of DO and COPCs at the groundwater-surface water interface, manipulation of DO within limnocorrals, and the deployment of in situ organism exposure chambers. COPCs were sampled in porewater along a vertical gradient at 6 inches (deep) and 2 inches (shallow) below the sediment surface, and in near-bottom surface water. DO was found to be significantly associated with organism survival, with observed DO minimums dropping below effects threshold concentrations at both the upwelling site and reference site. Elevated ammonia levels were observed in deep porewater and intermediate concentrations were found in shallow porewater within a localized portion of the upwelling. However, ammonia concentrations in near-bottom surface waters in this area were observed to be below levels of concern. These results indicate potential risks in a localized part of the upwelling site due to elevated ammonia in porewater and low DO in surface water. Overall, this study emphasizes the importance of assessing the influence of different stressors including natural factors like DO declines, to support informed management decisions.

2.02.T-02 Beyond the Culverts: Investigating How Stormwater Toxicity, Metapopulation Dynamics and Habitat May Influence Coho Salmon Abundance After Passage Barrier Removal

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Salmon conservation in urbanizing watersheds poses unique challenges related to habitat availability, water quantity, and water chemistry. Stream restoration practices have been developed in pristine or less impaired watersheds where precipitation naturally moves across the landscape through soils to streams. In contrast, urban watershed stormwater is often collected and directly discharged into freshwater habitats. A primary source of urban stormwater is road runoff, which is composed of thousands of compounds. Recently, the tire-derived chemical 6-PPD-quinone has been identified as the acutely toxic causative factor in the coho mortality syndrome in urban watersheds. Land use analyses identified relationships between the mortality syndrome and basin scale habitat conditions and identified the landscape characteristics associated with the syndrome and produced a predictive map to identify mortality rates in unmonitored basins. Thousands of sites in basins with water chemistry impaired by road runoff and predicted coho mortality are proposed for restoration and reconnection, particularly through removal of fish passage barriers (e.g., culverts). Coho salmon exhibit metapopulation dynamics via straying that can link populations inhabiting high and low quality habitats. Restoring access to habitat with impaired water chemistry urban runoff can create a drain on nearby populations and result in a net loss of abundance. Models show that poor quality habitat patches become a drain (sink) on populations in good habitat (source). Models examined factors that can influence the long-term population abundance after barrier removal. Prominent factors for coho are straying rates between populations and the amount and the quality of the newly available habitat, particularly the predicted stormwater mortality across different basins. Modest changes in stormwater mortality produce large differences in productivity, therefore implementing stormwater mitigation, such as green stormwater infrastructure in reconnected habitats may provide large gains in abundance. Reconnecting habitats that add large carrying capacity, have low stormwater

mortality and are closely linked to stable source populations provides the greatest opportunity for increasing coho abundance through passage barrier removal and restoration.

2.02.T-03 Assessing Remediation and Restoration Effectiveness in the Upper Arkansas River, Colorado

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Although it is recognized that improving both water quality and habitat are often necessary to restore degraded watersheds, the relative importance of these actions is generally unknown. Remediation and restoration activities in the upper Arkansas River, a historically mining-polluted watershed in Colorado, provided an opportunity to contrast the effectiveness improvements in water quality and instream habitat. Long-term (1989-2023) monitoring conducted before and after remediation and restoration treatments showed significant differences in responses of benthic macroinvertebrate communities and brown trout (*Salmo trutta*) populations. Both macroinvertebrates and brown trout responded significantly to improvements in water quality, with macroinvertebrate metrics returning to reference conditions within about 10 years. About 15 years after remediation was completed, a large-scale habitat restoration project that was designed to improve the brown trout fishery in the Arkansas River was initiated. Restoration treatments included planting riparian vegetation, bank stabilization, and installation of instream structures (e.g., boulders and large woody debris) to enhance pools and increase habitat heterogeneity. Habitat restoration significantly increased trout density and biomass; however, consistent with results of previous studies, we observed few changes in benthic macroinvertebrate communities after restoration was completed. Differences in the responses between macroinvertebrates and fish were likely due to different mechanisms that influenced recovery. Because high metal concentrations significantly degraded all biological compartments, we expected that remediation would improve macroinvertebrates and fish. In contrast, habitat restoration, which was designed to increase trout populations, also increased macroinvertebrate prey consumption. These results suggest that restoration of streams may be influenced by predator-prey relationships and potential top-down effects of fish on macroinvertebrate communities. Monitoring programs that assess changes in availability and utilization of prey resources by fish provide a more complete characterization of restoration effectiveness on stream ecosystems.

2.02.T-04 Water Quality and Ecological Integrity of the Mine Impacted Transboundary Kootenai River Basin

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The transboundary Kootenai River Basin, which contains Lake Koocanusa, is located in Montana, Idaho, and southern British Columbia and is the traditional territory of the Ktunaxa Nation. The river basin contains critical habitat for Burbot (*Lota lota*), Westslope Cutthroat Trout (*Onchorhynchus clarkii lewisi*), Bull Trout (*Salvelinus confluentus*) and White Sturgeon (*Acipenser transmontanus*) and also serves a vital role in maintaining adequate flows for several endangered salmon and steelhead runs in the Columbia River. Historic and present-day coal mining in the Canadian headwaters has degraded water quality within the rivers that drain into the basin. The US Geological Survey was authorized by the US Congress to monitor the water quality and ecological integrity of the watershed. Of particular concern are the effects of selenium and nitrate on the aquatic food web in Lake Koocanusa and the Kootenai River downstream of Libby Dam. Though selenium is an essential nutrient, at excess levels, it can cause oxidative stress, deformities, and mortality in fish and wildlife. Nitrate enrichment can alter food web structure and function. These issues are particularly concerning for transboundary fish

populations that have significant ecological, cultural, and socioeconomic value in the United States and Canada. To address these concerns, we developed a multi-agency integrated water quality and ecosystem research monitoring program to evaluate mining effects on water quality and aquatic ecosystems in the United States. This presentation will detail 1) increasing trends in contaminant loads from the Elk River over the last 30-years, 2) observed exceedances of aquatic life standards in water throughout the Reservoir, 3) fish tissue exceedances of aquatic life standards from Koochanusa Reservoir to the Kootenai River in Idaho, and 4) increasing trends in selenium concentrations at five sampling locations covering 400 river miles of the Kootenai River Basin. Investigations into potential impacts to fish and wildlife populations will be described.

2.02.T-05 The Integration of Microplastics and Nanoplastics into Large Scale Multiple Stressor Ecological Risk Assessments Using San Francisco Bay and the Delta Region as a Case Study

Wayne G. Landis, Cynthia C. Kuhn and Emma Sharpe, Western Washington University

It is now understood that micro and nanoplastics are part of the contaminant loading of marine, estuarine, and freshwater systems. This loading is only one of many other contaminants including pesticides, metals, and water quality parameters. Current risk assessments for micro/nanoplastics are based on regulatory tools such as species sensitivity distributions (SSDs) that are derived from regressions of the results of toxicity tests to a variety of species. SSDs are based on a mid-1980s effort to derive water quality standards that would be protective of aquatic communities. As demonstrated by Sharpe it is possible to build probabilistic Bayesian networks (BNs) to estimate risk due to tire wear particles. Kuhn has built BNs to estimate risk to a variety of types of microplastics and used mercury to provide a comparison to a well understood and common contaminant. Bayesian networks can incorporate specific pathways, be spatially explicit, incorporate other types of contaminants, include water quality parameters, and estimate risk to multiple endpoints derived from regulations and local stakeholders. San Francisco Bay serves as the model case study because of the extensive data base on chemical contaminants and the intense sampling program conducted by the San Francisco Estuary Institute to quantify microplastics at that site. The exposure-response data for microplastics are derived from the Brander and Harper labs at Oregon State University and toxicity are characterized using the *drc* package in R. Toxicity tests demonstrate that there are multiple modes of action in addition to the dilution of or reduction to the quality of food uptake. There are a variety of advantages to a multiple stressor approach using BNs. First, the conceptual model is explicit with the cause-effect pathways clearly delineated. Second, the BN approach has been demonstrated in multiple previous environmental assessments including the effects of climate change. The various BN software tools allow for the characterization of uncertainty and sensitivity. Finally, BNs can be used to calculate the management goals required for reducing overall risk and to prevent unintended consequences. Consequently, microplastics and eventually nanoplastics can be integrated into the overall evaluation and management of contaminants for the region. This material is based upon work supported by the National Science Foundation Growing Convergence Research Big Idea under Grants #1935028 and #1935018.

2.02.T-06 Assessing the Ecological Impact of Aquatic Pollution in a Rural Environment

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Rural aquatic ecosystems are simultaneously subjected to multiple sources of contamination such as pesticides and nutrients associated with agriculture, as well as bacteriological contamination associated with livestock and sewage effluents discharge. In the present study, pesticide, metal, and bacterial aquatic contamination were evaluated together with the trophic state of the river and the health and presence of pesticide residues in fish of the Pergamino River (Buenos Aires Province, Argentina) in order to study the anthropogenic impacts on the water quality of the river. The Pergamino River flows through productive fields dedicated mainly to extensive crop production and livestock pastures. The river also crosses through the City of Pergamino. Four sites were

sampled along the river in November 2021 and March 2022. Two of the sites were located upstream from the City of Pergamino, while the two other sites were located downstream from the city, so as to compare the impacts of agriculture and urbanization on water quality. Forty-four different pesticide molecules were detected in surface water samples, the herbicides saflufenacil and atrazine, and the fungicide carbendazim were detected in all water samples tested. Other frequently detected pesticides were triadimefon, tebuconazol, and cyproconazol, which were present in more than 80% of the samples. The Trophic State Index had values above 80 in all sampling sites, indicating hypertrophic conditions. The water concentrations of Cu, Pb, Hg, Mn, Cr, and Al frequently surpassed water quality criteria. Biological and chemical oxygen demands as well total coliforms values were greatly elevated in sites downstream from the municipal wastewater outlet. In fish, imazetapir, cyantraniliprole, chlorothalonil, fludioxinil, myclobutanil and atrazine were the pesticides with the highest detection rates, being present in more than 50% of the individuals. Thirteen pesticides were detected in 30 to 50% of the fish examined. The potential impacts of these findings on aquatic life are evaluated according to different approaches such as environmental risk assessment, indicators or multivariate statistics. Overall, results obtained point out the pervasive impact of the anthropogenic activities (agriculture and urbanizations) on the water quality of the Pergamino River and the potential negative effects on the aquatic life.

2.03.P-Mo Canada's Oil Sands and Dilbit

2.03.P-Mo-024 Measuring the Environmental Fate of Naphthenic Acids in Wetlands Using Polar Organic Chemical Integrative Samplers

Alexander M. Cancelli and Frank Gobas, Simon Fraser University

Treatment wetlands have emerged as a potential treatment option for oil sands process-affected waters (OSPW) produced from bitumen extraction. Of particular interest is the removal of naphthenic acids (NAs), which are widely acknowledged as the primary constituents of toxicity in OSPW. While removal of O2-NAs from OSPW in treatment wetlands have been demonstrated, the specific mechanisms for removal of NAs in wetlands is not well understood. The aim of this study was to quantify the rates of O2-NA removal from OSPW via sorption, microbial degradation, and plant uptake. The removal of O2-NAs from OSPW was evaluated in mesocosms consisting of 1) OSPW, 2) OSPW with sediment, and 3) OSPW with cattails and sediment. OSPW and sediment was collected from the Kearl Oil Sands site, and cattail seeds were obtained from northern Alberta and grown at the Simon Fraser University greenhouse, where the experiments were also conducted. The concentrations of 60 O2-NAs freely dissolved in OSPW were evaluated using Polar Organic Chemical Integrative Samplers deployed for 5-day increments over a 30-day study period. The analysis of POCIS extracts were performed by high performance liquid chromatography with triple quadrupole mass spectrometer detection (LC-MS/MS). O2-NA depuration in OSPW (without sediment or cattails) was slow, and similar to rates of O2-NA degradation in tailings ponds. With the addition of sediment (OSPW + sediment), microbial degradation and sorption of O2-NAs was found to significantly enhance O2-NA depuration compared to OSPW only, with average depuration rates of 0.01 (SD 0.001) day⁻¹. The addition of cattails further enhanced O2-NA depuration from OSPW with average rates of 0.04 (SD 0.004) day⁻¹. This increase in O2-NA removal from OSPW highlights the important role of wetland vegetation in supporting enhanced microbial degradation and plant uptake. The findings are used to test and evaluate a contaminant-fate model for ionizable substances in wetland environments. This research supports further evaluation of treatment wetlands as a potential option for OSPW remediation and improves environmental and human health risk assessments of NAs.

2.03.P-Mo-025 Source Apportionment of Polycyclic Aromatic Compounds in Air at Surface-Mining and In-Situ Mining Areas in the Alberta Oil Sands Region

Jasmin K Schuster, Tom Harner and Ky Su, Environment and Climate Change Canada

Under the Oil Sands Monitoring (OSM) Plan, Environment and Climate Change Canada (ECCC) operates a network of polyurethane foam (PUF) passive air samplers (PAS) for tracking spatial and temporal trends of

Polycyclic aromatic compounds (PACs) in ambient air. In the current study, the network was extended to assess differences in PACs levels in open pit mining areas versus areas dominated by in-situ mining. Canada's oil sands deposit in Northern Alberta is the largest bitumen deposit in the world and divided into the Athabasca (AOSR), Peace River (PROSR) and Cold Lake Oil Sands Regions (CLOSR). The AOSR is the largest deposit of the three. PACs were monitored from 2011-2015 to establish baseline levels in ambient air in the AOSR. PACs are chemicals of concern, which are released from the oil sand industrial activity. PACs such as polycyclic aromatic hydrocarbons (PAHs) have a wide range of sources (e.g., wildfires, traffic) in addition to the petroleum industry, whereas PACs such as alkylated PAHs (alkPAHs) and dibenzothiophenes (DBTs) are predominantly present in bitumen. Elevated PAC levels in the AOSR have previously been associated with fugitive dust from activities like open pit mining and heavy vehicle traffic. Passive air sampling was resumed in the AOSR in 2020 to assess changes to the baseline. Additional sampling sites in the PROSR and CLOSR were added to compare PAC levels in ambient air of regions dominated by in-situ mining and those impacted by open pit mining. Overall PAC levels reported for 2020-2022 are the same order of magnitude as 2011-2015. Substantially higher levels of PACs were observed at locations close to open pit mining activities in the AOSR ($57 \pm 17 \text{ ng/m}^3$) compared to locations in vicinity of in-situ mining in the PROSR and CLOSR ($5.9 \pm 7.1 \text{ ng/m}^3$), with concentrations of PACs associated with the petroleum industry being a factor of 10 higher. PAC levels reported for near-source and background locations in the PROSR and CLOSR are the same order of magnitude. The composition of PAC congeners between samples collected in the AOSR and PROSR/CLOSR are significantly different with the AOSR samples showing a higher content of semi-volatile and particle-phase PACs associated with bitumen. Positive Matrix Factorization was used for source apportionment of the different PAC profiles between the 13 sampling locations over summer and winter seasons.

2.03.P-Mo-026 Response Factors of Alkylated Phenanthrenes and Impacts on Data Quality

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Alkylated polycyclic aromatic hydrocarbons (APAHs) are a structurally diverse class of compounds that can enter the environment either from petrogenic or pyrogenic activities. By convention, the nomenclature of APAHs is done by adding a prefix to the name of parent PAH. The prefix is derived by summing the number of carbon atoms of the alkyl-moieties attached to the PAH-ring for example, a phenanthrene that has 3 carbon atoms alkyl-moieties attached to the ring is designated as C3-phenanthrene. Unlike their parent derivatives, APAHs contain numerous theoretical constitutional isomers within each grouping. For example, phenanthrene containing 3 carbon alkyl-moieties has 115 possible isomers. To date, quantitation of APAHs is done either by selected ion monitoring or multiple reaction monitoring. In both cases, the sum of the area of all the peaks in the ion chromatogram in a sample is comparing to the area to an external standard of a constitutional isomer run separately. Because commercially available isomers are limited, the choice of the isomer selected as the external standard will impact the quantitative values. Here we report on the response factors of 38 individual alkylated phenanthrenes, use their structural features to explain differences in response factors and discuss implications on the quality of environmental data.

2.03.P-Mo-027 Effects of Physical Removal of Residual Oil on Shoreline Environments Previously Treated with Non-Invasive Remediation Methods

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North Americans use oil every day for a myriad of purposes but the potential for spills remains an area of significant environmental concern for the public. There is much debate among industry professionals and regulators on the most efficient and least ecologically impactful remediation methods to be employed in the event of a spill. From 2018 to 2022 the IISD Experimental Lakes Area in northwestern Ontario has been evaluating the efficacy of minimally invasive methods of remediation, specifically enhancement of the natural

microbial community, in a freshwater shoreline environment. This was achieved by spilling conventional heavy crude oil in large 5 x 10 m enclosures sealed to the sediment in wetland shorelines in an oligotrophic boreal lake and then amending these areas with nutrients. The current study now aims to assess whether this response technique has left weathered oil bound to the shoreline and how physical removal of any recalcitrant oil in the shoreline material may affect the surrounding environment. Here we present changes in water quality (turbidity, nutrients, DOC, DIC) and primary production (chlorophyll-a) two years after nutrient addition and in the weeks following the physical removal of residual oil from the shoreline. We will also present how the concentrations of polycyclic aromatic compounds change in the water of the enclosures following excavation. The results from the Physical Removal of Oil to Validate In-lake Treatments (PROVE IT) study will continue to inform regulators and spill responders on the efficacy of microbial community enhancement as well as the potential effects of physical removal methods in a wetland shoreline ecozone.

2.03.T Canada's Oil Sands and Dilbit

2.03.T-01 Search for Novel Non-Halogenated and Halogenated Heterocyclic Aromatic Compounds in the Canadian Environment

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Canada is the world's fifth-largest oil producer and has the world's third-largest proven oil reserves, with large percentage present in Alberta Oil Sand Region (AOSR). Oil exploitation can leave traces of several categories of environmental pollutants including non-halogenated and halogenated heterocyclic aromatic compounds (HPACs and Halo-HPACs). HPACs are a subcategory of a widely known class of environmental pollutants: polyaromatic compounds (PACs), which include several persistent, bioaccumulative and toxic (PBT) compounds. Compared to other categories of PACs, HPACs analysis draws less attention mainly due to the challenges like the presence of a large number of theoretically possible isomers and lack of commercially available standards. However, the similar physical-chemical properties to the common PACs make HPACs have similar fates and behaviors in the environment. Gas chromatography coupled with a tandem mass spectrometer (GC/MS/MS) was used to develop a multiple reaction monitoring (MRM) method to analyze forty-five HPACs and Halo-HPACs in Canadian environmental samples. An extraction and purification procedure was developed to detect these compounds in sediment samples using the accelerated solvent extraction (ASE) technique. Method validation was performed for the constructed method under Eurachem guidelines. The recovery of the method ranged from 59% to 130% and limits of detection and quantitation ranged from 0.1 to 2.3 ng/g and from 1.5 to 7.6 ng/g, respectively. The method exhibited good inter- and intra-day repeatability for all the standards with percent relative standard deviation below 18.2%. The results suggest that the developed method is an effective and efficient approach for the targeted analysis of HPACs and their halogenated derivatives in abiotic samples from AOSR.

2.03.T-02 Current Water and Sediment Quality in the Lower Athabasca River Region: An Approach to Setting a Reference Condition

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The development of oil sands extraction projects in Alberta, Canada has been ongoing for several decades. Over that time period, water and sediment quality in the lower Athabasca River region has been monitored by different organizations. The goals of this monitoring have been to establish background or reference conditions, and to evaluate change over time and space. However, there have been limited attempts to compile water and sediment quality data from multiple programs and organizations into a standard format, or to establish a reference condition using that data for the lower Athabasca River, the Athabasca River Delta and Lake Athabasca. We present such a data standardization and compilation effort and an approach for setting the

'current condition' water and sediment quality in each of these areas. This current condition is separated by flow season and region, and incorporates methods to account for censored water and sediment quality datasets. The goal of this work is to facilitate a common understanding of what is currently normal in these aquatic ecosystems, which can be used as a reference point for future impact assessment or as anti-degradation targets for their management. The approach may also be useful for estimating historic change in water and sediment quality condition. Gaps and other issues in available monitoring data sets will be highlighted.

2.03.T-04 Assessing Naphthenic Acid Fraction Components: Metabolic Reprogramming of Rat Hepatocytes

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The potential toxicity of bitumen extraction on water quality and wildlife health in the Alberta Oil Sands Region (AOSR) is not yet fully understood. While toxicological assessments have focused on aquatic and invertebrate species, the effects of exposure in mammals remain largely unknown. Chemical constituents found in oil sands process affected water from the AOSR can impact endocrine signaling and result in aberrant lipid accumulation and altered glycemic control in mammals. The goal of this study was to determine the effects of exposure to naphthenic acid fraction components (NAFC), a subgroup of bitumen-derived dissolved organics, on energy homeostasis in a mammalian hepatocyte model. McA-Rh7777 cells, a rat hepatocellular carcinoma cell line, were exposed to NAFC (Frank et al., 2006) for 24h and 48h at environmental concentrations [0, 1.25, 25, 125mg/L]. We assessed the mRNA expression of key genes involved in glucose and lipid homeostasis: *Fasn*, *Acca*, *Cpt1a*, *G6pase*, *Pgc1a*, *Cd36*, *Angptl4*, and *Gdf15*. Additionally, we assessed triglyceride accumulation, and glucose and fatty acid uptake. As mitochondria coordinate cellular adaptations to stressors including bioenergetics, we further assessed mitochondrial function and mitochondrial membrane potential. NAFC treatment [1.25 and 125mg/L] significantly reduced triglyceride output at both 24 and 48h. The reduction in triglyceride level at 125mg/L NAFC matched decreased expression of the lipogenesis genes, *Fasn* (24h) and *Pgc1a* (24h and 48h). Similarly, 125mg/L NAFC significantly reduced glucose uptake. Interestingly, NAFC exposure [25 and 125 mg/L] increased expression of genes involved in mitochondrial fatty acid β -oxidation: *Cpt1a* and *Cd36* at both timepoints. In line with mitochondrial activation, we observed increased mitochondrial membrane potential at both 24 and 48h, following exposure to 125mg/L NAFC. Together, these data suggest that NAFC exposure disrupts transcriptional regulators of metabolic homeostasis. Cells have metabolic flexibility that allows them to adapt to changes in substrate availability. The observed reductions in triglyceride levels and glucose uptake suggests that NAFC exposure may lead to a period of glucose deficiency, which causes a metabolic switch from glucose substrate use to fatty-acid oxidation. Given altered energy homeostasis is critical to organism health, this study provides a potential mechanism by which bitumen-derived organic compounds may be affecting wildlife health.

2.03.T-05 Solar Photocatalytic Detoxification of Oil Sands Process-Affected Waters

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We present a compendium of 5 years of results evaluating solar photocatalytic treatment of oil sands process-affected waters (8 different samples from 3 oil sands operators), conducted as a series of joint industry projects through Canada's Oil Sands Innovation Alliance (COSIA). Oil sands process-affected water (OSPW) aquatic toxicity has been associated with a complex mixture of naphthenic acid fraction components (NAFCs). Passive treatment methods are commonly used for environmental remediation, but given the biological recalcitrance of some NAFCs, OSPW toxicity may not be adequately addressed through conventional passive technologies. In this work, solar treatment with buoyant photocatalysts (BPCs) was evaluated as a candidate passive advanced

oxidation process (P-AOP) for OSPW remediation. Treatment efficacy was assessed by both analytical chemistry and standard rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) whole effluent toxicity (WET) bioassays. Solar photocatalysis using BPCs fully degraded naphthenic acids (NAs) and acid extractable organics (AEO) in all OSPW samples, and fish toxicity was eliminated well before concentrations of dissolved organics had significantly diminished (within <7 days of sunlight exposure for all OSPWs), as described by tissue partitioning modelling. Interestingly, classical NAs and AEO, traditionally considered among the principal toxicants in OSPW, were not found to be correlated with OSPW toxicity herein; alternate techniques, such as biomimetic extraction by solid phase microextraction (BE-SPME) were instead evaluated to monitor treatment. Solar photocatalysis was also found to be effective at treating other OSPW contaminants of potential concern (COPCs), including polycyclic aromatic hydrocarbons (PAHs) and trace elements. Together, these results demonstrate that complete detoxification of OSPW to standard WET bioassays is achievable without requiring complete elimination of all dissolved organics, suggesting only a small fraction of the total AEO may be responsible for most of the toxicity of OSPW, and that this fraction is effectively treated by solar photocatalysis. These results have significant implications for OSPW release, for which a less extensive but more selective treatment may be required than previously expected.

2.03.T-06 Development and Testing of a Mechanistic Model for Wetland Treatment of Neutral and Polar Organic Contaminants in Oil Sands Process-Affected Water

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Bitumen extraction produces large volumes of Oil Sands Process-affected Water (OSPW) containing a suite of organic contaminants that include polycyclic aromatic hydrocarbons (PAHs) and O₂-naphthenic acids (NAs), which are chemicals primarily responsible for OSPW toxicity. To address this problem, there is a need for feasible and effective treatment technologies to better manage this wastewater. Treatment wetlands have been shown to remove PAHs and NAs from OSPW and continue to be a potential option for OSPW remediation. To further understand the role of treatment wetlands, the mechanisms of contaminant fate and removal from OSPW, and to evaluate the effects of wetland design and operation, a mechanistic model of the fate of neutral and polar organic contaminants in a free water surface flow wetland was developed. The model simulates wetland processes of chemical removal such as sorption, microbial degradation, plant uptake, and evapotranspiration. The model evaluates the removal efficiency of PAHs and from OSPW based on the physicochemical properties of contaminants, environmental conditions, and wetland design. Model performance was evaluated against data collected from the Kearl Treatment Wetland in northern Alberta, Canada. Good agreement between model estimated and observed concentrations of PAHs and NAs in OSPW in the wetland was observed over time. The model indicates that chemical removal is highly sensitive to rates of microbial and plant-mediated biotransformation. The efficient removal of PAHs and NAs therefore relies on a robust microbial community in the wetland. The greatest removal efficiency occurs for substances with a log K_{ow} or log D between 3.0 and 5.5. Substances a log K_{ow} or log D greater than 6.0 are not suitable for wetland treatment, even if they can be biodegraded. Evapotranspiration is not a significant pathway of chemical removal in wetlands for OSPW due to the very low log K_{AW} (less than -1.67) of PAHs and NAs evaluated in this study. This is the first treatment wetland model to evaluate the fate of both neutral and polar organic contaminants in a flow-through treatment wetland operation, making it a helpful tool to assess the feasibility of these treatment systems for the oil sands industry. The model is particularly useful to evaluate trade-offs in wetland design and operation and explore wetland optimization for more targeted contaminant removal.

2.04.P-Th Effect of Environmental Pollutants to Aquatic Organism Health: Linking Molecular Effects to Apical Endpoints

2.04.P-Th-017 High Content Screening to Predict Sublethal Effects on *Daphnia*: A New Tool for Rapid Assessment of Chemicals

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More than 86 million tons of chemicals, potentially hazardous to the environment, are produced annually in Europe. This makes it nearly impossible to evaluate the adverse effects of each chemical in a realistic time frame. Developing new tools capable of rapidly revealing adverse effects on relevant ecotoxicological endpoints is therefore fundamental. Mistra SafeChem is a research program for green and sustainable chemical with the objective to develop “safe by design” chemicals. As part of this programme, we are developing high content screening (HCS) protocols for sublethal adverse effects in *Daphnia magna*. Since these methods are *in-vivo*, it is necessary to find a suitable anaesthesia technique that keeps the *D. magna* still for the image acquisition without harming them. An ideal anaesthesia method would be a substance that make the *D. magna* reach a stage with fast total immobility; without cardiac or respiratory arrest; no signs of violent locomotor activity previous to the immobilization; and full recovery after anaesthesia. We compared the performance of Tricane, a common anaesthesia for fish and crustaceans, and ethanol as an anaesthesia for *D. magna* in our HCS workflow. Furthermore, a double staining using Calcein AM and Propidium Iodide (PI) was carried out after an exposure to the model compound Methoxychlor. Calcein AM, was used as a stain to image viable cells, as it transforms into a hydrophilic fluorescent compound when penetrating the cell membrane in living cells. PI was used to stain dead cells, with its potential to penetrate damaged cell membranes and intercalates into the DNA of the cells, thus staining only non-viable cells. The results demonstrated that only ethanol at 3% and 5% kept *Daphnia* still, with no signs of violent locomotor activity and full recovery post-anaesthesia. In addition, the staining method showed a decreasing fluorescence intensity from Calcein AM and an increasing intensity from PI in *D. magna* with increasing concentrations of Methoxychlor (50-200 µg/L), yet concentrations that did not cause increased mortality. This suggests that the developed approach, based on whole organism HCS and automated image analysis may offer a new, faster and more accurate tool to assess sublethal ecotoxicological effects of industrial chemicals in *D. magna*.

2.04.P-Th-018 Comparison of Zebrafish Toxicity Between Different Developmental Windows of Exposure to Three Environmentally Relevant PFAS Compounds

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Zebrafish have emerged as a sensitive and high-throughput model for measuring toxicity associated with chemicals, including PFAS, in a relatively short span of time. The current study's objective is to leverage zebrafish to explore effects of exposure to PFOS, PFOA, and PFHxS for 14 exposure lengths between 6 and 144 hours post fertilization (hpf). Zebrafish were exposed to eight concentrations of each PFAS chemical (0-100 µM), chlorpyrifos (positive control for behavior), and 0.33% DMSO (vehicle control) for varying lengths of time starting at 6, 24, 48, or 96 hpf, and ending at 24, 48, 96, 120, or 144 hpf. Morphology was assessed daily, zebrafish neurobehavioral responses to alternating dark and light cycles were measured at 120 hpf for windows ending at 120 or 144 hpf, and whole animal RNA was collected from windows ending at 24, 48, or 120 hpf for transcriptomics. Daily 50% chemical renewal was conducted, and media was analyzed by liquid chromatography-mass spectrometry at each window's beginning and end to validate exposure concentrations. Exposures ending at 24, 48 or 96 hpf did not lead to overt toxicity for any of the PFAS. When zebrafish were exposed to 100 µM PFOS, significant mortality was observed at all exposure windows ending at 120 hpf, with the exception of the 96-120 hpf exposure window. For windows 6-144 and 24-144 hpf, significant mortality at

144 hpf was identified at the two highest PFOS concentrations (100 and 31.6 μM). No significant mortality was observed from exposure to PFOA, PFHxS, or any other PFOS concentration. Zebrafish hatch showed no significant differences; however, we observed chemical concentration- and exposure start time-dependent alterations to zebrafish length over time, swim bladder inflation, and behavior responses. We are currently evaluating transcriptomic points of departure (tPOD) from the whole-animal RNA and plan to investigate their potential relationships to observed morphological and behavioral responses. Results of this study will provide information on 1) the hazard to zebrafish associated with varying exposure lengths to select PFAS chemicals, and 2) the variability associated with tPODs estimated under various exposure scenarios, specifically different exposure durations and at different zebrafish developmental stages. *Contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.*

2.04.P-Th-019 Reproductive Toxicity of an Estrogenic Polyfluoroalkyl Substance on Fathead Minnows

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In previous studies, male fathead minnows (*Pimephales promelas*) were exposed to four PFAS that were shown to have estrogenic activity in vitro. Based on results from these 4 d in vivo tests, 1H,1H,10H,10H-Perfluorodecane-1,10-diol (FC10-diol) (DTXID50369896) was found to be the most estrogenically-potent among the tested chemicals. Therefore, FC10-diol was selected to evaluate whether estrogenic PFAS could elicit responses consistent with a hypothesized adverse outcome pathway (AOP) linking estrogen receptor agonism to reproductive impairment in fish. To determine the robustness of the AOP and potential reproductive toxicity of FC10-diol, adult fathead minnows were exposed for 21 days to one of five nominal concentrations of FC10-diol (0.68; 2.1; 6.8; 21; or 68 $\mu\text{g/L}$) or E2 (1 $\mu\text{g/L}$) in a short-term reproduction assay. Estrogen receptor activation was again confirmed as estrogen-regulated genes were up-regulated (*vtg* and *esr1*) or down-regulated (*igf1* and *apoeb*) in a concentration-dependent manner in exposed males. Morphologically, FC10-diol reduced male secondary sex characteristics as reflected by a reduction in tubercle score and, at the highest dose of FC10-diol, both male and female fathead minnows experienced a reduction in gonadosomatic index (GSI). A hypothesized increase in oocyte atresia in exposed females was confirmed histologically by a significant increase in the incidence and severity oocyte atresia in females exposed to the high dose of FC10-diol. Cumulative fecundity was significantly reduced for fish exposed to either 6.8 or 68 $\mu\text{g/L}$ of FC10-diol. The results of this study verify both the reproductive toxicity of FC10-diol in vivo and the applicability of the hypothesized AOP for estrogenic PFAS. *The contents of this abstract neither constitute, nor necessarily reflect US EPA policy.*

2.04.P-Th-020 Effects of Exposure to a Primary Wastewater Effluent on Liver Lipid Metabolism and Oxidative Stress in Northern Pike

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Urban wastewater effluents are sources of complex contaminant mixtures to aquatic ecosystems. The Montreal's (Canada) primary wastewater treatment plant effluent was found to be a source of poly- and perfluoroalkyl substances (PFAS) and halogenated flame retardants (HFR) to the St. Lawrence River. The northern pike (*Esox lucius*), a sedentary predatory fish of St. Lawrence River, was found to accumulate tissue concentrations of a range of PFAS and HFR. Recent studies reported changes in the lipid composition in liver and in the transcription of several genes involved in the regulation of lipid metabolism and oxidative stress of northern pike downstream of Montreal's wastewater effluent outfall. Correlations were also reported between these variables and liver HFR concentrations in pike. The objective of the present study was to refine our

understanding of the effects of exposure to this major wastewater effluent on the liver lipid metabolism of pike. Lipid peroxidation, a marker of oxidative stress, measured using malondialdehyde levels, was not different between pike collected upstream (reference site) and downstream (effluent impacted site) of the effluent outfall. RT-qPCR data analysis of lipid metabolism related genes revealed that the mRNA levels of fatty acid desaturase 2 (*fads2*) and sterol regulatory element binding transcription factor 1 (*srebf1*) were not significantly different between the two sites, while transcription of peroxisome proliferator-activated receptor alpha (*ppara*) was downregulated. Other genes are currently being analyzed by digital droplet PCR, including several genes of the phospholipase A2 family involved in the conversion of lysophosphatidylcholines into phosphatidylcholines, for which the levels were found to be altered in a previous study. Moreover, new PFAS were quantified in pike liver, including perfluorooctadecanoic acid (PFOcDA) (n.d. - 6.6 ng/g ww) and 6:2 fluorotelomer sulfonic acid (6:2-FTS) (n.d. - 366 ng/g ww). Other analyses are in progress including the composition of liver lipids (target lipidomics) and HFR concentrations. Correlations between these biological variables and HFR and PFAS concentrations in pike liver will be assessed. This study will provide essential information on the effects of a major wastewater effluent on the energetic metabolism and health of an apex predator of a freshwater ecosystem, and provide insights onto potential effects of more novel PFAS and HFR on this species.

2.04.P-Th-021 Mixture Effects of Phthalate Esters and Their Subgrouping Based on Toxicity Profiles

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Dialkylesters of phthalates (phthalate esters) are the group of chemicals mainly used as plasticizers but have diverse hydrophobicity with different length of alkyl chains. The alkyl chains range from methyl (C1) to undecyl (C11) and some of these are branched, which caused the octanol-water partition coefficient ($\log K_{ow}$) to be widely ranging from 1.5 to 11.5. While the results of literature review suggest the monotonous increase of toxicity from congeners between C1 and C4 and inconsistent toxicity (nontoxic or slightly toxic) from congeners over C8, no series of studies to cover such wide range of compounds with identical species and testing methods in the same laboratory. Therefore, we conducted the short-term (sub-)chronic aquatic toxicity tests using three species, zebrafish (*Danio rerio*), *Ceriodaphnia dubia*, and green alga (*Raphidocelis subcapitata*) for 12 phthalate esters from C1 to C11. As a result, we found monotonous increase from C1 to C6 in *C. dubia* and *R. subcapitata* while the increase was from C1 to C5 for zebrafish and the toxicity was the strongest for zebrafish among three species for C1 to C5. For the phthalate esters of over C8, the no significantly consistent toxicity was detected in the soluble fraction after the filtration through PTFE membrane filter (0.4 μm) while slight toxicity with gentle dose-response curve was found for the colloidal suspension through the glass fiber filter (0.7 μm). These completely different toxicity profiles of congeners over C8 compared to those between C1 and C6 (or C5) suggests the subgrouping of these two. In addition, the trend analysis of ToxCast data for these congeners also suggests slight difference in shorter and longer alkyl chains although the applicability of these dataset to the aquatic toxicity testing may be limited. Relative potency factor (RPF) was determined for C1-C6 and C8-C11 separately based on C4 (DBP) and C8 (DEHP) as index compounds, respectively. The toxicity of various binary mixtures of DBP and DEHP was investigated for the three species based on the combined exposure profiles derived from the monitoring data by Ministry of the Environment, Japan to assess the combined ecological risk of this group of chemicals.

2.04.P-Th-022 Assessment of the Effects of Cadmium, Samarium, and Gadolinium on the Blue Mussel (*Mytilus edulis*): A Biochemical, Metabolomic, and Transcriptomic approach

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Diverse contaminations from continental and local anthropogenic pressures are occurring in estuaries and coastal areas, which present both over 40% of the world population and a particular biological relevance (feeding, spawning, nursing zones and migratory passages). In addition to a chemical stress, the global changes

make the temperature increase and the ocean acidification be additional stressors to the living organisms. Achieving and maintaining a good ecological status of the marine environment requires an assessment regarding the different environmental stresses. In this context, the ambition of this project was to assess health status of a sentinel marine species, the blue mussel *Mytilus edulis*, exposed to two Rare Earth Elements and a metal, respectively Gadolinium (Gd), Samarium (Sm) and Cadmium (Cd), based on the use of biochemical, metabolomic and genomic methods. These organisms have been exposed in aquaria of artificial seawater, for 8 days, with doses of each pollutant representative of environmental levels (50 and 500 µg/L), or with water for control conditions. Digestive gland, gills and mantle have been selected, dissected, frozen and analyzed. We found that both Cd, Gd or Sm exposure tend to show an impact on two biochemical markers of oxidative stress (Glutathione-S-Transferase, Superoxide dismutase) at in a tissue dependent way. A lipidomic analysis showed that some phosphoinositides were upregulated in several conditions or tissues. Interestingly the mitogenic phosphatidylinositol trisphosphate (PIP3) appear to be overexpressed for the three tested elements in the digestive gland. To fine tune the impacts of Cd, Gd and Sm on *Mytilus edulis* metabolism and physiology, subsequent RT-qPCR analysis were focused on signaling pathways potentially modulated by oxidative stress or PIP3. Preliminary results indicates modulation of proliferation, apoptotic and stress signaling pathways. Finally, this work suggests a possible toxicity of Cd, Gd and Sm exposure at environmental dose during 8 days on *Mytilus edulis*.

2.04.P-Th-023 Developmentally-Related Differences In Sensitivity To Propranolol In Early Life Stage Fathead Minnow (*Pimephales promelas*)

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Propranolol is a non-specific beta-adrenoceptor antagonist originally designed to treat cardiovascular related illnesses, such as angina pectoris and high blood pressure. Subsequently, propranolol has also been used to treat a diverse array of seemingly unconnected disorders, from PTSD to infantile hemangioma, which reflects the broad systemic distribution of the beta-adrenoceptors. Propranolol ranks among the most prescribed pharmaceuticals and is commonly found in wastewater effluents, prompting concern over its potential adversely affect exposed organisms. Early developmental periods are generally considered to be of greater risk to the negative effects of chemical contaminants. In the present study we use the magnitude of the transcriptional response as an estimate of sensitivity across different developmental windows in the fathead minnow (FHM). Three different ages of post-hatch FHM (4, 5, and 6 dpf +/- 1h) were exposed for 6, 24, or 48h to two nominal concentrations of propranolol. Transcriptional responses were characterized using RNA-seq and the number of differentially expressed genes (DEGs) was used as a measure of reactivity. A clear trend toward increased reactivity with age was observed, with fish >7 dpf at the end of exposure being particularly reactive to propranolol. DEGs overlapped to a large extent among treatment groups, suggesting a highly consistent response that was independent of age. Cluster analysis was performed on the transcriptional profiles of exposed fish. Prior to 7 days post fertilization (dpf), clustering was primarily driven by age, regardless of the concentration or duration of propranolol exposure, whereas fish > 7 dpf clustered based on propranolol exposure. Increased reactivity at 7 dpf coincided with developmental milestones that may act to alter the pharmacokinetic and dynamic properties of propranolol, such as the switch from endogenous to exogenous feeding, an increase in the systemic expression of beta-adrenoceptors, and the onset of gill functionality. These results have potential implications beyond propranolol, as toxicity testing in early life stages typically utilize fish < 4 dpf, prior to the onset of these potentially important developmental milestones, and may underestimate risk for some chemicals.

2.04.P-Th-024 Characterization of Growth and the GH-IGF1 Pathway in Adult and Juvenile Mummichog (*Fundulus heteroclitus*) Exposed to Environmental Contaminants

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Mummichog (*Fundulus heteroclitus*) are small-bodied killifish that inhabit the east coast of North America.

Environmental contaminants ammonia and 17 α -ethinylestradiol (EE₂) cause developmental and reproductive endocrine effects in mummichog in lab studies; however, little is known about the mechanism(s) by which ammonia and EE₂ disrupt fish growth. Experiments were conducted to develop a standardized growth bioassay and to investigate the effect and potential mechanism(s) of ammonium chloride (NH₄Cl) and EE₂ on juvenile and adult mummichog. Throughout flow-through exposures, weight measurements were taken every three days, with average individual weights as an apical endpoint representing growth. At termination of the experiments, gene expression was examined by qPCR in brain, liver, muscle, and gonad for genes known to regulate growth, including growth hormone (*gh*), insulin-like growth factor 1 (*igf1*), and selected receptors. In an experiment where 6-month-old mummichog were exposed for 21 or 33 days to 0 (control), 125, or 200 mg/L NH₄Cl, average individual weights of NH₄Cl treated fish were significantly less than controls after 33 days. After 21 days, 200 mg/L NH₄Cl treated fish showed significantly lower liver expression levels of *ghra* and *ghrb* compared with controls. However, in the livers of the 33-day fish, the gene expressions of *igf1* and *ghrb* were significantly elevated for the 200 mg/L NH₄Cl group compared with the control group. When exposed to NH₄Cl at 9 or 11 months, higher concentrations (250 & 312 mg/L) were required to cause significant changes in weight, indicating that younger stages are more vulnerable to growth inhibition. As temperature and EE₂ are known to stimulate reproductive growth, adult post-spawning females were exposed to 250 ng/L EE₂ or 250 mg/L NH₄Cl at two temperatures (20 and 25°C) for 21 days. The EE₂ group exposed to 25°C had significantly higher final body weights, while all 25°C groups had significantly higher average gonad weights relative to body weights (gonadosomatic indices) compared to fish held at the lower temperature. A final experiment will assess effects of EE₂, NH₄Cl and temperature on 3-month-old fish. Together, these exposures are supporting the development of a standardized growth bioassay for mummichog. Gene expression endpoints for the GH-IGF1 pathway may reveal whether mechanisms are similar or vary for different classes of contaminants.

2.04.P-Th-025 Comparative Toxicity Profiles of Environmentally Relevant Alkylated Naphthalenes Using Early Life Stage Zebrafish

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With an increasing demand for fossil fuels and a growing frequency of wildfires, polycyclic aromatic hydrocarbons (PAHs) are an environmental contaminant of growing concern. While PAHs are typically present in complex mixtures, our knowledge is primarily focused on unsubstituted PAHs. Understanding the toxicity of alkylated PAHs is important for a comprehensive understanding of the hazard potential, as they often occur in higher abundance than the parent PAHs or other substituted PAHs. 103 alkylated PAHs were screened for morphological and behavioral effects in a high-throughput developmental zebrafish assay. Embryos were exposed to 12 concentrations from 0 to 100 μ M (n =14) and screened for morphological effects using 10 morphological endpoints, and behavior using a larval photo motor response. The aryl hydrocarbon receptor (AHR) is often implicated in the toxicity of PAHs and the induction of cytochrome P4501A is an excellent biomarker of AHR activation. Embryos were evaluated for Cyp1a expression using an AHR-responsive reporter line. Our results demonstrated that naphthalenes are challenging to screen in aquatic systems due to their volatility. To overcome this challenge, we developed a methodology for testing volatile compounds in our system. Using this method, we exposed our series of 30 naphthalenes for phenotypically anchored targeted transcriptomics at 48 hours post-fertilization. To our knowledge, this is the largest set of alkylated PAHs yet to be screened and the first series of naphthalenes to be tested in any system. Results from this study will enhance our ability to identify structure-activity relationships to move toward predictive hazard assessment of PAHs. This research was supported by the NIEHS of the National Institutes of Health under Award Number P42 ES016465, P30 ES030287, and T32 ES007060.

2.04.P-Th-026 Examining the Impact of Insecticide Treated Mosquito Net Fishing on Aquatic Organisms

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The distribution of insecticide treated mosquito nets (ITNs) is a great public health success story, with studies suggesting these nets reduced the global burden of Malaria by approximately 40%. ITNs are typically treated with pyrethroids, which are known to have a low toxicity to mammals and high toxicity to aquatic organisms. Recent studies have shown that ITNs are being used for alternative practices, including fishing. In a study from a waterside community located on Lake Tanganyika, 87.2% of people utilized their mosquito net for fishing as opposed to malaria protection. Coinciding with reports of off label fishing, there are reports of a decrease in fish quality and quantity. Quantitative data is missing to support these claims, so we designed experiments in both vertebrates, *P. promelas*, and invertebrates, *D. magna* to investigate the toxicity of ITNs. Organisms were exposed to varying sizes of nets, 1cm², 5cm², 10cm², 20cm², and non-treated 20cm² sized nets. We tested six exposure scenarios for 7 days and mortality was recorded. To test for sub-lethal effects, we focused on oxidative stress endpoints that have been linked to pyrethroid exposure. Following sublethal exposures, we measured Superoxide Dismutase (SOD1 & 2), Catalase (CAT), P53, PXR, and CYP3A4 expression using qPCR in the *P. promelas* larvae to expand our understanding of oxidative stress and sublethal impacts. For our last experiment we placed *P. promelas* into mesocosms and exposed them to a scaled-up equivalent of our smallest and largest size netting (now 4x4cm² and 9x37cm²). Our purpose for this experiment is to investigate fate and transport of the ITNs following submersion into mesocosms. We do not expect to see overt toxicity in this study but do expect to see sub-lethal effects in the fish including ROS production and gene expression changes for the aforementioned genes. We also expect to see pyrethroids leaching from the net and sorbing to sediments, reducing toxicity in our sediment treated tanks. We expect to see bioaccumulation of alpha-cypermethrin over the course of our 4 week (4 exposure time point) study. Results from these studies indicated that ITNs are highly toxic to invertebrates and vertebrates and can lead to sub-lethal alterations following exposure.

2.04.P-Th-027 Chronic Atorvastatin Exposure Increases the Intermolt Duration of Juvenile Red Swamp Crayfish (*Procambarus clarkii*)

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Atorvastatin is one of the most prescribed pharmaceuticals in the United States. As a statin, it lowers cholesterol in humans by competitively inhibiting hydroxymethylglutaryl-CoA reductase in the mevalonate pathway. The mevalonate pathway is also integral to arthropod development, since it is the primary biochemical pathway to produce juvenile hormones that aid in growth and reproduction. So far, atorvastatin has been detected in the environment in the parts per billion range and may pose a threat to aquatic invertebrates. Crayfishes (Astacoidea and Parastacoidea) are a large group of aquatic benthic invertebrates that can occupy several niches and function as keystone species in many environments, and this group may be sensitive to atorvastatin pollution. In this study, juvenile red swamp crayfish (*Procambarus clarkii*) were used as a model organism in a controlled lab environment to assess the sublethal, chronic effects of atorvastatin on molting. Crayfish were reared and exposed to the contaminant for one full molt cycle after a two-week acclimation period. Out of the four treatment (0.15ug/L, 1.5ug/L, 15ug/L, and 100ug/L), solvent control, and control groups, the highest dosed group (100ug/L) showed a significant increase in intermolt duration from all other treatments (ANOVA: F = 8.036, p = 3.02 x 10⁻⁰⁵), averaging 91.875 days (max 120 days, min 76 days) compared to an average of 60.806 days for all other groups combined (max 84 days, min 25 days). All other treatments were not statistically different from controls.

2.04.P-Th-028 Bioaccumulation of PFAS Compounds and Biological Responses in Aquatic Biota Exposed In Situ to Tertiary Treated Wastewater Effluent

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Wastewater treatment plant (WWTP) effluents discharged into aquatic receiving environments contain mixtures of contaminants, including perfluoroalkyl substances (PFAS), known to adversely affect aquatic life. This study investigated PFAS concentrations in river water and effluent, and bioaccumulation in the tissue of fish (Rainbow Trout (*Oncorhynchus mykiss*), Rainbow Darter (*Etheostoma caeruleum*)), and freshwater mussels (Flutedshell Mussel (*Lasmigona costata*)). Oxidative stress in trout, darters, and mussels, liver transcriptome changes in fish, and microbiome changes in the amphipod *Hyaella azteca* and darters were evaluated after *in situ* exposure to effluent from recently upgraded tertiary treated municipal WWTP. Invertebrates and fish were deployed in cages at an upstream reference site and two exposure locations downstream of a WWTP for two (fish and *Hyaella*) or four weeks (mussels). Targeted PFAS analysis was conducted in fish whole body homogenates, mussels (composite of gills, gonad and digestive gland), surface water collected from *in situ* locations, and in final effluent released from the WWTP. PFAS were detected in relatively low concentrations in biota from this region compared to other studies. In trout, the highest PFAS concentration was for PFOS (5.38 ng/g) while the highest concentrations in mussels was 7:3 FTCA (0.16 ng/g). PFAS concentrations in river water for C4 to C16 PFCA ranged from 17.65 to 25.85 ng/L. Maximum concentrations of PFAS in treated effluent were generally higher than those measured in river water, with the exception of PFUnDA, PFDoDA, PFTeDA, PFECCHS, FHxSA, FDSA, and 6:2 Cl-PFAES. There was limited impact of WWTP effluent on the gene expression of darter and trout liver tissues, with only few genes significantly up-, or down-regulated. Correspondingly, we did not detect any significantly enriched pathways in either organism in response to effluent exposure. Likewise, oxidative stress was not consistently induced in biota tissues and there were limited effluent effects on the gut content microbiome of darter and *Hyaella*. This study has implications for other WWTPs globally by demonstrating the value of treatment upgrades on reducing the impact on biota in the receiving environment.

2.04.T Effect of Environmental Pollutants to Aquatic Organism Health: Linking Molecular Effects to Apical Endpoints

2.04.T-01 Integrated ‘Omics to Connect to Higher Levels of Biological Organization

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New and rapidly evolving high-throughput techniques are becoming more available and affordable for characterizing the molecular-level effects of environmental stressors on aquatic organisms. Advances in ‘omic-based approaches provide useful tools for identifying potential targets of xenobiotics, with available *in silico* software commonly applied to develop hypothesis-driven research. Although these approaches are becoming more widely used and accepted in the field of toxicology, there remains a limitation in data analysis, interpretation, and links with higher levels of biological organization. Shortcomings in whole genome sequencing of target species, characterized metabolites, and annotated software for lipidomic analyses emphasizes the importance of anchoring molecular approaches to histological, physiological, and behavioral endpoints. This talk will address how ‘omic-based analyses are being incorporated into laboratory and field studies, the current gaps and limitations of ‘omic-based approaches, and how these tools can be used to compliment and guide the use of appropriate apical endpoints. This talk will also serve to introduce the session as a whole and provide a future outlook of how integrated approaches can be implemented to assess risk in the face of a changing climate.

2.04.T-02 Use of Molecular Tools and Behavioral Assessments to Understand The Developmental and Multigenerational Adverse Outcomes of Benzo[A]Pyrene Exposure

Kristie L. Willett and Cammi Thornton, University of Mississippi

Benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH), represents a class of ubiquitous environmental contaminants derived from the incomplete combustion. Exposures to PAHs are associated with developmental, reproductive, and multigenerational deficits, but linkages between the underlying molecular mechanisms and these apical endpoints have not been fully elucidated. Our experimental goal is to anchor BaP-mediated molecular endpoints with adverse outcomes in zebrafish using RNASeq, DNA methylation analyses, CYP19 morpholino and AhR null transgenic technologies. For example, following embryo-larval waterborne BaP exposure, the interaction of CYP19a1b and negative effects on growth, edemas and body shape was established with morpholino approaches. To assess the role of the aryl hydrocarbon receptor (Ahr), zebrafish (Ahr2^{+/+}, Ahr2^{+/-}, Ahr2^{-/-}) were exposed to DMSO (0.01%) or 100 µg/L BaP from 6 to 120 hours post-fertilization (hpf) and behavior and growth were assessed at 120 hpf and 3 weeks pf. BaP treatment caused significant hypoactivity in all Ahr2 genotypes at 120 hpf compared to their respective controls during the dark phase of the photomotor response assay, with the least activity in BaP-exposed Ahr2 null fish. Altered behavior did not persist to 3 wpf, however growth was significantly decreased at 3 wpf following BaP exposure. When we studied multigenerational responses and sex-dependence of preconceptional parental dietary exposure to BaP, significant hyperactivity was detected in F1 96 hpf fish resulting from both crosses with paternal BaP exposure. Gene expression and DNA methylation (RRBS) was measured in F0 gonads and 10 hpf F1 embryos resulting from each cross. F1 embryos from the BaP male x control female cross had the most differentially methylated regions and differentially expressed genes. Bioinformatic analysis highlighted impacts on genes associated with epigenetic processes and those critical during early development and provide new molecular pathways to mechanistically validate to further anchor BaP's various behavioral and developmental adverse outcomes. *Supported by NIEHS R21ES030154, R21ES019940, and R03ES018962.*

2.04.T-03 Molecular Insight into EDC-Induced Infertility in Aquatic Model Systems

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Developmental exposure to low, environmentally relevant levels of polycyclic aromatic hydrocarbons (PAHs) and other aryl hydrocarbon receptor (AhR) agonists can result in persistent disease outcomes in aquatic organisms with a lasting generational impact. Thus, it is crucial to characterize not only the extent and duration of such health effects, but also the underlying mechanisms through which the initial AhR ligand exposure perturbs the trajectory of development. We previously exposed juvenile zebrafish to sublethal levels (50 pg/ul) of the highly potent AhR ligand TCDD during sensitive windows of gonadal development and maturation (3 and 7 weeks post fertilization [wpf]), resulting in transgenerational outcomes of reduced fertility (F0-F2), as examined through reproductive assays and transcriptomic/methylomic analysis of gonadal tissue. Due to the highly heterogeneous cell- type and -stage landscape of the testes, we hypothesized the various cell types in the testes contribute markedly different profiles towards the pathology of TCDD exposure. To investigate the contributions of the diverse cell types in the adult zebrafish testes to TCDD-induced pathology, we turned to single-cell RNA-Seq and the 10x Genomics platform. The method successfully captured every stage of testicular germ cell development from spermatogonial stem cells to spermatozoa. We found that the testes of adult fish exposed during sexual differentiation to TCDD contained sharply decreased populations of late spermatocytes, spermatids and spermatozoa. Spermatogonia and early spermatocyte populations were, in contrast, enriched following exposure. Pathway analysis of differentially expressed genes supports previous findings that TCDD exposure resulted in male infertility, and suggested this outcome is due to apoptosis of spermatids and spermatozoa, even years after exposure cessation. Apoptosis of mid to late germ cells was confirmed by immunohistochemistry. This suite of disruption at the cellular and subcellular level provides support for the environmental explanation of idiopathic male infertility in aquatic organisms.

2.04.T-04 Sublethal Effects of Photo-Induced Crude Oil Toxicity to Early Life Stage (ELS) Red Drum (*Sciaenops Ocellatus*) Across Multiple Levels of Biological Organization

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Ultraviolet (UV) radiation from sunlight can significantly increase the toxicity of polycyclic aromatic hydrocarbons (PAHs) from crude oil in the aquatic environment through photo-induced toxicity. Increased mortality due to photo-induced PAH toxicity is well documented in early life stage (ELS) fish and invertebrate species. However, little is known about the sublethal impacts of photo-induced PAH toxicity. At the sub-organismal level, our previous research in ELS red drum (*Sciaenops ocellatus*) has shown that sublethal co-exposure to UV and crude oil can induce cell death in eye and skin tissue and can alter transcriptomic pathways relating to phototransduction, eye development, and dermatological disease. Because eye tissue and vision-associated pathways can be sensitive targets of photo-induced toxicity, the present study investigated the impact of sublethal co-exposure to UV and crude oil on visual acuity and visually-mediated prey capture and predator avoidance behaviors in larval red drum. Larval red drum (21 days post fertilization) were exposed to a low (<1 µg/L tPAH₅₀) concentration of crude oil in the presence or absence of UV. Visual acuity was assessed using an optomotor response (OMR) assay. Prey capture ability was assessed by determining the number of prey items (*Artemia* sp. nauplii) individuals could capture within 10 minutes. Predator avoidance behavior was assessed using a visual startle response assay. Across treatments, visual acuity, prey capture behavior, and predator avoidance were not significantly impacted by UV, oil, or their co-exposure. These results suggest that photo-induced PAH toxicity did not alter whole-organism behavioral responses at the oil concentration and larval life stage tested, despite the possibility of toxic effects at the cell and molecular levels. Assessing responses to contaminant exposure across multiple levels of biological organization is important for better understanding potential impacts to survival and ecological performance.

2.04.T-05 Linkage Between Mercury-Induced Epimutations and Transcriptomes in Zebrafish Brain and Retina Associated with Abnormal Neurobehavior

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Methylmercury (MeHg) is a ubiquitous environmental toxicant that is often detected in predatory fish and fish-consuming species, including humans. Developmental neurotoxicity yielding abnormal neurological phenotypes (e.g., hyperactivity, cognitive deficits, and visual impairment) in individuals via direct prenatal MeHg exposure has been well-established. We have previously demonstrated that direct developmental MeHg exposure of F0 generation zebrafish can promote hyperactivity and visual deficit in ancestrally exposed F2 generation zebrafish via epigenetic transgenerational inheritance. The objective of the current study was to investigate signatures of dysregulated gene expression and coding sequence-associated DMRs in the brain and retina of F2 generation zebrafish ancestrally exposed to MeHg (30 nm) compared to control. We have demonstrated linkage between CNS transcriptome changes (brain and retina) and neurobehavioral abnormalities. However, the mechanistic linkage between gene dysregulation and epimutations (i.e., differential DNA methylation regions [DMRs]) in transgenerational inheritance is unknown. We have demonstrated that the transgenerational transcriptome is not closely associated with coding sequence-associated DMRs induced by direct developmental MeHg exposure. This suggests that DMRs associated with other epigenetic mechanisms (e.g., long non-coding RNAs) may play a more significant role, increasing the mechanistic complexity.

2.04.T-06 Inhibition of Fin Regeneration in Fathead Minnow (*Pimephales promelas*) Following Exposure to the Synthetic Glucocorticoid, Fluticasone Propionate

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Fluticasone propionate has been previously established as both a potent glucocorticoid receptor agonist and is

commonly detected in effluents and surface waters. Prolonged use of pharmaceutical glucocorticoids in humans has been linked to apical outcomes such as osteoporosis, and impairment of bone growth. Unlike mammalian species, teleost fish naturally regenerate extremities following damage or amputation. Inhibition or delayed regeneration of fins could plausibly reduce an individual's ecological fitness, making affected fish less capable of obtaining food, avoiding predation, and/or migrating within their home range. In the current experiment, we examined whether glucocorticoid receptor agonism induced by fluticasone propionate elicited inhibition of fin regeneration. The caudal fins of juvenile (~30 days post-hatch) fathead minnows (*Pimephales promelas*) were amputated during brief anesthesia. Following recovery, fish were exposed to fluticasone propionate (0.037, 0.11, 0.33, 0.99, 2.96, 8.89, 26.7, and 80.0 µg/L) for 7 days. Measured endpoints included length, weight, and caudal fin area. Fin measurements were normalized to the length of each fish to account for differences in size. We observed fluticasone propionate to significantly impair fin regeneration at concentrations as low as 8.89 µg/L (n=6) compared to a Lake Superior water control group. Ongoing research aims to provide a weight of evidence for adverse outcome pathway 334 (AOP:334) – Glucocorticoid Receptor Activation Leads to Impaired Fin Regeneration – and elucidate the quantitative relationship between fluticasone propionate and glucocorticoid receptor agonism by evaluating mRNA endpoints associated with receptor activation and fin regeneration. *The contents of this abstract neither constitute nor necessarily reflect US EPA policy.*

2.05.P-Th Emerging Contaminants as Agents of Global Change: Prioritization of Whole Ecosystem and Multi-Stressor Research

2.05.P-Th-030 Comparison of Priority Substance Content in Effluents of Advanced Wastewater Treatment vs. Mechanical Treatment in Istanbul

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Urban wastewaters are the main anthropogenic point sources of micropollutants entering the environment, and the determination of the ones posing high risk to the environment is critical. Those showing bioaccumulative, toxic, persistent and ubiquitous presence in aquatic environments of Europe were identified as priority substances (PSs) (45 PSs in total) and their environmental quality standards have been defined by the European Union Water Framework Directive and was adopted by the Turkish Regulation on Surface Water Quality. This study examines the occurrences of PSs in the effluents of four advanced wastewater treatment plants (WWTPs) and three mechanical treatment facilities (MTFs) of the megacity of Istanbul. It consists of their year-long seasonal monitoring where samples were characterized by analyses of PSs as well as of their physicochemical parameters. Analyses on the organic PSs indicated that, the detection frequencies of di(2-ethylhexyl)-phthalate, fluoranthene, dioxin and dioxin-like compounds, trichloromethane were more than 80% in both WWTP and MTF effluents. Excluding some compounds, the concentration of group of PSs in MTF effluents were up to 12 times higher than their concentrations in WWTP effluents. The highest difference was found among total concentrations of polycyclic aromatic hydrocarbons. Potential risks related to discharge of organic PSs in WWTP and MTF effluents were estimated calculating their risk quotients (RQs). PSs with RQs above 1.0 were recommended to be monitored in the effluents of WWTPs and MTFs. The number of PSs recommended to be monitored in WWTPs and MTFs were surprisingly identical. The lower concentrations of PSs in the effluents of WWTPs showed the superiority of advanced biological treatment. Hence, to reduce the contamination by PSs entering the Sea of Marmara and the Black Sea, it is recommended to upgrade the mechanical treatment with biological treatment. At the same time, to treat PSs to a larger extend, currently available biological treatment process needs to be further improved.

2.05.P-Th-031 Temperature Mediation of PFAS Toxicity for Estuarine Fish in the Long Island Sound Watershed

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Per- and polyfluoroalkyl substances (PFAS) are a group of thousands of different chemicals characterized by at least one carbon containing two or more fluorine molecules. Introduced into manufacturing in the 1940s and 50s, PFAS are now ubiquitous environmental contaminants of global ecological concern. Publicly owned treatment works (POTWs) have been a focus areas for PFAS in the environment because POTWs effluents can be enriched relative to influents, though POTWs are not themselves direct sources of PFAS contamination. Along the Connecticut (CT) and New York coastlines, there are 44 POTWs discharging > 3 billion liters of wastewater effluent into the Long Island Sound (LIS) ecosystem each day. A recent study examining 12 POTWs near CT's shoreline estimates that 70–315 kg of perfluoroalkyl acids (a sub-class of PFAS) are loaded into the LIS every year via POTWs effluents. Previous research on PFAS in aquatic environments has overwhelmingly examined PFAS as singular stressors in freshwater ecosystems, but research on other chemical groups demonstrates that abiotic factors, such as temperature, can mediate organismal responses to contaminants. Climate change is predicted to warm the Long Island Sound by 0.45°C/decade. This coupled with the lack of methods to remediate PFAS contamination, underscores the need for multi-stressor research to address how increasing temperatures paired with chronic PFAS exposures will impact LIS organismal and ecosystem health. This research examines concentrations of PFAS in water and tissues of small-bodied estuarine fish collected during the summer of 2023 both upstream and downstream of four different POTWs proximate to the Long Island Sound. These data will be used to generate laboratory experiments exposing small-bodied fish to environmentally relevant PFAS concentrations under increased temperature scenarios to inform how fish fitness will be impacted. This work will directly generate knowledge to inform which sites are a high priority for remediation to mitigate anthropogenic stressors and enhance the health and sustainability of the Long Island Sound. *This presentation will provide an overview of the study design, research questions, and initial results.*

2.05.T Emerging Contaminants as Agents of Global Change: Prioritization of Whole Ecosystem and Multi-Stressor Research

2.05.T-01 Can Agrochemicals Have an Effect on Greenhouse Gas Production in Freshwater Ecosystems?

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Wetlands play an important role in the environment by providing various ecological benefits, such as capturing and storing carbon and regulating the cycling of greenhouse gases (GHGs). Agrochemical use may indirectly contribute to increased GHG production in freshwater ecosystems. Glyphosate and 2,4-D are the two most widely used herbicides, and are often combined into pesticide mixtures due to weed resistance. While the individual effects of glyphosate and 2,4-D on aquatic organisms are well documented, understanding their combined effects are challenging due to complex ecosystem interactions. Previous studies have shown both glyphosate and 2,4-D can affect microbial communities, which in turn disrupt biogeochemical processes, potentially leading to increased GHG emissions from wetlands. To investigate this further, we conducted a microcosm experiment using various concentrations and combinations of glyphosate and 2,4-D. We predicted that the two herbicides would have synergistic impacts on GHG production. The results demonstrated that herbicide treatment, particularly the mixture of glyphosate and 2,4-D, significantly increased the emission rates of methane (CH₄) and carbon dioxide (CO₂) compared to the control or individual herbicide treatments. Additionally, fluxes of CH₄, CO₂, and nitrous oxide significantly increased over time. However, only the cumulative flux of CO₂, representing the total amount of CO₂ emitted throughout the experiment, was

significantly affected by the herbicide treatments. Specifically, treatments with high concentration of glyphosate led to elevated CO₂ emissions. These findings indicate that glyphosate and 2,4-D runoff into wetlands can contribute to GHG production, which can have considerable implications for climate change. The results of this research suggest the need for policy and management strategies aimed at reducing agrochemical use to mitigate GHG emissions from wetlands.

2.05.T-02 The Effects of the Direct Discharge of Coalbed Methane Produced Water on the Headwater Streams of Colorado's Largest State Wildlife Area

Keli Kringel

The process of extraction of coalbed methane (CBM) brings massive quantities of water from coal formations to the surface. Disposal of this wastewater directly into nearby streams is permitted in some CBM basins. The CBM produced water effluent has elevated salinity and sodicity and can also contain heavy metals, trace elements, and organic compounds that are harmful to aquatic life. Aside from the chemical mixture, the discharge brings physical, physicochemical, and temporal changes to the ecosystem. Field studies assessing the ecological impacts of this discharge on the receiving aquatic ecosystems have been few and have yielded equivocal results. In a 30,000-acre State Wildlife Area in the Purgatoire River watershed of Las Animas County, Colorado, ten contaminated streams (small headwater streams comprised of CBM produced water) and six comparable reference streams (small natural intermittent streams) were sampled and analyzed for differences in macroinvertebrate community structure and water quality. Non-metric multidimensional scaling ordinations showed significant separation between the two stream types. The biodiversity metrics Taxa Richness, EPT Richness, and Shannon-Wiener Diversity were all significantly reduced in the produced water streams versus the natural streams. Stoneflies and oligochaetes were significantly reduced in both taxa richness and relative abundance in the produced water streams. Mayflies and caddisflies showed significantly decreased richness but unchanged relative abundance levels, due to certain tolerant taxa in the produced water flows. Based on their concentrations and published regulatory/safe levels, the water quality parameters of concern in the CBM produced water streams were alkalinity, conductivity, chloride, pH, fluoride, aluminum, iron, temperature, dissolved oxygen, ammonia, and the sodium adsorption ratio (SAR). The variables showing the strongest correlation to biodiversity and community composition were, surprisingly, calcium, SAR, and magnesium, with calcium appearing to have a protective effect on the communities. The surface discharge of CBM produced water is not regulated by EPA effluent guidelines, but this study shows that it has significant and possibly long-lasting effects on the small intermittent/ephemeral streams that receive it.

2.05.T-03 Potential Synergistic Effects of Nitrogen and Copper on Periphytic Algae Growth and Community Structure in an Alpine Lake of the Sierra Nevada Mountains, California

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Alpine mountain lakes are oligotrophic systems and are often considered to be 'pristine, reference' environments that are relatively unaffected by anthropogenic activities. However, these aquatic ecosystems can be exposed to nitrogen and copper contaminants through atmospheric drift and deposition. Furthermore, observations of increased algal growth and blooms in mountain lakes are increasing globally. Such observations have been made at lakes in the Tokopah Basin of Sequoia National Park, which are exposed atmospherically to agricultural contaminants from the Central Valley, CA. Recent research has identified that copper from pesticides is likely drifting to these ecosystems, which may have consequences for algal growth as a micronutrient. Despite this predicted risk, copper is poorly studied in these aquatic ecosystems. Given the known effects of nitrogen deposition on algal growth dynamics, we designed an in-situ nutrient-diffusing substrate (NDS) experiment to investigate the potential for nitrogen and copper to synergistically influence algal

growth and alter community structure. Using a full factorial NDS design, we allowed periphyton to grow on substrates placed in Pear Lake, which were exposed to a range of three and six environmentally relevant concentrations of nitrogen and copper, respectively. After 21 days, we collected samples for ash-free dry mass (AFDM), copper concentrations in periphyton, and community structure using 16S and 18S metabarcoding methods. Preliminary results indicate that copper and nitrogen had interactive effects on AFDM, copper concentrations in periphyton increased with exposure levels, and that initial exposure concentrations affected the composition of the periphyton community. The combination of an NDS experimental design with metabarcoding endpoints is a novel approach to understanding effects of agricultural contaminants on periphytic algal communities in remote alpine lakes. These results improve our understanding of (1) the effects of combinations of agricultural contaminants on geographically isolated ecosystems through downwind drift, rather than downstream runoff, (2) the importance of including micronutrients, such as copper, in addition to macronutrients (nitrogen and phosphorus) in studies of algal growth dynamics, and (3) the dynamics of algal growth and blooms in remote, oligotrophic aquatic ecosystems.

2.05.T-04 Effects of Nanocopper Antifouling Paint on Benthic Community Diversity

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Copper-based antifouling paints are used to prevent the settlement of aquatic sessile organisms. A primary concern of copper-based antifouling paints are the adverse effects to non-target aquatic organisms, particularly in sediment where copper accumulates after ablating from boat hulls. Recently, some manufacturers have adopted nanocopper as the active ingredient instead of cuprous oxide or cuprous thiocyanate. However, less is known about the effects of nanocopper paint on benthic communities than conventional copper antifouling paints. Therefore, the aim of this research was to evaluate the differences between nanocopper particles, nanocopper in a paint matrix, and dissolved copper (copper sulfate) on eukaryotic benthic community diversity with an environmental RNA metabarcoding approach. Using sediment cores collected from the Narrow River Estuary (Rhode Island, USA) as mesocosms, 3 concentrations of nanocopper paint dust (20, 120, and 240 mg Cu/kg sediment), nanocopper particles (120 mg Cu/kg sediment), copper sulfate (120 mg Cu/kg sediment), or paint dust without nanocopper (0 mg Cu/kg sediment) were added to the mesocosms via spiking a reference sediment. After a 14-day exposure, sediment was collected for RNA extraction and reverse transcribed to cDNA. Amplicon PCR was performed for two markers: cytochrome oxidase subunit 1 (COI) and 18S rRNA (18S). Libraries were sequenced on an Illumina MiSeq and bioinformatic analyses were conducted in QIIME2. Preliminary results show that copper sulfate had the greatest impact on benthic communities, resulting in the lowest species richness and evenness of any group. No significant differences were observed in species richness or evenness among any of the nanocopper paint dust treatments and the controls, although there was a trending decrease in richness and evenness in the high nanocopper paint dust group compared to lower concentrations. Several organisms comprised of ciliates, cercozoa, nematodes, and arthropods, were less abundant in the highest nanocopper paint dust treatment compared to controls. This investigation suggests that copper sulfate dissolves rapidly, exposing the benthic community to high levels of ionic copper. Paint dust and nanocopper particles, by design, release dissolved copper more slowly, resulting in less substantial impacts on the benthos. These results broaden our understanding of the adverse effects of different forms of copper and identification of taxa sensitive to nanocopper.

2.05.T-05 Effects of Nitrapyrin on Nitrogen Procession and Microbial Community Structure in Aquatic Microcosms

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Nitrapyrin is an additive applied to farmland to stabilize ammonia-nitrogen fertilizers by inhibiting microbial nitrification. Nitrapyrin is characterized as both a bactericide and a nitrification inhibitor that down-regulates

microbial enzymes and methanotrophic activity. Although nitrapyrin is widely used, its mode of action is not well-understood. But understanding is important because field-applied nitrapyrin has been documented to transport to streams, where it could alter microbial function and interrupt the natural nitrogen cycle by. To address this data gap, we exposed a nitrifier-enriched microbial community to environmentally relevant concentrations of nitrapyrin (nominal doses of 300 or 1000 ng/L) in the presence of ammonia and nitrate. Two experiments were conducted in 18 L glass jars; one for 72 hours with ammonium added only at the beginning; and the second for 96 hours, with ammonium added at the beginning and again after 48 hours. We monitored for total ammonium, nitrite, nitrate, total dissolved nitrogen, nitrapyrin, dissolved oxygen, pH, temperature, conductivity, and turbidity. Additionally, genetic sequencing was used to assess the microbial community following exposure to nitrapyrin. In both experiments, nitrapyrin substantially altered the microbial community structure and increased the relative abundance of heterotrophs. Unexpectedly, 1000 ng/L nitrapyrin enhanced ammonia loss in the first 48 hours and after the 48-hour ammonia addition, but without a concomitant rise in nitrite. Additionally nitrate in the jars with 1000 ng/L nitrapyrin was completely lost by 48 hours in both experiments. In the jars with 300 ng/L nitrapyrin, ammonia loss was also enhanced compared with controls, but was followed by nitrite and nitrate accumulation. Overall, addition of nitrapyrin did not stabilize ammonia and, in fact, nitrification was apparent at 300 ng/L nitrapyrin. The unexpected depletion of ammonia, nitrite, and nitrate at 1000 ng/L nitrapyrin is likely due to changes in the microbial community structure, possibly through increased heterotrophic nitrogen consumption in the time frame of the experiment. These experiments documented that nitrapyrin can change both microbial community structure and nitrogen dynamics in aquatic environments in complex ways that are not limited to inhibition of nitrification.

2.05.T-06 The Crayfish Mercury Project: A Community-Based Approach to Environmental Monitoring at the River Basin Scale

Tate Libunao and Alan S. Kolok, University of Idaho

Recently, there has been a push toward large scale environmental monitoring that is done at the magnitude of major river basins or entire countries. Such monitoring can be used to prioritize areas for subsequent contaminant remediation followed by ecosystem restoration. The challenge however, is that monitoring at this geographic scale would require a considerable amount of personnel and financial resources to acquire a sufficient sample size for an adequate environmental assessment. To address environmental monitoring at the river basin scale, we propose using a community-based participatory research approach featuring a charismatic sentinel species. Specifically, we are in the process of determining mercury contamination across the Columbia River Basin in the northwestern United States by evaluating tissue levels of mercury in crayfish. Tribal and non-tribal communities throughout the Columbia River Basin were identified and engaged with to assist in the collection of local crayfish. From 2021-2022, over 800 crayfish of three species have been captured across 30 unique sites throughout Idaho, Montana, Washington, and Oregon. Collectively, this effort has documented a fairly uniform body burden of mercury in crayfish tissue (roughly 100 µg/kg) across the basin with a relatively small number of sites having either significantly higher or lower mercury concentrations. While the development of a capable citizen-science network has been integral in conducting mercury reconnaissance efforts across the Columbia River Basin, further development of innovative sampling methods to streamline crowdsourcing will further expand our geographic reach and increase collection sites and sample sizes. This research to date is providing a case study on how community organizations can supplement professional sampling efforts to meet the growing demand of large-scale environmental monitoring.

2.06.P-Mo Exposure and Effects of Micro- and Nanoplastics in the Environment

2.06.P-Mo-028 Trends in Microplastics (MPs) Indoor Settling in the Winter and Summer Months

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Microplastics (MPs) have polluted various environmental media, including water, air, soil, food, snow, honey,

etc. Their presence has been considered a nuisance, with varying degrees of impact on public health and the environment. Airborne MPs have been shown to cause various health effects when inhaled. The most recent form of microplastic (MP) pollution that has attracted the attention of the global community is indoor air pollution. Indoor air pollution is of great concern because most modern human activities occur indoors, and urban dwellers have been shown to spend 90% or more of their time indoors. To date, few studies have investigated the presence and characteristics of MPs in indoor air and dust, especially those that extend over a long period. In the present study, the deposition of MP in dust fallout in three indoor environments (i.e., a dormitory, an office, and a laboratory) was tracked over six months during weekdays and weekends in 2021. Passive and active sampling methods were adopted from previous studies to collect MPs in indoor air and dust fallout in Qingdao. Additional sampling was conducted to determine the exchange of MPs between indoor and outdoor air during ventilation. The analysis showed that the three indoor environments were polluted with MPs at an average of 921 ± 806 MPs/m²/day. Seasonal variations in the abundance of MPs were also observed, with the average abundance in summer (1092 ± 993 MPs/m²/day) being higher than the average MP abundance (749 ± 514 MPs/m²/day) in winter months. Of the three sampling sites, the highest average MP abundance was observed in the dormitory (1719 ± 945 MPs/m²/day), followed by the office (587 ± 250 MPs/m²/day), and finally, the laboratory (456 ± 195 MPs/m²/day). Fibers (90%) and polyester were identified as the main shape and polymer type, respectively. The results of this study suggest that indoor environments are contaminated with MPs at higher concentrations, especially in homes, making indoor environments one of the main media through which people are exposed to MP. Policymakers can adopt the results of this study to initiate MPs' control via social campaigns, consumer movements, or regulatory actions.

2.06.P-Mo-029 Copper Adsorption to Microplastics and Natural Particles in Seawater: A Comparison of Kinetics, Isotherms, and Bioavailability

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The growing use of plastics has led to microplastics (MPs) being ubiquitously distributed in marine environments. Although previous studies have emphasized MPs as important metal-transport vectors, few have considered the differences between these anthropogenic particles and their coexisting natural counterparts in sequestering metals in seawater. Here, we compared Cu adsorption to pristine and naturally aged MPs (polystyrene and polyethylene) with that to algae particles and sediments and assessed the bioavailability of the adsorbed Cu by a gut juice extraction assay. Adsorption kinetics and isotherms consistently showed that natural particles bound far more Cu to their surfaces than MPs. The rougher surfaces, greater specific surface areas, and lower ζ -potentials of natural particles contributed to their stronger Cu adsorption capacity than pristine or aged MPs. Natural particles also contained more diverse functional groups for binding Cu, with oxygen-containing groups playing a dominant role. Adsorbed Cu on natural particles was less extractable by sipunculan gut juice than that on MPs, indicating their higher Cu affinity. Overall, our study suggests that natural particles outcompete MPs in carrying metals in the water column and transferring them to marine organisms in today's environmental context. This work provides new insights for assessing the risks of MPs in marine environments.

2.06.P-Mo-030 Toxicity of Aged and Non-Aged Polyethylene Microplastics Toward The Estuarine Bivalve *Scrobicularia plana*

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Human activities, and more particularly agricultural and horticultural activities, generate plastic pollution of soils, particularly by microplastics (MP). This pollution by MP is now ubiquitous and raises the question of its impact on the health of ecosystems in different compartments, in particular along the soil/aquatic continuum. The objective of this study is to assess the toxicity of polyethylene (PE) microplastics on a key organism of the

estuarine compartment, an environment at the soil/water interface. The estuarine bivalve, *Scrobicularia plana*, was exposed for 21 days to three environmental concentrations (0.008µg/L, 10µg/L, 100µg/L) of aged, non-aged or field-sampled macro-waste generated polyethylene microplastics (<400µm). The responses of organisms were analysed at different levels of biological organisation. Preliminary results show differences in DNA damage and burial behaviour of organisms depending on the conditions tested. Data collected in the present project combining soil and aquatic effects of MPs will help enlarge the database of the toxicity of MP generated since several years by BIOSSE laboratory that will be useful for future regulatory needs.

2.06.P-Mo-031 The First Volunteer Research of Distributions of Microplastics Greater Than 1µm in Global Oceans by a Japanese Giant Ship Company

Yutaka Kameda and Emiko Fujita, Chiba Institute of Technology

Microplastics (MPs) is recognised as one of the pollutants with a vast number of environmental impacts. There are lots of studies of MPs in the focused area of the ocean with different methods and different polymers and sizes. This is the first study of distribution of MPs greater than 20µm in global oceans with a harmonised method. We are investigating the numbers, sizes and polymer types of MPs greater than 20µm in the global oceans since March 2020. Sampling of MPs in the ocean was conducted by 14 volunteer ships with the cooperation of a Japanese shipping company. Two hundred samples were collected as of November 2022. Approximately 1 m³ of sea water samples at the depth of 3 - 14m were obtained from the hydrant of the ships. Particulate matters were collected by plankton nets (10 µm mesh) on site. The filtered samples were pretreated by H₂O₂ digestion and NaI density separation. The MPs polymers were characterized by a spectrum imaging method using micro-Fourier transform infrared spectroscopy. The observed concentrations of MPs ranged from 22 to 4,660pieces m⁻³ in the ocean. Their geometric mean value was 260 pieces m⁻³. Remarkably high concentrations higher than 1,000 pieces m⁻³ were found at Kuroshio Current, California Current, Kamchatka current and North Atlantic Gyre. These results indicated that distribution of MPs was heterogeneous in the oceans. The median diameter of MPs size distribution was approximately 60 µm. The dominant polymer is polyethylene(PE), polypropylene(PP), polyethylene terephthalate(PET) and polymethyl methacrylate(PMMA). PE and PP are the most produced plastics in the world and PE was the first produced plastic. PET was the most common fibrous polymer. PMMA was detected mostly in the Pacific Ocean. There were some PMMA hotspots at Kuroshio Current. Polymer types and shapes may be affected by land-based input, ocean currents and wave-driven. This project will continue several years later and can provide data on MPs abundance to further the understanding of MPs pollution in the ocean. This data will also be used to develop numerical models to predict MPs in the global ocean.

2.06.P-Mo-032 Effects of Microplastics Containing Additives on Blue Mussel (*Mytilus edulis*): Study of the Ageing and the Sorption of Polyaromatic Hydrocarbons as Influencing Factors

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The effects of microplastics at environmental levels have yet to be fully examined taking into account the factors of ageing and sorption of some ubiquitous environmental contaminants, such as polycyclic aromatic hydrocarbons (PAHs). Current hypotheses indicate that microplastics which have been degraded by ultra-violet (UV) radiation and/or sorbing PAHs could have a greater impact on the organism, for example by the transfer of additional contaminants to the organism. This study aimed to assess the influence of these factors on the sentinel species, *Mytilus edulis*, combining biochemical marker analyses and a lipidomic approach. The microplastics used in the experiment were polyethylene microplastic beads (MP). Then, some of them were irradiated for 1000 h in an accelerated ageing chamber (AMP). To simulate PAH sorption, raw microplastics and irradiated microplastics were added to a solution of 4 PAHs (MP-PAH and AMP-PAH respectively). The contamination of tanks was done with the 4 types of microplastics at 10 µg/L, with a treatment without

contamination (Control). Fifteen individuals were exposed for 8 days in 15-L tanks filled with artificial seawater per treatment and in triplicates. The potential of microplastics to induce neurotoxicity was determined by Acetylcholinesterase (AChE) measures. Superoxide dismutase (SOD), catalase (CAT), and glutathion S-transferase (GST) served to monitor oxidative stress. These biomarkers were measured on gills, digestive gland and mantle after dissection. Acid Phosphatase (AcP) was used as biomarkers for immunotoxicity and measured in hemolymph. Finally, lipidomic analyses were performed using flow injection analysis coupled to high resolution mass spectrometry (FIA-HRMS) on lipid extracts of digestive glands. All the experiments were performed from 5 individuals per tank, i.e., 15 par condition. The combination of artificial ageing and sorption of PAH factors for microplastic exposures showed effects on the blue mussel after 8 days of exposure at environmental doses (10 µg/L) with the detection of oxidative stress and neurotoxicity which were organ dependent. The mantle, not usually analyzed in ecotoxicological studies, seemed to be an interesting organ to monitor the biochemical effects of microplastics. None marked influence of either the ageing or the PAH sorption was highlighted through the lipidomics. Biochemical and lipidomic approaches are complementary tools in the environmental risk assessment.

2.06.P-Mo-033 The Application of Tannic Acid-Coated Magnetite Nanoparticles for Recovery of Microplastics from the Water System

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The presence of microplastics is rapidly increasing in millions of tonnes in the environment which is posing a huge threat to public health and ecosystems. Harmonized detection methods and microplastic remediation techniques are essential in monitoring the impact of microplastics on the environment. Therefore, this study aimed to apply the tannic acid-coated magnetite nanoparticles approach for the recovery of microplastics, Polystyrene (PS), and polyethylene terephthalate (PET), from water bodies. A facile method of microplastic recovery was established and the tannic acid-coated magnetite (TA-Fe₃O₄) was used for magnetic separation. Tannic acid, iron (II) sulfate heptahydrate, and iron (III) chloride hexahydrate were used to prepare adsorbent via a one-pot co-precipitation method. Then, this adsorbent material was characterized using SEM-EDS, XRD, TGA, FTIR, Raman spectroscopy, zeta potential, and BET techniques. This indicated that the developed adsorbent material is promising and successfully synthesized. The maximum adsorption efficiencies of PS 83% and PET 98% were recorded at the experimental condition of pH 6 -7, contact time 300 min, adsorbent dose, TA-Fe₃O₄, 5 g/L, and initial microplastics concentration of 1.25 g/L. The study also indicated that the effect of ions on microplastic recovery was found to be insignificant. However, in terms of microplastics recovered from the spent adsorbent, the maximum recovered microplastics were found at pH 3 - 4 in the acidic medium under the sonification condition. In conclusion, the tannic acid-coated magnetite is efficient and promising for the recovery of microplastics at an industrial scale. However, further investigation on the optimization of the method and extended application for multi-micropollutant performance should be expected.

2.06.P-Mo-034 Effects of Polylactic Acid (PLA) Microplastics on Reproduction, Antioxidant Defense Cellular Energy Production-Related Genes in *Daphnia magna*

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The concern for biodegradable microplastics (BMPs) is increasing as an alternative to conventional plastic, emphasizing the necessity for ecotoxicity investigations specifically focused on aquatic organisms exposed to BMPs. However, previous studies still focused on non-biodegradable microplastics (BMPs). This study aims to evaluate the toxicity of water fleas *Daphnia magna* (*D. magna*), towards BMP polylactic acid (PLA) and compare it with the impact of non-BMPs polyethylene terephthalate (PET). The acute toxicity of PLA MP fragments was similar compared to toxicity of PET MP fragments in *D. magna* ($p \geq 0.05$). Chronic exposure results showed that the 5mg L⁻¹ PLA MP fragments exert adverse reproductive toxicity (the survival rate, sex ratio, and deformed embryos) in *D. magna*. The survival rate of *D. magna* exposed to 5mg L⁻¹ of PLA MP fragments was significantly ($p < 0.05$) lower (52.38%) than those exposed to 5mg L⁻¹ of PET fragments

(85.71%), while those exposed to 1mg L⁻¹ was not significant ($p \geq 0.05$). Total offspring in *D. magna* exposed to PLA MP fragments was significantly decreased compared to control groups, and this effect was also similar to PET MP fragments. The rate of undeveloped embryos in *D. magna* exposed to 5 mg L⁻¹ of PLA (15.70% ± 20.71%) was higher than that of PET (12.64% ± 7.10%). The sex ratio of *D. magna* exposed to 5 mg L⁻¹ of PLA (40.90% ± 37.80%) was higher than that of PET (51.70% ± 39.21%). Furthermore, the reproduction (VTG), antioxidative defense (SOD) and cellular energy production (AK) related gene was significantly down-regulated in *D. magna* exposed to 5mg L⁻¹ PLA MP fragments. Overall, the results revealed similar toxicity (immobilization and reproduction) in individual levels of *D. magna* exposed to PLA and PET MP fragments, however, significant differences in molecular level during long-term exposure. These findings suggest that BMPs exposure to aquatic organisms can cause similar reproduction damage and adverse molecular effects compared to non-BMPs, which needs to be given sufficient attention for ecotoxicological risks with their usage. Collectively, the present study will advance our knowledge regarding the toxicity of BMPs on zooplankton, and builds a foundation for freshwater environmental studies on toxicity to BMPs.

2.06.P-Mo-035 Interstate Technology Regulatory Council (ITRC) Microplastics Outreach Toolkit

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The Interstate Technology and Regulatory Council (ITRC) is a state-led environmental coalition working to create innovative solutions and best management practices (BMPs) for the environmental sector. ITRC produces guidance documents and trainings that broaden and deepen technical knowledge and expedites quality regulatory decision making while protecting human health and the environment. ITRC is a program of the Environmental Research Institute of the States (ERIS), a 501(c)(3) organization incorporated in the District of Columbia and managed by the Environmental Council of the States (ECOS). ITRC represents all 50 states with membership from state, federal, tribal, and international agencies, as well as members from academia, the private sector, and the public. A recent ITRC activity was to develop a microplastics (MP) guidance document. The guidance introduces the topic of MP (Section 1), information on how they move and where they can be found in the environment (Section 2), sampling and analysis considerations (Section 3), information on human health and environmental effects (Section 4), a summary of current laws and regulations (Section 5), and technologies that can be used to abate and mitigate MP in the environment (Section 6). Additionally, there is a discussion regarding the current data gaps and recommendations for future research and regulatory actions (Section 7). Online trainings on the guidance document are provided quarterly through USEPA CLU-IN platform. In February 2023 the ITRC started to develop an outreach toolkit to provide resources for environmental professionals to use in communicating MP issues to lawmakers and members of the public. Here is a link to the Microplastics Guidance: <https://mp-1.itrcweb.org/>.

2.06.P-Mo-036 First Quantification of Nanoplastic Bioaccumulation and Tissue Distribution in fish at Sub-ppm Exposure Levels: The Role of Intrinsic Labelling

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Radiolabelled nanoparticles (NPs) have proven effective in overcoming limitations associated with quantifying carbon-based contaminants in environmental and biological media, particularly at low detection limits. Nuclear techniques such as quantitative whole-body autoradiography (QWBA) enable direct visualization of radiolabelled particle concentrations within organs. In this study, we synthesized ¹⁴C-labeled nanopolystyrene particles (nPS) with sizes of 20 and 250 nm, radiolabelling the carbon-backbone of the NPs. Rainbow trout were exposed to these particles, and their biokinetics and NP tissue distributions were measured using QWBA.

The objective was to compare the toxicokinetics and tissue distribution of nanopolystyrene at different dosing levels and exposure durations. Environmentally realistic nPS concentrations of 15 ppb and concentrations seventeen times higher (250 ppb) were used for low and high dosing, respectively. This allowed us to investigate whether Rainbow trout, a commercially important fish species, exhibited different uptake, absorption, and depuration patterns of NPs under different exposure conditions. Our findings revealed rapid accumulation of nPS within the digestive tract of fish, even at a concentration of 15 $\mu\text{g L}^{-1}$, while accumulation in internal tissues such as skeletal muscle (fillets). Toxicokinetics in tissues and faeces indicated differences between acute and short-chronic exposures, suggesting variations in accumulation and depuration. Upon transferring the fish to clean food pellets, the radioactive particles were rapidly depurated from the fish. QWBA results indicated the presence of nPS (albeit at minimal detection levels) in bones, eyes, skin, and kidneys, suggesting the potential for both sizes of nPS to enter the circulatory system. These findings highlight the advantages of employing radiolabelling techniques in the study of Contaminants of Emerging Concerns (CEC). Radiotracers contribute to a better understanding of the bioavailability, bioconcentration, bioaccumulation, biotransformation, and biomagnification of organic CEC, such as NPs, p-phenylenediamine (PPD) additives, Perfluoroalkyl and Polyfluoroalkyl Substances (PFAs), and Tributyl tin, in biota. The data generated using this approach is critical for informing risk assessments and developing policies pertaining to these chemicals.

2.06.P-Mo-037 Ingestion and Trophic Transfer of Microplastics through Aquatic to Terrestrial Organisms

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Microplastics are important emerging contaminants in marine, freshwater, and terrestrial environments. As microplastic pollution continue to increase, it is important to understand the occurrence, fate, and transport of microplastics in these environments. Although the trophic transfer of microplastics within ecosystems has been studied, empirical evidence of the trophic transfer of these pollutants across ecosystems is lacking in the literature. The present study aims to characterize a pathway of microplastic movement from aquatic to terrestrial ecosystems using *Tachycineta bicolor* (Tree Swallow) as a model terrestrial organism on the top trophic chain and aquatic insects as the lower trophic organisms. Analyses of microplastics in water, sediment, aquatic insects, and lower gastrointestinal tracts of *T. bicolor* nestlings from three locations along the St. Louis River in Duluth, MN, USA and three locations in Wisconsin, USA were conducted to investigate the possibility of microplastic movement from aquatic to terrestrial ecosystems. Plastic composition was identified with Fourier-Transform Infrared Spectroscopy (FTIR). Microplastics were found in sediment, water, aquatic insects, and the lower GI tracts of *T. bicolor* in all six sites. Additionally, a correlation analysis revealed that microplastics in *T. bicolor* nestlings are significantly correlated to microplastics concentrations in insects ($r=0.81$) and microplastics in insects were significantly correlated to microplastic concentrations in water ($r=0.92$) but not sediment ($r=0.18$). The results suggest that microplastics in the lower GI tract of *T. bicolor* nestlings were derived from aquatic insects - an indication of microplastic movement from aquatic to terrestrial ecosystems. Once ingested by birds, microplastics can be regionally transferred as birds migrate. The findings of the present study suggest that microplastics can be transferred in the natural ecosystem following a closed cycle of inland-aquatic-inland environment.

2.06.P-Mo-039 Employing spICP-MS to Measure Uptake, Distribution, and Toxicity of a Heterogenous Mixture of Nanoplastics in Zebrafish Embryo-Larvae

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Nanoplastics ($< 1 \mu\text{m}$) are potentially the most hazardous fraction of plastic litter in the environment. Unlike microplastics, nanoplastics have been shown to translocate across biological membranes and therefore pose an

environmental and human health risk. Although a number of studies have employed pristine mono-disperse spherical nanoplastic beads to assess toxicity, these studies rely on high dosage and qualitative assessments of nanoplastic uptake and distribution as it is difficult to quantify nanoparticle numbers and size distribution in tissue. To overcome this disadvantage, we have developed a method that combines synthesis of metal-tagged, polydisperse nanoplastics with single particle ICP-MS (spICP-MS). The presence of a small amount of metal (0.1-1%) enables the use of spICP-MS to assess size-dependent nanoplastic uptake and distribution in an organism. The high sensitivity of spICP-MS enables us to perform uptake and distribution experiments at environmentally-relevant concentrations that also reduce the chance of unwanted NP aggregation. The usefulness of our approach for toxicological studies was explored by assessing the uptake and distribution of tantalum tagged polymethyl methacrylate (Ta-PMMA) nanoplastics (100 nm-1 μ m) in the zebrafish (*Danio rerio*) embryo-larvae model. Dechorionated embryo-larval zebrafish were exposed to PMMA-Ta suspensions at 0.1 ppb, 10 ppb and 1 ppm in embryo water starting at 6-h post-fertilization (hpf). PMMA size uptake and distribution was measured at 24 hpf and 5 dpf through spICP-MS and oxidative stress was measured to assess dose-specific toxicity endpoints. Preliminary spICP-MS data has shown size dependent uptake of metal tagged PMMA in a 24 hpf zebrafish larvae. The results from this study will demonstrate utility of metal-tagged nanoplastics with subsequent spICP-MS analysis in nanoplastic toxicity research.

2.06.P-Mo-040 Sublethal Impacts of Field-Collected Microplastics with Biofilms, Perfluorooctane Sulfonate, and Suspended Sediments on Benthic Invertebrates

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As the body of literature grows around the impacts of microplastics (MP) on invertebrate species, observed responses range from none to mortality. Biological, chemical, and physical variables often associated with MP in the environment may have sublethal effects that indirectly influence the survival of benthic invertebrates. Research is needed on the combined effect of additional stressors and environmental variables and MP on organisms. Understanding sublethal effects can help give an indication of underlying mechanisms of MP toxicity. Feeding behavior is a major influence on MP uptake with indications that biofilms on MP increase consumption, therefore we evaluated MP accumulation, behavioral impacts, and oxidative stress biomarkers in three organisms representing different feeding types (filter-feeder, detritivores, and grazer). Microplastics for these studies were extracted from field-collected sediments in a river. For all studies we exposed invertebrates to 1.6 microplastics/ml in addition to suspended sediments, perfluorooctane sulfonate (PFOS), and the MP with and without biofilms. Species-tests were conducted for 7 days with culture water and sediment obtained from a reference site. The results from laboratory experiments reveal that MP can prolong burrowing time for detritivores and MP uptake is influenced by the MP concentration rather than the presence of biofilms on the plastics. Notably, grazers exhibit higher consumption of MP when biofilms are present compared to when they are absent. Detritivores also accumulate PFOS-laden MP at similar concentrations as treatments without PFOS, contrary to previous studies suggesting that PFOS reduces feeding activities in organisms. Lastly, suspended sediments had effects similar to MP. Overall, these results indicate that it is necessary to consider other dominant contaminants and environmental variables when studying the effects of MP on benthic invertebrates.

2.06.P-Mo-041 Comparative Toxicity of Different Micro- and Nanoplastics Obtained from Human Consumer Products on Human Cell-Based Models

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The pollution of micro- and nanoplastics has been a global concern. As global plastic production continues to increase, more plastic waste will be released into the environment and fragmented into small particles, called secondary micro- and nanoplastics, over time. However, most toxicology research with plastic particles used primary micro- and nanoplastics, such as plastic micro- and nanobeads, which is not environmentally relevant. This limits our ability to conduct ecological and health risk assessments to support the management of plastic

pollution. The present study aims to determine the potential effects of micro- and nanoplastics made from the physical beaking of human consumer products, such as plastic cups, forks, straws, and cloth fibers, on human cell lines using a cytotoxicity approach. The plastic particles were a mixture of micro- and nanoplastics of polystyrene, polyvinyl chloride, polypropylene, polyethylene, polyethylene terephthalate, and polyamide with a size distribution of $< 10\mu\text{m}$ for the 25% centile, $< 20\mu\text{m}$ for the 50%, and $< 90\mu\text{m}$ for the 90% centile. Human cell lines representative of three different tissue types, including colon (CaCo-2), liver (HepaRG), and endothelial cells (HMEC-1), were exposed to the plastic particle mixture for 24 hours. Results showed that HMEC-1 cells exhibited a significant decline in cell viability when exposed to shredded fork particles at concentrations $\geq 10\mu\text{g/mL}$. However, this particular cell line demonstrated a statistically significant increase in cell viability as the concentration of shredded cup particles increased, indicating a proliferative capacity specific to this type of particle. No significant change compared to the control was observed in CaCo-2 cells following exposure to either type of plastic particle. Additional sublethal effects of these two types of plastic particles and straw and fiber particles on the cell lines, such as determining oxidative stress levels and assessing mitochondrial dysfunction, are under investigation. Although data presented here are the results of the initial stage of the study, the effects are interesting and have implications for health risk assessment for micro- and nanoplastic exposure that have not been published in the literature, especially for human cell lines exposure to real plastic particles.

2.06.P-Mo-042 Fiber Length Influences Microplastic Ingestion by the Zooplankton *Daphnia magna* *Conner Simon and Risa Cohen, Georgia Southern University*

Surface waters receive megatons of plastic waste each year that ultimately accumulate in rivers, lakes, and oceans. Once in the environment, plastics degrade into microplastics in a variety of shapes including spheres, fragments, and fibers. Microplastic fibers (MFs) are most common, resulting from textile processing and laundering and maritime equipment degradation. Existing data suggest that MF exposure decreases zooplankton feeding and survival, which may adversely affect higher trophic levels that depend on planktonic food resources. It is unclear which characteristics are responsible for MF toxicity, but length may be an important factor because some lengths may be preferentially ingested while others may have high propensity to cause gut blockage. To determine whether length affects zooplankton MF ingestion rates and mortality, *Daphnia magna* were exposed to different lengths of a commonly observed MF polymer, polyethylene terephthalate, for 7 days in the laboratory. The experimental treatments were: 0 (control), 45, 70, or 100 μm lengths at a concentration of 50,000 MFs L^{-1} . Fiber lengths were selected to occur within the range of *D. magna* food particles. Mortality was assessed daily while the concentration of ingested MFs was measured only at the conclusion of the experiment. The proportion of zooplankton containing MF fibers was similar across treatments regardless of length. However, zooplankton consumed different amounts of each length according to the following order: 70 μm > 45 μm > 100 μm . There were no differences in mortality after 7 days across all treatments. This investigation demonstrated that zooplankton ingest MFs across a range of sizes and that fiber length influences the number of MFs consumed. Although survival was unaffected, long-term accumulation of MFs in zooplankton may lead to reduced feeding, growth, and reproduction with potential population-level effects.

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

2.06.T-01 Microplastic Fibers Enhance Copper Uptake in the Ribbed Marsh Mussel (*Geukensia demissa*) *Risa Cohen and Alina Tucker, Georgia Southern University*

Coastal ecosystems receive particulate and dissolved pollutants simultaneously via surface water transport. Microplastic fibers are particulate contaminants that upon ingestion by marine invertebrates cause internal abrasion, intestinal blockage, and decreased survival. In addition, microplastic fibers adsorb dissolved chemical contaminants such as copper to their surface. In trace amounts, copper is essential for metabolic processes, but in excess, it is toxic to marine organisms. Therefore, the possibility that microplastic fibers enhance copper

uptake is particularly problematic for filter feeding marine bivalves that ingest microplastic fibers from the water column. We hypothesized that microplastic fibers increase copper concentration in tissues of the ribbed marsh mussel (*Geukensia demissa*). Mussels were collected from a location with low microplastic fiber and copper concentrations and returned to the laboratory where they were exposed to one of four experimental treatments for 8 days: control (no addition), environmentally relevant copper (18 $\mu\text{g L}^{-1}$) or microplastic fiber (500 fibers L^{-1}) concentrations, and the combination of both copper and microplastic fibers. The concentration of microplastic fibers in mussel tissue increased in a similar manner when microplastic fibers were added, regardless of the presence of copper. In contrast, there was an interaction between microplastic fiber and copper treatments on tissue copper concentration, with the microplastic fiber plus copper treatment having the highest average tissue copper concentration. The findings from this study suggest that the presence of microplastic fibers increased mussel copper concentration, with the potential for adverse effects on bivalves and the organisms that rely on them for food resources in salt marsh ecosystems.

2.06.T-02 Developmental Phenotypic, Transcriptomic, and Multigenerational Consequences of Exposure to Nanoplastics in Zebrafish

Tracie R Baker and Danielle Meyer, University of Florida

Nanoplastics (NPs), or synthetic plastic particles $<1 \mu\text{m}$ in size, are likely ubiquitous in environmental waterbodies and drinking water sources. Our lab group has quantified micro- and nanoplastics in the environment, including remote areas of the Florida Everglades. While microplastics (MPs) have been extensively studied, the toxicity and associated health risks of large-scale human and animal exposure to NPs have not been well documented. Thus, larval zebrafish were utilized to investigate the health effects of developmental exposures to 50 and 200 nm polystyrene fluorescent NPs, specifically evaluating accumulation of plastics, behavioral response, and whole-body transcriptomic outcomes. From 6 to 120 h post-fertilization (hpf), zebrafish were exposed to a range of NPs (10-10,000 parts per billion), resulting in dose-dependent increases in NP accumulation. Exposure to higher concentrations of larger sized NPs (200 nm) altered larval swimming behavior, as evidenced by hyperactivity during dark periods. Notably, exposures did not impact mortality, hatching rate, or deformities; however, transcriptomic analysis suggests dysregulation of neuromotor pathways at both high and low concentrations. These fish were raised to adulthood and exhibited reproductive deficits and continued developmental phenotypes in the next generation. Results of this study suggest that NPs can accumulate in the tissues of larval zebrafish, alter their transcriptome, affect behavior and physiology, reproduction, and the next generation potentially decreasing organismal fitness in contaminated ecosystems. 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.

2.06.T-03 Alterations to Organismal Behavior due to Micro- and Nanoplastic Exposures

Andrew Barrick and Tham C. Hoang, Auburn University

Plastics are ubiquitous substances with approximately 42,000 tons entering the environment annually. Once entering the environment, plastics can be broken into microplastics ($1 \mu\text{m} < 5 \text{mm}$) and nanoplastics ($<1 \mu\text{m}$), which increases their potential for bioaccumulation and transfer through the food chain in the natural ecosystem. Since plastics are hydrophobic to many chemicals, especially organic pollutants, plastics can ab/adsorb pollutants in the aquatic environment. In addition, most plastics contain chemical additives that have been reported to be toxic to living organisms. Therefore, ingestion of micro, and nanoplastics are a potential vector for co-pollutants, enhancing their ecotoxicity towards aquatic organisms. Much of the ecotoxicological research has focused on plastics as particles with endpoints targeting bioaccumulation, subcellular and developmental effects, and mortality. The presence of plastics can alter the behavior of organisms through either neurotoxicological effects or changes to behavior to avoid contamination, which can lead structural changes in a population and ecosystem communities. At present micro- and nanoplastics and their relationship to neurotoxic additives, such as phthalates and phenols, is poorly understood. This presentation supplies a

synopsis of current research of how micro- and nanoplastics can influence the behavior of exposed organisms. The research focuses on three main categories: 1) changes in behavior due to plastic exposure, 2) changes in behavior due to exposure to chemical additives, and 3) alterations to behavior due to micro- and nanoplastics presence in the environment. The purpose of this presentation is to highlight behavioral research as an emerging research topic for micro- and nanoplastic research and provide perspectives on future research directions. As micro- and nanoplastic exposure studies are beginning to focus on smaller scale plastics, the presentation highlights the need for future research on zooplankton, such as copepods and daphnia, to fully understand environmental risks associated with micro- and nanoplastics.

2.06.T-04 Comparison of Species Sensitivity Distribution Methods for Risk Assessment of Microplastics

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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 scientific 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, there has been an increase in research and regulatory activity directed at supporting MP RAs that utilize the SSD approach. For example, in 2018 the state of California passed legislation requiring a comprehensive literature review to determine if a RA could be conducted for both human health (via drinking water) and aquatic organisms. California concluded there was insufficient data to conduct a formal RA for human health, but sufficient data were available to generate SSDs based on no observed effect concentrations (NOECs) or lowest observed effect concentrations (LOECs). The SSDs 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 first developed by USEPA's Office of Pesticide Programs to support ecological RAs of pesticides, and subsequently applied to site assessments of legacy contaminants (e.g., PCBs). This method produces a composite DRC and confidence interval that reflects the variability and uncertainty in the DRC for each study. 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 conducting RAs.

2.06.T-05 Role of Environmental Weathering on Micro- and Nano- Plastics Surface Reactivity:

Implications on Sorption of Legacy Pollutants and Bioaccumulation and Toxic in Fish Intestinal Cells

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Microplastics (MPs) and nanoplastics (NPs) are ubiquitous in the environment and are exposed to weathering conditions, including photodegradation, that may modify surface charge and reactivity, and release plastic impurities such as trace metals, additives or solvents. Moreover, MPs/NPs ability to act as vectors of legacy pollutants and modulate the bioavailability, bioaccumulation and toxicity of these contaminants has been highlighted in recent studies. Here, rainbow trout (*Onchorhynchus mykiss*) intestinal cells (RTgutGC) were exposed to high density polyethylene (HDPE) MPs (pristine and oxidized) and NPs, UV aged or not. Moreover, to assess the role of MPs/NPs on the bioaccumulation and toxicity of heavy metals and organic pollutants, cells

were exposed to MPs/NPs in presence or absence of cadmium and a mixture of lindane and DDE as a proof of concept. Particles were characterized by Dynamic Light Scattering, zeta potential, Attenuated Total Reflection – Infrared Spectroscopy (ATR-IR), and scanning electron microscopy/energy X-ray spectroscopy. Toxic effects of MPs and NPs at different concentrations (12.5-200 mg/L) in absence or presence of Cd (EC10= 449.64 µg/L) or of Lindane/DDE (2000 and 25 µg/L, respectively) were examined on RTgutGC cells using a multiple endpoint viability assay, simultaneously measuring metabolic activity and lysosomal/ cell membrane integrity. Moreover, bioaccumulation of legacy pollutants in RTgutGC cells was evaluated. Characterization demonstrated that all particles present an incipient stability (-8 to -23 mV), except for the particles coated with Cd that rapidly aggregated (-1 to -12 mV). ATR-IR analyses indicated oxidation and modification of the surface chemistry after exposure to UV radiation. SEM analysis showed that 6 weeks UV aging of the plastic particles notably altered its surface morphology, presenting more roughness visible in surface morphology. Data showed no toxicity on RTgutGC cells exposed to MPs/NPs with Cd or with the mixture of Lindane and DDE. Moreover, the presence of MPs/NPs (25 mg/L) on the exposure medium resulted in a reduction of cadmium and lindane/DDE bioavailability in RTgutGC cells. Overall, these studies indicated that photo degradation and oxidation increase surface reactivity and sorption capacity of HDPE MPs/NPs which resulted in a reduction of dissolved chemicals bioaccumulation and toxicity.

2.06.T-06 Unveiling the Fate of Nanoplastics: Coupling Accelerator Mass Spectrometry and Radiolabeling for Ultra-Sensitive Toxicokinetic Analysis at ppt Levels

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Radiolabelling of nanoparticles (NPs) has emerged as a powerful technique to overcome challenges associated with quantifying carbon-based contaminants in environmental and biological samples. Traditional nuclear techniques face limitations in detecting low concentrations but radiolabelling has enabled exposure experiments at the upper range of predicted concentrations, such as 15 µg L⁻¹. However, the natural background of ¹⁴C restricts radiation-based measurements to sub-ppb exposure levels, impeding risk assessment of current wildlife concentrations. To address this challenge, we employed Accelerator Mass Spectrometry (AMS) for precise ¹⁴C mass analysis, enabling the first exposure experiments at a realistic predicted concentration of 800 ppt. In this study, we synthesized ¹⁴C-labeled nanopolystyrene particles ([¹⁴C]nPS) with sizes of 20 nm and 250 nm, effectively radiolabelling the carbon-backbone of the NPs. Mussels (*Mytilus edulis*), a commercially important bivalve species, were exposed to these particles at a concentration of approximately 800 ppt for 48 hours. Our investigation aimed to determine whether mussels exhibited different uptake, absorption, and depuration patterns for NPs under various exposure conditions. Results revealed rapid accumulation of nPS in mussels, with notable differences between the two particle sizes. The smaller particles exhibited more rapid uptake, while the larger particles displayed slower depuration. The bioaccumulation model indicated that 95% of the 20 nm particles were absorbed within 7 hours, while the 250 nm particles reached the same level within 2 hours. Depuration occurred in two distinct steps, with 95% of the compartments being depurated after 5 days for 20 nm particles and 15 days for 250 nm particles. In the slower second depuration step, it would take approximately 600 days for both particle sizes to depurate 95% of the compartments in clean, nanoplastic-free water. These findings underscore the persistent accumulation of nanoplastics, even at an exposure concentration of 800 ppt. This innovative approach, combining radiolabelling and AMS, represents a significant breakthrough in the risk assessment of plastic pollution. It enables precise quantification of nanoplastic toxicokinetics within a wide range of predicted concentrations, specifically at ppt levels. This novel methodology holds promise for informing regulatory frameworks surrounding emerging contaminants of concern.

2.06.V Exposure and Effects of Micro- and Nanoplastics in the Environment

2.06.V-003 A Bioassay-Based Assessment of The Potential Ecological Risks of Microplastics in the Diep River (Milnerton), Western Cape, South Africa

Asmat Begum Khan, Omoniyi Kolawole Pereao and Beatrice Opeolu, Cape Peninsula University of Technology

Plastic pollution is a major global issue which poses serious environmental and human health risks. Rivers may serve as a pathway for microplastic (MP) litter. The Diep River (DR) runs through the City of Cape Town via different land use types into the ocean. In this study, microplastics burden in the Diep River was assessed with the use of biotests. This study assessed the impacts of microplastics on river water quality. Surface water samples were analysed for physicochemical parameters, and ecotoxicological tests using 3 organisms that represent different trophic levels - *Raphidocelis subcapitata* (microalgae), *Daphnia magna* (crustacean), and *Tetrahymena thermophila* (protozoan), both in the absence and presence of microplastic standards. For microalgae, an algal density measurement of 1×10^6 cell/mL was prepared from the concentrated algal inoculum, added to samples, incubated for 72 hours, and an optical density measurement was then taken. For crustaceans, young daphnids were pre-fed before the experiment, and transferred into test wells which were covered and incubated in darkness at 20°C for 48 hours. The test plate was scored to determine the amount of immobilised daphnids. For protozoan, a ciliate inoculum and food suspension was prepared, added to samples, incubated in darkness at 30 °C for 24 hours, and an optical density measurement of the turbidity was taken. The percentage effect (PE) was determined for the 3 organisms. Growth inhibition was observed for *R. subcapitata* and *D. magna* at all sites with the addition of microplastic standards. Three sites - DR 2, DR 3 and DR 5 had higher PE values for *R. subcapitata* relative to samples without virgin microplastics. The PE values for samples with microplastics were DR 2(25.0%), DR 3(13.0%) and DR 5(21.0%); corresponding values for samples without microplastics were DR 2(17.3%), DR 3(10.0%) and DR 5(13.2%) respectively. All 5 sites in the presence of microplastics; DR 1 (25%), DR 2 (30%), DR 3 (30%), DR 4 (25%) and DR 5 (25%), showed *D. magna* PE increase of $\geq 100\%$ compared to samples with no microplastics (DR 1(0%), DR 2(15%), DR 3(0%), DR 4(5%) and DR 5(0%). This study provides insights into possible policy development, policy implementation and management strategies for the Diep River.

2.07.P-Mo Fate and Effects of PFAS in Coastal Ecosystems

2.07.P-Mo-044 Biomonitoring of Emerging PFAS in Wildlife and Domestic Animals in North Carolina

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Over the last decade, applications of non-target analysis (NTA) for environmental monitoring have detected dozens of novel PFAS in the Cape Fear Region (CFR) of North Carolina including GenX (HFPO-DA), Nafion byproduct 2 (BP2), and perfluoroether carboxylic acids with four (PFO4DA) or five (PFO5DA) ether linkages. Many of these compounds were first identified in surface water and then subsequently detected in biota. Detection of these novel PFAS in humans prompted the first toxicological investigations, which identified significant adverse health effects at elevated exposure levels. However, three findings from CFR monitoring have led our lab to reanalyze banked blood and tissue samples for additional novel PFAS. First, although PFO5DA was nearly below the method reporting limit in previously investigated surface water, the compound was determined to have one of the highest bioaccumulation rates in a recent bioaccumulation study.¹ Second, the same bioaccumulation study identified the novel PFAS Nafion by-product 6 (BP6) in both surface water and dosed rodent tissues. Lastly, a reanalysis of wildlife NTA data revealed suspect peaks of three novel PFAS that showed evidence of belonging to the PFO5DA homologous series (PFO6, PFO7 and PFO8) but have not been

observed in surface water. As a result, our lab sought to determine concentrations of these additional novel PFAS by reanalyzing banked wildlife and domestic animal blood and tissue samples from the CFR (horse, dog, alligator, fish, and seabirds). Both Nafion BP6 and PFO6 were quantitated using matching available standards while concentrations of PFO7 and PFO8 were estimated using semi-quantitative NTA methodologies. Results show that none of the four PFAS were observed above method reporting limits in horse and dog serum; however, PFO6, PFO7, and PFO8 were observed at varying frequencies and concentrations in alligator, fish, and seabird tissues from the region. These results show that PFAS found in surface water near or below method detection limits may be a concern for bioaccumulation in biota. Further investigations into the toxicological impacts of these new perfluoroethers are warranted.

2.07.P-Mo-045 Partitioning, Bioaccumulation and Bioavailability Evaluation Using Passive Samplers of Sediment-spiked PFAS Aged for up to Eight Weeks

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Sediment has been considered a relevant source of per- and polyfluoroalkyl substances (PFAS) bioaccumulation to aquatic invertebrates and a source of PFAS to higher trophic levels, including humans. However, the effect of aging on bioavailability and the relative contribution of the sediment and aqueous phases for the benthic bioaccumulation of sediment spiked PFAS is largely unknown, particularly for estuarine and marine sediments. To address this knowledge gap, estuarine sediment from a relatively pristine site was spiked via coated sand (solvent-free) with a mixture of PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFOS, PFDS, 8:2 FTS, and 6:2 FTS at a target concentration of 5 mg/kg dw for each chemical. The spiked sediment was aged for 1, 7, 14, 28 and 56 d at room temperature. After each aging period, spiked sediment and clean overlying water were added to 1 L beakers, followed by one each of integrative and equilibrium passive samplers and 50 adult amphipods (*Leptocheirus plumulosus*). Exposures lasted 14 days, during which overlying water was not exchanged. At exposure termination, PFAS concentrations were measured in whole sediment, porewater, overlying water, passive samplers, and amphipods. Chemical analysis is ongoing. Measured porewater concentrations will be compared with passive-sampler-predicted concentrations and the distribution of PFAS in sediment particles, porewater and overlying water will provide insight for exposure pathways relevant to each PFAS investigated. Preliminary results indicate that 100% of the PFHxA and over 60% of the PFOA and PFHxS remains associated with the aqueous phase of the exposure system. Results from this experiment will be used to optimize analyte-specific exposure approaches for investigating PFAS toxicity and bioaccumulation to benthic invertebrates.

2.07.P-Mo-046 Bioaccumulation and Toxicity of Field-collected PFAS-Impacted Sediment to *Leptocheirus plumulosus* and *Chironomus dilutus*

Paige M Krupa, Guilherme R. Lotufo, David W. Moore and Ashley N Kimble, U.S. Army Engineer Research and Development Center

Per- and polyfluoroalkyl substances (PFAS) are currently the focus of much attention due to their ubiquitous occurrence, environmental persistence, and implications to human and ecological health. However, data gaps exist regarding the fate and effects of these substances in aquatic environments, particularly for estuarine and marine systems. Most aquatic toxicity and bioaccumulation studies to date focus on water-column exposures, with less data available for sediment-based exposures. Furthermore, few studies describe effects of sediment-based PFAS at environmentally relevant concentrations. To address this knowledge gap, the current studied collected sediment from 16 estuarine and 10 freshwater sites suspected of having elevated levels of PFAS. Estuarine sediments were evaluated using 28-d bioaccumulation and 10-d toxicity tests with the amphipod *Leptocheirus plumulosus* while freshwater sediments used 20-d midge (*Chironomus dilutus*) bioaccumulation and toxicity tests. Though the sediment chemistry results indicated long-chain compounds

accounted for the majority of total PFAS in both estuarine and freshwater sediments, predominant analytes varied between sites. 6:2 FTS, 8:2 FTS, PFHpS and PFDoS were the analytes measured at the highest concentrations in estuarine sediment while preliminary results show high levels of NETFOSAA and PFOS in freshwater sediment samples. Bioaccumulation and toxicity test results will provide insight on the potential of these compounds to affect aquatic organisms.

2.07.P-Mo-048 Evaluating the Influence of PFAS Structure on Behavior in Early Life Stage Mummichog (*Fundulus heteroclitus*)

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Per- and polyfluoroalkyl substances (PFAS) are widely used and long-lasting environmental contaminants that have been linked to adverse impacts in fish and other species. Previous work has shown neurobehavioral impacts of some PFAS, but further work is needed to understand the magnitude and underlying mechanisms of these effects, and the potential to incorporate them in predictive modeling efforts. Therefore, we exposed embryos of an ecologically important estuarine minnow, the mummichog (*Fundulus heteroclitus*), to three long-chain PFAS (perfluorooctanesulfonamide, FOSA; N-ethylperfluorooctanesulfonamide, N-EtFOSA; or perfluorononanoic acid, PFNA). Beginning at 1 day post fertilization (dpf), embryos were exposed to FOSA, N-EtFOSA, or PFNA for 6 days, then followed until 30 days post fertilization, with a multitude of biological endpoints collected (including survival, morphological phenotypes, and growth). At 24 dpf/10 days post hatch (dph), individuals were assessed in a light/dark behavior assay in which larvae experienced alternating 10-minute light/dark photoperiods over a period of 1 hour and 20 minutes. During this time, EthoVision software was used to track larval movement. Larvae exposed to FOSA showed significant behavioral differences in movement, velocity, and acceleration in comparison to control groups, especially in periods of darkness. In contrast, larvae exposed to N-EtFOSA showed significant behavioral differences in swimming distance and turn angle. Additionally, larvae exposed to PFNA exhibited abnormal behavior in acceleration rates during periods of both light and dark. The results from the light/dark behavior assays show that despite belonging to the same class of chemicals, the concentration and structural differences of compounds may induce different effects within organisms. For these reasons, the light/dark behavior assay is a useful tool in discovering potential visual, motor, and neurobehavioral effects of PFAS exposures. Results from behavioral assays can provide insights into the toxicity of PFAS and help inform efforts to incorporate sensitive sublethal effects into predictive toxicity models for fish populations.

2.07.P-Mo-050 Ecotoxicological Assessment of Multi-Stressors Perfluorooctanesulfonate (PFOS) and Temperature in Two Estuarine Fish Species

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Perfluorooctanesulfonate (PFOS), part of the PFAS chemical group, resists biodegradation due to its long fluorine-carbon chain, making it ideal for oil and water repellent products and fire-fighting foams. These characteristics also make PFOS detrimental to the environment as it bioaccumulates and biomagnifies. PFOS has been detected in South Carolina estuarine fish tissues, but information is lacking regarding potential impacts to estuarine fish health and survival. In addition, climate change and rising temperatures may alter both organism physiology and the bioavailability of chemical contaminants. This study is examining the effects of PFOS on two species of fish found abundantly in South Carolina estuaries, the red drum (*Sciaenops ocellatus*) and the sheepshead minnow (*Cyprinodon variegatus*), under different temperature conditions. Red drum, a recreationally important fishery in S.C., spend their early life stages in estuarine systems such as the Charleston Harbor, S.C., with optimal temperatures between 10°C to 20°C. Sheepshead minnows are used as a standard toxicity test organism. They are an ecologically important species, serving as prey for larger fish species (including red drum) and exhibiting a wide thermal tolerance (0-45 °C). The ongoing study is conducting acute

(96h) toxicity exposures with juvenile red drum and adult sheepshead minnows to determine the effects of PFOS under optimal and elevated temperatures. Fish survival (LC50 values), respiration rate, and endocrine response (cortisol and thyroid hormone levels) are being assessed using respirometry methods and LC MS-MS. These findings will offer insights into the mechanisms of PFOS toxicity on two estuarine fish species and may be applied to other fishes to different degrees. Knowledge of how an important fishery responds to the combination of elevated PFOS and temperature will be essential for creating conservation and management plans.

2.07.P-Mo-051 Evaluation of Ex-Situ Passive Samplers to Measure Bioavailable Per-/Polyfluoroalkyl Substance (PFAS) Concentrations in Marine Sediments

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Per-/polyfluoroalkyl substances (PFASs) are highly persistent organic compounds used in numerous consumer products. The pervasiveness of PFASs has raised concerns on a global scale. Contaminated sediments are a potential exposure pathway for aquatic organisms, and consequently to higher trophic predators, including humans. However, partitioning and bioavailability of PFASs in benthic systems is still poorly understood. Assessing the ecological consequences of sediment-associated PFASs requires a thorough understanding of the interactions between sediment and water quality, PFAS bioavailability, and resulting tissue concentrations in benthic species. Various factors impact PFAS bioavailability such as electrostatic interactions, organic carbon content of the sediment, salinity, PFAS chain length, etc. There is a critical need for low-cost sampler materials that can rapidly separate out and sequester diverse PFASs from complex environmental matrices with an affinity that is comparable to that of living tissues. Here, we will evaluate synthetic materials for their ability to select for the bioavailable fraction of sediment-associated PFASs and predict bioaccumulation by marine benthic biota, focusing on a marine polychaete, *Alitta Succinea*. Materials, including a surface-modified graphene monolith, will be used for *ex-situ* passive sediment sampling and evaluated for rapid characterization and quantitation of bioavailable PFASs from sediment-seawater slurries. The use of passive sampling devices avoids dealing with complex environmental samples and helps bypass complicated sample preparation and extensive cleanup steps which can result in loss of analyte, thereby providing a better environmental picture. *Ex-situ* passive sediment sampling will yield new information on the bioavailable fraction of PFASs in sediment and advance our ability to forecast tissue loads in benthic creatures.

2.07.P-Mo-052 Sublethal Impacts of Perfluorooctane Sulfonic Acid (PFOS) Exposure on *Acropora tenuis* Coral Larvae

Kaylie Anne Costa, *Camden G. Camacho* and *John A Bowden*, *University of Florida*

Per- and polyfluoroalkyl substances (PFAS) are an expanding group of man-made chemicals with emerging health concerns in humans and other species. Upon their creation in the 1940s, PFAS have been used in a variety of applications including but not limited to: fire-fighting foams, textiles, clothing, stain repellent, surfactants, food packaging, and cosmetics. The widespread use of PFAS has made them ubiquitous in the environment, with especially high levels in the marine environment. Drastic declines observed in coral reefs in response to changes in climate and other anthropogenic stressors, have hinged the survival of these ecosystems largely on the overall health and reproductive success of stony corals. There is no available published information on the impacts of PFAS on stony corals, despite repeated incidence of high PFAS levels in coastal areas near coral reefs worldwide. This presents a major research gap in coral ecotoxicology that requires further study. The purpose of this experiment was to characterize the impact of perfluorooctane sulfonic acid (PFOS) on *Acropora tenuis* larvae. Symbiotic and aposymbiotic *A. tenuis* larvae were exposed for 72 hours to varying levels of PFOS at the Tropical Biosphere Research Center in Okinawa, Japan. Exposure concentrations were verified by targeted liquid chromatography tandem mass spectrometry. At 24, 48, and 72 hours of exposure, the impact on larval symbiont acquisition and the larval lipidome (utilizing nontargeted LC-MS/MS) were

analyzed. Lipids are the main energy source for coral larvae; and therefore, represent a novel endpoint to assess larval health. At the conclusion of the 72-hour exposure, impacts on larval settlement were recorded. Understanding the impact of specific PFAS on the health of coral larvae will increase the knowledge of anthropogenic influence on coral reefs. The data gained from these experiments is of utmost importance not only for the future health of coral reefs, but also, to coral restoration practices. Coral restoration practices are increasing in abundance and size in many regions, in response to coral reef declines, with coral restoration projects now documented in over 56 countries. These projects mainly utilize branching species, such as *Acropora*, due to their rapid growth rate in comparison to branching corals. Therefore, understanding the impact of ubiquitous chemicals such as PFOS on corals will be necessary to maintain healthy reefs in Okinawa, Japan and worldwide.

2.07.P-Mo-053 Unravelling The Complexity Of Per- And Polyfluoroalkyl Substances (PFAS) Contamination In Marine Organisms

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Per- and polyfluoroalkyl substances (PFAS) are a family of thousands of synthetic organofluorine compounds of high environmental concern. Some of these compounds have proven to be persistent, mobile, bioaccumulative and able to biomagnify. The stability and mobility of PFAS in waters make the oceans the largest environmental reservoir of PFAS and as a result, marine ecosystems are widely contaminated with these compounds. The combined evidence of their ubiquitous distribution and toxic properties has led to the ban of some of them (e.g., perfluorooctanesulfonic acid and long chain perfluorocarboxylic acids called PFOS and PFCA respectively). However, many alternative compounds and precursors of unknown environmental fate or effect on living organisms remain in use. By applying a non-specific method for PFAS analysis in biota (the Total Oxidizable Precursor or TOP assay), we evidenced that unknown PFCA precursors were by far the major contributors to the total PFAS burden of bivalves of the French coasts. Thus, beyond the ~20 PFAS traditionally studied and monitored in the environment, a large number of PFAS are overlooked. In this context, there is now a crucial need to identify the compounds responsible for this contamination. It is also necessary to further our knowledge of their environmental behavior, including bioaccumulation potential and toxicokinetics. The objective of this work is to go beyond the traditionally investigated PFAS in order to obtain a more exhaustive vision of the contamination of French coastlines by PFAS. Our specific objectives are i) to provide a definitive identification of these substances using a non-targeted approach, ii) to determine if they are accumulated by marine organisms from contrasting ecosystems, such as bivalves of the French coasts from a biomonitoring program, organisms from sea bass and sole trophic networks of the Seine estuary, and top predators such as marine mammals from the Bay of Biscay. Finally, the last objective is iii), to assess the biomagnification capacity of newly identified compounds from zooplankton to sea bass and sole. This presentation describes both the samples we investigate, and the methods used to achieve the aforementioned objectives. A detailed example of the annotation of putative PFAS found in real samples is also provided.

2.07.P-Mo-054 PFAS Exposure in Orca (*Orcinus orca*) Food Sources

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Orcas, also known as killer whales, are top predators in the ocean and have come to be a symbol for the Puget Sound in Northwest America. However, a recent study found that orca samples collected from stranded/injured individuals along the coast of British Columbia had high levels of PFAS in their liver and skeletal muscle. To further understand possible routes of PFAS exposure to these orcas, we analyzed PFAS levels in Chinook and Coho salmon from the Puget Sound region, which are a part of the orca food chain. Since fishing restrictions limit the number of samples that can be collected in a given year (Chinook Salmon in the Puget Sound are listed as endangered, and Coho salmon on the Oregon coast are listed as threatened under the Endangered Species

Act), we have also included Chinook homogenates collected in 2016 by the Washington Department of Fish and Wildlife. To gain insight into possible PFAS exposure routes for the salmon, we also analyzed PFAS levels in Atlantic Herring, which is a food source for Chinook and Coho salmon. Twenty herring samples caught in the winter of 2021-2022 in the Puget Sound by the Washington Department of Fish and Wildlife were analyzed. PFAS in fish tissues were extracted using an amended version of the Second Draft EPA Method 1633, and analyzed with LC-MS/MS. Additionally, stable nitrogen isotopes were measured in a subset of samples to give insight on the trophic level of the fish in our study. Preliminary results indicate that concentrations of most of the detected PFAS were about 1 ng/g dry weight. PFHxDA (perfluorohexadecanoic acid), PFOS, and GenX were frequently detected in samples. These results will be compared with data from fish samples from the Great Lakes where median PFOS levels in fish filet, gut, egg, and liver was 4.5, 16, 23, and 38 ng/g wet weight, respectively. This study will contribute to the body of scientific knowledge on not only orcas, but also the contamination status of their food source in the Puget Sound.

2.07.P-Mo-055 Assessment of the Bioaccumulation and the Effects of Per- and Polyfluoroalkyl Substances (PFASs) on Blue Mussel (*Mytilus edulis*): A Biochemical and Lipidomic Approach

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The increasing popularity of per- and polyfluoroalkyl substances (PFASs) in industrial and commercial applications has led to the release of several fluorinated organic compounds into aquatic systems. Environmental toxicity due to potential bioaccumulation, high persistence, and ubiquity is a concern with the PFAS releases. To assess the bioaccumulation, biochemical and molecular responses of marine mussels (*Mytilus edulis*) due to PFAS exposures, they were exposed to spiked food with a mixture of 24 homologs at two concentrations: C1 (625 ng/ g dw) and C2 (6250 ng/ g dw), in parallel of controls. Three 20-litre glass tanks per concentration and for the controls contained 20 mussels in artificial and aerated water changed every 2 days before feeding. The 24 PFASs were chosen to represent a wide variety of carbon chain lengths: 5 perfluoroalkyl sulfonates (PFASs, C4-C12), 14 perfluoroalkyl carboxylic acids (PFCAs, C4-C18), 5 precursors (C8-C12). Dietary exposure was carried out for 8 days and followed by 5 days of a depuration period. The PFAS bioaccumulation kinetics were determined in the whole individuals sampled every 2 days. The measure of a battery of biomarkers was carried out in 3 different organs of the mussels (gills, digestive gland and mantle) after exposure, depuration and dissection. Lipidomic analyzes were performed using flow injection coupled to high resolution mass spectrometry on lipid extracts from the mussel digestive glands after exposure. The bioaccumulation pattern of PFASs highlighted increasing levels in organisms with increasing exposure concentration and their carbon chain length. The results expressed oxidative stress with elevated superoxide dismutase (SOD) activity in digestive glands at both exposure concentrations compared to controls. Glutathione S-transferase (GST) activity was significantly increased in the digestive glands of mussels exposed to C1. After the 5-day depuration period following the 8-day exposure, the oxidative response had been recovered for SOD and GST activities. Regarding the catalase activity, no significant difference was observed between the exposed and control mussels after exposure, as well as after depuration. The same results were obtained for the neurotoxicity studied through the acetylcholinesterase activity in the 3 tissues studied.

2.07.P-Mo-056 Mixture Effects of PFOS and PFOA on Embryonic and Larval Sheepshead Minnows (*Cyprinodon variegatus*)

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Per- and polyfluoroalkyl substances (PFAS) are ubiquitous environmental contaminants originating from firefighting foams, cookware, and many other everyday products. Due to their amphiphobic properties and chemically inert carbon-fluorine bonds, PFAS have long environmental half-lives and are considered to be “forever chemicals”. Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are two PFAS

that are often found at high concentrations in aquatic environments. Both chemicals have previously been shown to be toxic to fish, causing both lethal and sublethal effects. Furthermore, additive and synergistic toxicity has also been observed in fish exposed to a mixture of the two chemicals. However, the majority of PFAS studies have utilized freshwater species and there is little information available on brackish and saltwater species. Salinity has been shown to influence the fate of PFAS. With the ocean being the terminal sink for PFAS, further studies of PFAS toxicity on saltwater species are warranted. Previous studies have assessed the toxicity of PFOS on sheepshead minnows (*Cyprinodon variegatus*), a brackish teleost, but information on other PFAS, as well as mixture toxicity, is lacking. In this study, embryonic and larval sheepshead minnows were exposed to PFOS, PFOA, and several mixtures of the two chemicals to assess for lethal and sublethal effects. PFOS was found to be acutely toxic to larvae, with a 96h LC50 of 1.94 mg/L. Embryonic and larval PFOA LC50 were >100 mg/L and embryonic PFOS LC50 was >10 mg/L. While the LC50 of 1:1 mixtures of PFOS and PFOA did not differ from the LC50 of PFOS only, PFOS mixtures with 100 mg/L PFOA resulted in a larval LC50 of 2.74 mg/L, suggesting a protective effect of PFOA. These observations are supported by significant reductions in malondialdehyde concentrations in 2 mg/L PFOS + 100 mg/L PFOA exposures relative to PFOS only groups. No significant differences were observed in glutathione concentrations, but a downwards trend was seen with increasing PFOS concentrations. These results indicate that PFOA may be providing a protective effect against PFOS by reducing lipid peroxidation. Future work will further explore the protective mechanism of PFOA and the role of nuclear receptors in PFAS toxicity.

2.07.P-Mo-058 Per- and Polyfluoroalkyl Substances (PFAS) in Aquaculture Feeds and Dietary Exposure to and from Aquaculture Fish

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Human exposure to PFAS has been linked to numerous health concerns, including immune disorders, developmental toxicity, and endocrine disruption. One route of human exposure is through the consumption of contaminated food, particularly seafood. Previous studies looking at PFAS in seafood have focused on wild-caught fish, but few studies have looked at PFAS in aquaculture. Aquaculture fish are typically fed diets high in marine protein and other agricultural products, which could be a possible route of PFAS exposure. Objectives of this study are to (1) quantify PFAS in commercial aquaculture feeds and test the application of a Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method to this new matrix, (2) quantify PFAS in fillets of aquaculture fish, and (3) model potential dietary exposure of PFAS to humans through consumption of aquaculture fish. Thirteen commercial feeds were selected and analyzed for PFAS concentrations. Next, an eight-week feeding trial on juvenile red drum (*Sciaenops ocellatus*) was conducted to assess the effects of different feeds and ration levels on PFAS accumulation in the tissue of fish. The trial consisted of 24 tanks divided into six groups. Two commercial feeds with varying PFAS concentrations and compounds were fed at three ration levels: 2% total body weight (BW), 4% BW, and 6% BW for the first four weeks, then 1% BW, 2% BW, and 3% BW for the last four weeks. At the conclusion of the trial, fillets were sampled and analyzed for thirty PFAS. Based on results, potential weekly dietary exposure of PFAS to humans who eat aquaculture fish was then calculated. The method worked well for the analysis of PFAS from commercial fish feeds. Of the thirty PFAS analyzed for, sixteen PFAS were detected across the thirteen commercial feeds tested. PFAS are present in commercial feeds at varying levels, with perfluoroalkyl sulfonic acid (PFOS) being detected in all thirteen feeds analyzed. We hypothesize that fish fed a higher PFAS diet and higher ration will exhibit greater PFAS accumulation in their fillets compared to fish fed lower PFAS diets and smaller rations and thus present a higher dietary source of PFAS. Results from this experiment will provide baseline data on dietary contribution of PFAS to aquaculture fish and will allow for informed consumption recommendations on aquaculture fish to better safeguard human health.

2.07.P-Mo-059 All PFAS and No Pearls: Preliminary Findings of Per- and Polyfluoroalkyl Acids in Great Bay Bivalves

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Per- and polyfluoroalkyl substances (PFAS) are chemicals of emerging global concern due to their adverse effects on human and environmental health. In New Hampshire's Great Bay, they are of particular concern because of PFAS-containing fire-fighting foams used at the former Pease Air Force Base and other emerging sources. In this study, seven sites around Great Bay were sampled to investigate PFAS concentrations across different abiotic and biotic media in the estuarine environment including water, sediments, and biota. We sampled five commonly collected bivalve species (*Mya arenaria*, *Mytilus edulis*, *Ostrea edulis*, *Crassostrea virginica*, *Ensis leei*) to evaluate risk from PFAS for recreational harvesters. Of the five compounds (PFBS, PFHxS, PFOS, PFOA, and PFNA) for which there are screening levels pertaining to shellfish, concentrations in all species fell below the screening threshold. The only exception was for PFOS in *Ensis leei*, the Atlantic jackknife or razor clam, for which concentrations were significantly higher than the other species and above the threshold. By understanding the PFAS compound profile in biotic versus abiotic matrices, we can better understand the uptake pathway of these contaminants from the environment into lower trophic level marine organisms. These results can better aid our understanding of bioaccumulation and biomagnification in marine systems to benefit future food web modeling and provide data for the state of New Hampshire's public health agencies and stakeholders.

2.07.P-Mo-060 Determining Transport of PFAS from Airfields and Urban Centers to the Near-Shore Marine Environment in Oahu, Hawaii

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Per- and polyfluoroalkyl substances (PFAS) have been shown to be present across the globe and have the potential for biomagnification in the food chain. PFAS detections are often correlated with fire-fighting foams at airfields, wastewater treatment plant (WWTP) effluents, and urbanization. The island of Oahu, Hawaii is home to multiple military bases and airfields. Many of these are located along the shoreline resulting in the potential for direct surface runoff into the ocean. Oahu has the highest population of the Hawaiian Islands with multiple WWTPs across the island. There is a large concern focused on the potential runoff of PFAS, especially from airfields, entering the nearshore environment posing a threat to sensitive coral habitats and overall aquatic life which could impact fisheries and eventually human exposure. Passive sampling devices can provide a measure of the exposure of aquatic organisms to bioavailable chemicals such as PFAS. The polar organic chemical integrative sampler (POCIS) were deployed at 15 near-shore sites surrounding Oahu between November of 2019 and May of 2020. These sites were situated near WWTP outfalls and areas of runoff from the several military airfields on the island. Extracts from the POCIS were analyzed using liquid chromatography/tandem mass spectrometry (LC/MS/MS) for a suite of 28 PFAS. Between 10 to 20 of the targeted PFAS were detected at each site (mean of 14). Six PFAS (PFHpA, PFOA, PFHxS, PFNA, PFOS, and PFDA) were detected at every site. PFHxS, PFOA, and PFOS were the PFAS generally measured at the highest levels across the sites. Total PFAS levels were generally highest near airfields followed by WWTP outfalls and urban areas and lowest around rural and undeveloped areas of the island.

2.07.P-Mo-061 National Mussel Watch Program: A National Perspective of PFAS Contamination in US Coastal Waters from 2015 – 2021

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The national Mussel Watch Program (MWP) chemical stressors survey in the nations' coastal waters measures a suite of over 600 chemical contaminants, including per- and polyfluoroalkyl substances (PFASs), in bivalves

along US coastlines on a regionally rotating monitoring schedule. Bivalve filter-feeders such as oysters and mussels are used as sentinel organisms for chemical contaminants because they bioaccumulate chemical compounds in their soft tissue to levels that reflect ambient concentrations. PFASs are a group of fluorine-containing compounds used in industrial processes related to surface protection/coatings, fire-fighting foam, and insecticides. In recent years, PFAS compounds have garnered increasing interest as potential ubiquitous and toxic chemical contaminants in aquatic environments. There are thousands of PFAS pollutants, but only a few are becoming more routinely monitored in the environment. The MWP measures PFAS compounds which are considered toxic and for which methodologies are well developed. This presentation illustrates the national perspective of the magnitude and distribution of PFAS survey data from the nations' coastal environments. From across the different coastal regions, the most frequently detected PFAS compounds were PFDODA, PFDS, PFHXA, PFHXS, PFNA, PFOA, PFOS, and PFOSA, with PFOS being the most ubiquitous across regions. PFOA and PFOS, in particular, are linked to serious health concerns such as liver damage, negative immunological effects, and cancer. Correlation analysis with land-use/percent impervious surface indicated that PFAS concentrations were found to be significantly higher at urbanized sites and with higher percent impervious surface. The MWP will continue to survey PFASs and other contaminants in US coastal environments to contextualize recent regional monitoring data in both time and space and to provide a perspective that may not be attainable at the local level. The program provides actionable information on "hot spots" of chemical stressors with the aim to trigger in-depth studies and mitigation through management actions at regional and local levels.

2.07.P-Mo-062 Prevalence of PFAS Compounds in Commercial Alaska Fish

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There are over 9,000 per- and polyfluoroalkyl substances or PFAS, which have been used in a wide range of commercial applications from stain-resistant coatings, to foams commonly used in firefighting exercises and incidents at airports. PFAS break down very slowly in the environment and as a result are now ubiquitous environmental contaminants with the potential to bioaccumulate. A number of PFAS have been linked with reproductive, developmental, and immunological effects, and at least two PFAS are probable carcinogens. NOAA's National Centers for Coastal Ocean Science (NCCOS), along with the Auke Bay Laboratories of NOAA's Alaska Fisheries Science Center, are working to assess PFAS in juvenile and adult pink and coho salmon in Alaska. A suite of 28 PFAS are being analyzed in an outgoing juvenile cohort and then in returning, mature adult pink and coho salmon associated with the same cohort sampled as juveniles. Over a 3-year span, tissues are being collected on site in Sashin Creek at the Little Port Walter Marine Research station (LPW) and Auke Creek Research Station (ACRS), and analyzed for PFAS. The goal of the project is to better understand the transport potential of these man-made chemicals across industrial complexes to near-pristine environments, assess their bioaccumulation potential in coastal and open ocean environments using commercial fish stocks, and elucidate biomagnification pathways through food chain processes. This work will provide resource managers with a clearer understanding of what lies ahead, so that mitigation policies can be developed and applied. Results from the analysis of the outgoing juvenile cohort during the first year indicated elevated levels of PFOS in some coho salmon from Sashin Creek, in some cases above established consumption guidelines. Samples from Year 2 of the project are currently being analyzed and the results will be presented.

2.07.P-Mo-064 Chronic Exposure of Larval Grass Shrimp (*Palaemon pugio*) to Four Per- and Polyfluoroalkyl Substances

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Per- and polyfluoroalkyl substances (PFAS) are a group of fabricated chemicals that includes such compounds

as perfluorooctane sulfonate (PFOS), perfluorohexanoic acid (PFHxA), perfluorohexane sulfonate (PFHxS), and perfluorobutanoic acid (PFBA). PFOS is no longer manufactured in the United States, but it is still produced internationally and can be imported into the United States in consumer goods. PFHxS has been used in non-stick and stain-resistant coatings, fire-fighting foams, and as a surfactant in industrial processes. PFHxA and PFBA are breakdown products of fluorinated products used in stain-resistant fabrics, paper food packaging, and carpets. The objective of this research is to characterize the effects of these four compounds on the larval grass shrimp life cycle. Grass shrimp (*Palaemon pugio*) play a major role in nutrient cycling, are prey for many commercially valuable fishes, and are often the dominant macrofauna in estuarine creeks. Any effects of contaminants on the sensitive larval stage can have repercussions on the grass shrimp population as a whole. The parameters measured, in addition to mortality, will include average days to postlarval status, number of molts to postlarval status, and growth. The research with these PFAS compounds will add much needed data to assess whether these contaminants pose a risk to estuarine organism health.

2.07.P-Mo-065 Exploring Coastal Foam as a Novel Exploratory Matrix for PFAS

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Per- and polyfluoroalkyl substances have become one of the most difficult anthropogenic contaminant classes to manage, in large part due to their extremely high mobility, ubiquitous presence and myriad of sources and their resistance to degradation. For routine surface water monitoring, hand grabs have become commonplace but are often limited by volumes that can pass through traditional solid-phase extraction. This consequence often makes detection of trace PFAS a challenge. It is well-known that some PFAS act like surfactants and aggregate near the water surface layer. In previous work in our laboratory, we have shown that when foaming landfill leachate, we can remove approximately 90% of the PFAS in the generated coalesced foam. Coastal foam (for saltwater locations) is commonly and abundantly observed in Florida. In this study, we investigated the presence of PFAS (by species and concentration) in coastal foam samples collected in Florida. In addition, we also collected freshwater foam to compare, as well as several foam samples outside of Florida (across locations within the US). Overall, we have 125 sites where we collected coastal/river/pond bank foam and a corresponding surface water sample to compare (collected near but away from the foam). Initially, the idea of examining foam was directed to looking at foam near areas with high PFAS sources (as foam has been observed near locations with AFFF release); however, eventually the project focused on looking at foam away from obvious sources to examine its usefulness regardless of site history. Interestingly, using a targeted LC-MS/MS workflow, we observed orders of magnitude higher concentrations in foam when compared to the surface water. Furthermore, the foam samples also exhibited an enhanced potential to identify new PFAS via nontargeted analysis (which were not realizable with the surface water samples).

2.07.P-Mo-066 Predicting PFAS Bioaccumulation in a Complex Assemblage of Freshwater Fish: Do We Need To Consider Fish Physiology and Ecology?

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Per- and polyfluoroalkyl substances (PFAS) are a diverse group of persistent anthropogenic chemicals, some of which can rapidly bioaccumulate in organisms. In aquatic animals, the two major exposure routes of PFAS are through diet and directly from water. However, predicting PFAS bioaccumulation across diverse organisms within a heterogenous ecosystem where PFAS vary across spatial and temporal scales remains challenging. Here, we adapted a previously published mechanistic food web PFAS bioaccumulation model to predict bioconcentration and bioaccumulation from diet of five PFAS in eleven co-occurring fish species. To evaluate model performance, we used empirically measured concentrations of PFAS in sediment, surface water, and collected fish tissues, all sampled from a currently (and historically) PFAS-contaminated stream. The tissue PFAS concentrations in fish predicted by the model successfully fell within a 2-fold variation from the empirically measured values in two seasons, Spring and Summer. However, the empirically measured variation

of PFAA bioconcentrations within and across species was consistently greater than predicted by the model. Therefore, we hypothesize that additional physiological and ecological properties of each species need to be considered to advance the development of robust predictive models at a whole ecosystem level. With further refinement, this model may be effectively implemented across ecosystems and used to predict concentrations in aquatic biota of selected PFAS from water and sediment samples only, without direct tissue sampling in fish. Moreover, further model development and vetting will improve our overall understanding of the many factors that influence PFAS bioaccumulation.

2.07.T Fate and Effects of PFAS in Coastal Ecosystems

2.07.T-01 Developmental Toxicity Across a Suite of Structurally Diverse Per- and Polyfluoroalkyl Substances (PFAS) in Mummichog (Atlantic Killifish)

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Per- and polyfluoroalkyl substances (PFAS) are increasingly at the forefront of environmental concern. Despite their frequent detection in environmental media, the highly persistent nature of many PFAS, and growing evidence demonstrating adverse health effects in a variety of species, toxicity data for marine and estuarine fish is severely lacking. A better understanding of organismal effects is needed to adequately assess the ecological hazards of PFAS, and ultimately to predict population-level effects. To address this knowledge gap, we conducted systematic developmental toxicity assessments for a structurally diverse suite of priority PFAS in mummichog (*Fundulus heteroclitus*, Atlantic killifish). The mummichog is a non-migratory, ecologically important estuarine fish that is amenable to the laboratory and enables evaluation of morphological and behavioral endpoints. Mummichog embryos were exposed to 11 PFAS spanning carboxylic acid, sulfonic acid, fluorotelomer sulfonic acid, and sulfonamide functional head group categories, with fluorinated chain lengths ranging from 5-8. Aqueous 6-day exposures (0, 1, 10, or 100 μM) were performed beginning at 1 day post fertilization (dpf), and a suite of developmental endpoints including morphology, heart rate, growth, swim bladder development, and light/dark behavior were assessed between 10-30 dpf. Within the tested concentrations, only the sulfonamide PFAS adversely affected overall survival; 100 μM perfluorohexane sulfonamide (PFHxSA) and perfluorooctane sulfonamide (PFOSA) exposures caused 79% and 100% mortality, respectively, compared to <20% in controls. PFHxSA also induced higher incidence of sublethal effects (i.e., abnormal body and head size, and minor cardiovascular effects). Other PFAS that elicited concentration-dependent abnormal phenotypes were perfluorohexanoic acid (PFHxA) and perfluorooctanoic acid (PFOA). These four compounds (i.e., PFHxSA, PFOSA, PFHxA, PFOA) and several other PFAS also induced abnormal larval behaviors in the light/dark assay. Preliminary analysis suggests that the functional head group of PFAS compounds is a primary driver of acute overt toxicity in mummichog, but PFAS across structural categories and chain lengths alter behavior. Ongoing analysis of more subtle, sublethal endpoints will further contribute to our understanding of PFAS bioactivity, facilitate prioritization of compounds for 'omics investigations, and inform population scale predictions of ecological impacts.

2.07.T-02 PFAS in Estuarine Fishes Collected Along the Atlantic Coast of Florida

Erin Pulster¹, Devon Firesinger² and Steven A. Murawski², (1)U.S. Geological Survey, (2)University of South Florida

Per- and polyfluoroalkyl substances (PFASs) are a class of more than 9,000 anthropogenic chemicals considered to be *forever chemicals* due to their environmental persistence. These chemicals have been widely used for a broad range of industrial and consumer applications. Extensive contamination of PFAS has been detected in all environmental media, including air, water, terrestrial wildlife, and various freshwater species. However, there is a data gap assessing levels of PFAS in estuarine and marine species and how these levels may

impact human health from consumption. Objectives of this study include (1) quantifying the concentration of PFAS in ecologically and economically important estuarine fishes collected along the Atlantic coast of Florida, and (2) characterize potential “hot spots” of PFAS concentrations by conducting site-specific assessments of contaminant levels. Fish samples ($n = 422$) consisting of 20 species were collected from four major estuaries and lagoons along the eastern seaboard. Samples include those species often targeted by anglers in this region (e.g., red drum, snapper spp., spotted seatrout, etc.). Fish were collected from the St. John’s River estuary, Indian River Lagoon, St. Lucie River Estuary and Lake Worth Lagoon. Forty target PFAS were analyzed in the edible tissues of fish using high-performance liquid chromatography tandem mass spectrometry (LC/MS/MS). Levels and compositional profiles are compared across species and estuary. This study is part of a larger effort to assess the need for specific estuary and lagoon fish consumption advisories in Florida.

2.07.T-03 Developing Bivalves as Biomonitorers of Per- and Polyfluoroalkyl Substances (PFAS) in Coastal Ecosystems

Shannon Jones, Ashley Pavia and Mi-Ling Li, University of Delaware

Per- and polyfluoroalkyl substances (PFAS) are a large group of chemicals used for manufacturing various commercial products. Nearshore ecosystems are particularly vulnerable to PFAS, due to the water solubility of PFAS and the high input of contaminated water. Bivalves have been used to indicate time-integrated exposure of other pollutants, but little is known about how they reflect PFAS contamination in the environment. Here, we assess the utility of bivalves as a tool to monitor environmental PFAS contamination. We collect two bivalve species (Eastern oyster and Ribbed mussel), water, and sediment from eleven locations along the shoreline of Delaware Bay, where prior research has detected widespread PFAS contamination in water, marine plankton, and fish. Our results show oysters and mussels collected at the same location exhibit distinct PFAS concentrations and compositions. This indicates that biological and ecological factors play a major role in varying PFAS accumulation between species. We find a high burden of PFAS precursors (i.e., Perfluorobutane sulfonamide, Perfluorohexane sulfonamide, and Perfluorooctane sulfonamide) in the Eastern oyster, which is likely driven by the active industries manufacturing and using PFAS in the region. The spatial pattern of total PFAS concentration in these oysters resembles that of water, implying the promise of oysters as biomonitorers for PFAS contamination in the coastal environment. We also investigate how biotic and abiotic factors (e.g., DOC, bivalve size) affect the relationship between PFAS in bivalves and their surrounding environment (i.e., water and sediment). The results of this study will help develop a quantitative method for utilizing bivalves as biomonitorers in coastal environments.

2.07.T-04 PFAS-Related Developmental Immunotoxicity using Marine Medaka (*Oryzias melastigma*) as a Model

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Both legacy and replacement per- and polyfluoroalkyl substances (PFAS) have been found in the blood of humans during the prenatal and postnatal developmental stages, and these PFAS exposures have been linked to a reduced vaccine response. While PFAS have been associated with changed immune function, little data is available on how replacement PFAS compare to their legacy counterparts at concentrations relevant to human blood exposure levels (0.5 ng/ml & 5 ng/ml) during critical windows of innate immune development which are hypothesized to be more vulnerable periods of immune development. Here marine medaka (*Oryzias melastigma*) is used as a model to evaluate PFAS-related developmental immunotoxicity during three major critical windows of immune development (7-11 days post fertilization (dpf), 3-5 days post hatching (dph), 12-19 dph). The exposure windows encompass key immune development milestones including lymphocyte progenitor cell migration and thymus colonization (7-11 dpf), medulla development in the thymus (3-5 dph), and establishment of immune competence of the thymus (12-19 dph). *O. melastigma* embryos and larvae were exposed to one of 6 different PFAS (PFOS, PFHxS, PFBS, PFOA, PFHxA, GenX) for the duration of the predefined critical window of immune development. Organismal level changes in immune competence were

then assessed using a *Edwardsiella piscicida* (5×10^7 cfu) host resistance assay. PFAS shown to impact immune competence at the organismal level were then used to assess transcriptome level changes after exposure. The 7-11 dpf critical window revealed lower survival after exposure to PFHxA (5 ng/ml), while critical window 3-5 dph revealed a trend for lower survival after PFOS, PFHxS, PFBS, PFOA, and GenX exposure at both low and high levels (0.5 & 5 ng/ml). Similarly, 12-19 dph critical window revealed significant reduction in survival after exposure to PFOS, PFHxS, PFBS, PFOA, and GenX at low and high concentrations. For transcriptome analysis of the 12-19 dph critical window, PFOS, PFHxS, PFBS, PFOA, and GenX were used in exposures at the low concentration (0.5 ng/ml). Whole larvae were pooled and used for Total-RNA sequencing. Pathways related to immune function are expected to be impacted in relation to PFAS exposure. The preliminary data indicate variability in PFAS development immunotoxicity which may be compound-specific and critical window-specific.

2.07.T-05 Toxicity of 10 Per- and Polyfluoroalkyl Substances (PFAS) to Five Standard Marine Species

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Per- and poly-fluoroalkyl substances (PFAS) are emerging contaminants of concern that 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) have been shown to be chronically toxic to aquatic organisms at observed concentrations in aquatic systems near PFAS sources, resulting in concerns over the ecological risk posed by these compounds. There is a paucity of effects data for marine aquatic life, limiting the assessment of ecological risks and challenges in developing 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) to five standard marine laboratory toxicity testing species, encompassing nine endpoints are being evaluated. These 10 PFAS compounds have been identified as important due to their presence at AFFF-impacted sites. 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 (*Strongylocentrotus purpuratus*), and 5) 48-h germination and growth for giant kelp (*Macrocystis pyrifera*). All species were tested using standard USEPA methods. Results from the initial range finding toxicity testing demonstrated observed toxicity typically above 1 mg/L PFAS concentrations, with *M. galloprovincialis* generally as the most sensitive species and *M. pyrifera* as the least sensitive. As expected, longer chained compounds were more toxic than shorter chain compounds and, when chain length was the same, perfluorosulfonic acids (PFSAs) were more toxic than perfluorocarboxylic acids (PFCAs) or fluorotelomer sulfonates. Results from completed definitive testing and 7 and 28-d bioaccumulation testing with *A. affinis* and *A. bahia* will be presented. Understanding the effects of PFAS exposure on marine ecological receptors will lead to better remediation outcomes at impacted sites.

2.07.T-06 Derivation of Marine Surface Water Criteria for Perfluorooctanesulfonic Acid (PFOS) and Perfluorooctanoic Acid (PFOA) for the Protection of Aquatic Life and Wildlife

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Aquatic ecotoxicity studies for per- and polyfluoroalkyl substances (PFAS) in estuarine and marine environments are underrepresented in the toxicology literature and are generally limited to a few PFAS compounds, primarily perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). As a result, marine aquatic life criteria are lacking relative to freshwater aquatic life criteria in the U.S., Australia, and other international jurisdictions. Further, marine aquatic life criteria may not account for indirect exposure through the ingestion of dietary items that may bioaccumulate or biomagnify PFAS through the food web. This work

presents the derivation of ecological guideline values for PFOS and PFOA in marine surface water in accordance with the framework for developing Australian and New Zealand Fresh and Marine Water Quality Guidelines. Ecological guideline values for marine surface water were derived for the protection of direct exposure to aquatic life and indirect exposure to wildlife using a species sensitivity distribution (SSD) approach. Ecotoxicity endpoints used as SSD inputs for direct exposure were based on no observed effect concentrations (NOEC) or 10 percent effect concentrations (EC₁₀) for growth, development, reproduction, or survival endpoints for pelagic invertebrates, benthic invertebrates, phytoplankton, or fish. Ecotoxicity endpoints used as SSD inputs for indirect exposure were based on marine surface water concentrations that were back-calculated from toxicity reference doses (growth, development, reproduction, or survival endpoints) using dose rate models and assumed bioaccumulation rates. Ecological guideline values for marine surface water were identified for direct and indirect exposure SSDs at concentrations corresponding to 99%, 95%, 90% and 80% species protection levels. SSDs developed for PFOS and PFOA indicate that 95% protection levels for indirect exposure are two orders of magnitude lower than comparable protection levels for direct exposure. These findings indicate that indirect exposure to PFOS and PFOA through bioaccumulation and wildlife ingestion is an important and potentially more sensitive pathway relative to direct exposure to marine surface water. Therefore, the derivation of marine aquatic life criteria for PFAS, as well as guidelines for other exposure media, should include endpoints for indirect exposure via bioaccumulation and ingestion to ensure adequate protection of aquatic life and wildlife.

2.08.P-Mo One Health of Planktonic, Pelagic and Benthic Harmful Algal Blooms (HABs): The Detection, Fate, Effects, Monitoring, and Management of Blooms

2.08.P-Mo-068 Quantifying Exposure of Amphibians to Toxic Cyanobacteria Across Michigan's Inland Lakes

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Freshwater harmful algae blooms (HABs) across global temperate regions commonly occur in lakes beginning in early summer and continue through late fall. The majority of, and most severe, HABs often occur in late summer and early fall. Since many aquatic species also reproduce or rear young offspring at this time, there is potential for different organisms to be exposed to cyanobacterial toxins during early life stages. Research has shown that these toxins may be harmful to young fish and mammals, but little is known about the effects of the cyanobacterial toxin microcystin on early developing amphibians. We hypothesized that peak HAB occurrence may overlap in space and time with pond-breeding amphibians. We investigated these potential species interactions by measuring the historic overlap in HAB spatial and temporal distribution with previous survey and novel field observations of certain frog and toad species. Public observation records of 13 amphibian species in Michigan from community science portals like iNaturalist and Herpmapper were verified and collated by month between years 2000 – 2022. Trends in these data were compared with HAB occurrence data collected by the Michigan Department of Environment, Great Lakes, and Energy over the last seven years. Preliminary results from our meta-analysis show that 62% of all HABs and 33% of *Rana clamitans* (Green frogs) are observed between August and September on an average year. These trends were further investigated through auditory field surveys in 2021 and 2022 for *R. clamitans* in 30 lakes with historic and/or ongoing blooms. Following these surveys, we identified *R. clamitans* at 57% (n=28) of lakes with repeated, severe (>10 ug/L total microcystin concentration) blooms and 67% (n=3) of lakes with ongoing toxic blooms at the time of sampling. Our results identify clear examples of interactions between HABs and amphibian populations across spatial and temporal scales. While we found that certain amphibian populations persist in lakes following severe blooms, we also identified clear exposures of breeding amphibians to active, toxic cyanobacteria blooms. These interactions must be explored further with continued monitoring and advanced exposure experiments to fully understand the impact of HAB toxins on developing amphibians.

2.08.P-Mo-069 Enhanced Structural Elucidation of Microcystins by Electron-Activated Dissociation (EAD)

Karl Oetjen, Holly Lee and Kendra Adams, SCIEX

Over 250 congeners of hepatoxins derived from cyanobacteria have been identified to date. The observed structural diversity between congeners stems from variable amino acid substitutions within the cyclic peptide structures of microcystins (MC) and nodularin (NOD). Conventional immunoassay-based methods typically lack the specificity to unambiguously identify individual congeners, while collision-induced dissociation (CID)-based MS/MS analysis often produces non-selective fragments due to fragmentation primarily occurring at a common side chain present in all MC peptides. As a result, generating fragment-rich MS/MS spectra using alternative dissociation pathways, such as electron-activated dissociation (EAD), is critical to identify and differentiate known and new microcystins, which are likely numerous based on the possible amino acid configurations in the cyclic peptide. A suite of MC and NOD standards were introduced into the ZenoTOF 7600 system by infusion and acquired using both CID and EAD fragmentation modes. In EAD mode, the kinetic energy (KE) was ramped from -10 to 25 eV, while similar ramping experiments were performed to optimize the collision energy (CE) from 0 to 150 V in CID mode. For some MC congeners, doubly charged $[M+2H]^{2+}$ parent ions were monitored as the precursor, due to higher fragment intensities observed compared to those generated from singly charged $[M+H]^+$ precursor ions. In general, the MS/MS spectra generated from EAD fragmentation showed additional fragments that were not observed during CID fragmentation. Some of these novel fragments were associated with modifications of the amino acid side chains and cleavages of the amide bonds throughout the peptide to open the ring structure. For example, while CID fragmentation typically consumes most of the precursor ions, the doubly charged $[M+2H]^{2+}$ precursor peak and its corresponding charge-reduced species (CRS), $[M+H]^+$, that results from electron-capture detection (ECD) were preserved in the EAD MS/MS spectra of the MC-YR peptide. The softer approach of EAD produces more diagnostic fragments that can supplement the lack of structural information whenever CID produces non-selective fragments. A comparison of the fragments produced by EAD and CID showed that EAD fragmentation consistently produces more unique fragments than CID fragmentation for the MC peptides tested.

2.08.P-Mo-070 Comparison of Two Invertebrates Sensitivity to Algaecide and Cyanotoxin at Elevated Surface Water Temperatures

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Cyanobacteria harmful algal blooms (cyanoHABs) are a problem of increased concern across freshwater bodies in the United States, especially when taking climate change into consideration. CyanoHABs can be detrimental to public health, aquatic ecosystem health, and economic health of an area. The most common chemical treatment methods of cyanoHABs include copper sulfate and hydrogen peroxide. Hydrogen peroxide has been shown to be less toxic to non-target organisms because it breaks down into water and oxygen and does not produce heavy metal byproducts like copper sulfate. While hydrogen peroxide is generally a safer choice algaecide, its lethal and sublethal effects on zooplankton have not been examined at predicted summer surface water temperatures or in combination with cyanotoxins, which can be released during cell lysis. This study exposed two freshwater cladocerans, *Ceriodaphnia dubia* and *Daphnia magna*, to *Microcystis aeruginosa* lysate containing nominal concentrations of 0 to 256 $\mu\text{g/L}$ of microcystins as well as a daily 3 mg/L dose of hydrogen peroxide to mimic repeated treatment of a cyanoHAB event over 7 and 4 days, as the hydrogen peroxide was seen to break down to <0.2 mg/L after 24 hours. These assays were performed at 27°C after a period of organism culture acclimation from normal culture conditions of 25°C. The most recent results with both species will be presented. This study will help better understand how aquatic organisms interact with their environment during cyanoHAB events and if interacting effects are present between temperature, microcystin, and algaecide.

2.08.P-Mo-071 Microcystin Levels in Water, Sediment, and Invertebrates from the Stones River in Central Tennessee, USA

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Certain conditions of temperature, nitrogen and phosphorus concentration, light level, and water flow can lead to proliferation (blooms) of cyanobacteria in aquatic systems. When these blooms contain cyanobacteria that are actively producing toxins such as microcystin, the blooms are known as cyanobacterial Harmful Algal Blooms (cyanoHABs). CyanoHABs can negatively impact aquatic organisms, humans, and other animals as well as recreational activities. Due to the occurrence of algae blooms in the Stones River near Murfreesboro, TN, USA, a monitoring study was undertaken at sites on the river to assess the following: Is microcystin 1) detectable in water and/or sediments of the Stones River? 2) moving from the aquatic to the terrestrial system through emerging insects?, 3) level related to nutrient concentrations in the water? Microcystin was determined in water, sediment, and invertebrates by ELISA. Prior to analysis of sediment and invertebrates, a methanol extraction was performed to separate and concentrate microcystin. In summer 2021 only water was sampled, and most samples were found to contain between 0.1 and 0.3 ug/L microcystin (EPA drinking water limit = 0.3 ug/L). In summer 2022, all sediment and water samples were below detection for microcystin. However, at one site in August, >50% of Tetragnathid spiders (riparian spiders that feed on emerging insects) were found to contain >0.3 ng/g, indicating a possible transfer of microcystin from the aquatic to the terrestrial system. Sampling will be ongoing throughout summer 2023. These data indicate at least the periodic presence of microcystin, a cyanotoxin, in Stones River water and the detection of microcystin in riparian spiders even when it was not detectable in water suggests that there may be food chain transfer and accumulation of microcystin which could lead to negative impacts in the system.

2.08.P-Mo-072 Occurrence of Microcystin-LR Photoisomerization Products in a Shallow Eutrophic Lake Bofan Wei and Gregory L Boyer, State University of New York

The hepatotoxin, Microcystin-LR (MC-LR) is common in New York State lakes. Microcystin-LR undergoes a photochemical isomerization of the ADDA sidechain to form several photoisomerization products (iso MC-LR). These photoisomerization products were generated in the laboratory, characterized by high-field NMR spectroscopy, and an in-house LC-MS microcystin method modified for their detection in natural waters. Samples were taken weekly from Lake Neatahwanta (Oswego County, NY) during the 2016 and 2017 bloom season. Iso MC-LR were detected in ~95% of MC-LR positive samples. These photoisomerization products were observed in both the dissolved phase and the particulate phase. The ratio of iso MC-LR to MC-LR was not correlated with the total MC-LR concentration in either the dissolved or particulate fraction. The ratio of iso MC-LR to MC-LR in dissolved phase and particulate fraction were correlated with the light intensity. The formation of photoisomerization products over the course of the day increased with cumulated light intensity ($R^2=0.65$). This was not observed over the course of the season, suggesting other factors were also important. This study provides important information on the fate of microcystins in natural waters.

2.08.P-Mo-073 Evidence Map and Synthesis of Ecological Toxicity of Cyanotoxins Using ECOTOXicology Knowledgebase Systematic Protocols

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Cyanobacteria associated with Harmful Algal Blooms (HABs) can release toxins known as cyanotoxins, which can be deadly to humans, pets, and livestock. There are U.S. federal and state human health advisory thresholds and recreational and ambient water quality criteria for several cyanotoxins; however, it is unclear if these guidelines are protective of health for aquatic and terrestrial organisms in which acute and chronic effects are not well characterized. Presented here is the development of an evidence map for ecological toxicity of

cyanotoxins, including a literature inventory of studies of cyanotoxin exposure on aquatic and terrestrial organisms and a structured database of toxicity effects of priority cyanotoxins. The well-established protocols of the ECOTOXicology Knowledgebase (ECOTOX, www.epa.gov/ecotox) were leveraged to systematically identify and screen studies for relevance, focusing on 15 priority cyanotoxins along with over 100 additional cyanotoxins from HAB publications and government reports. Over 7,800 references were screened at the title and abstract level, followed by full-text review of over 1,500 publications. Of these, 1,125 publications met the ECOTOX inclusion criteria (e.g., verifiable CAS registry number, verifiable species, and control reported) and were annotated with test compound(s), test species, exposure type(s), and effect type(s). This literature inventory forms the base of the evidence map with diverse effects measured in over 100 different cyanotoxins and 600 biological species, including aquatic and terrestrial vertebrates, invertebrates, and plants. Detailed data extraction was prioritized for 15 cyanotoxins, which were identified based on production by the most common HAB-forming cyanobacteria in U.S. freshwater and marine ecosystems. This data extraction is largely complete and includes pertinent study details for test species, test compound, test methods and conditions, and toxicity results. The literature inventory informs future cyanotoxin research through identification of data availability and gaps for species, toxins, and endpoints. The structured effects data can be used to identify the most potent cyanotoxins and sensitive species and characterize the variability in responses across species and cyanotoxin classes, thus supporting evaluation of ecological risk from HABs and the most commonly occurring cyanotoxins in the environment. *This abstract does not necessarily reflect US EPA policy.*

2.08.P-Mo-074 Efficacy of Algaecides for the Preventative Management of Overwintering Cyanobacteria in Sediments

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Multiple common freshwater cyanobacteria that form harmful algal blooms (HABs) remain quiescent in sediment as either akinetes or vegetative cells, collectively termed overwintering cells, during non-ideal growth conditions (e.g., winter). Research has demonstrated the critical role of these overwintering cells to provide an inoculum for HABs. An attractive strategy to decrease this inoculum is to implement preventative *in situ* algaecide treatments targeting overwintering cells in sediments prior to the formation of a HAB. However, this strategy is novel and laboratory toxicity data are needed to support efficacy of this approach. Specific objectives of this research were to 1) evaluate copper- and peroxide-based algaecides, applied as single and repeat treatments in the laboratory, and 2) compare correlations between cell density and other response measurements (i.e., *in vivo* chlorophyll *a* and phycocyanin concentrations and percent benthic coverage), to identify informative metrics to assess overwintering cyanobacteria responses. Twelve treatment scenarios using copper- and peroxide-based algaecides were applied to sediments containing overwintering cyanobacteria prior to a 14-d incubation that simulated favorable growing conditions and cyanobacteria responses were measured. HAB-forming cyanobacteria present after a 14-d incubation were: *Aphanizomenon*, *Dolichospermum*, *Microcystis*, *Nostoc*, and *Planktonthrix*. Successive treatments of copper sulfate (CuSulfate) followed by sodium carbonate peroxyhydrate (PeroxiSolid) (second algaecide applied after 24 h) as well as repeat applications of a single algaecide, PeroxiSolid (second treatment applied after 24 h) resulted in statistically significant ($p \leq 0.05$; $\alpha = 0.05$) declines in cell density relative to untreated controls. Planktonic cyanobacteria responses measured in terms of phycocyanin were strongly correlated with cyanobacteria density measurements ($r = 0.89$). Chlorophyll *a* and percent benthic coverage did not correlate with planktonic cyanobacteria density measurements ($r = 0.37$ and -0.49 , respectively) and therefore, were unreliable measurements. These data provide initial evidence of the efficacy of algaecides for treating overwintering cells in sediments and contribute to our overarching hypothesis that preventative treatments may decrease the severity of HABs.

2.08.P-Mo-075 Acute and Chronic Toxicity of Microcystin to Four Standard Toxicity Test Organisms Using Lysates From Large Cultures of *Microcystis aeruginosa*

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There is a lack of information to estimate safe exposure levels to toxins produced by cyanobacteria for freshwater aquatic life. The uncertainty in concentrations and purity of commercially available standards for cyanotoxins, and potentially their cost, challenge their use for conducting acute and chronic toxicity tests. Therefore, we developed a large lab-scale batch culture method to generate lysate concentrates of cyanotoxins. Microcystin-producing *Microcystis aeruginosa* with confirmed *mcy* genes by qPCR and RT-qPCR was used in these cultures. The *M. aeruginosa* culture method starts with 250-mL cultures that are used to start 1-L cultures tests, which in turn are used to start 20-L carboy cultures. BG-11 Medium is added weekly. For the microcystin method, after 28-days and a *M. aeruginosa* cell density of 9.5×10^6 cells/mL, the cultures were centrifuged to isolate the cyanobacteria, then resuspended in test water and freeze-thawed three times. The resultant lysate was centrifuged again to remove particulate cell material. This procedure was repeated three times and resulted in solutions containing microcystin concentrations of 7200, 9400, and 7500 $\mu\text{g/L}$. Large batch culture acute toxicity tests resulted in 48-hr LC_{50s} for *C. dubia* and *D. magna* ranging from 563-687 $\mu\text{g/L}$ and 560-1265 $\mu\text{g/L}$, respectively. Microcystin 48-hr LC_{50s} for *N. triangulifer* was 1850 $\mu\text{g/L}$ and for 72 & 96-hr LC_{50s} ranged from 296-1456 $\mu\text{g/L}$ and 26-791 $\mu\text{g/L}$, respectively. Microcystin IC_{25s} for *C. dubia*, *N. triangulifer*, and *P. promelas* ranged from 5-57 $\mu\text{g/L}$, 134-183 $\mu\text{g/L}$, and 74-683 $\mu\text{g/L}$, respectively. The lysates used for testing were analyzed for congener combinations to see if there were any differences in speciation over the course of the study. Results from saxitoxin-producing cultures will also be presented.

2.08.P-Mo-076 In Ovo Exposure to Cylindrospermopsin, but Not Microcystin, Reduces Chick Embryonic Growth and Metabolic Rate

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The occurrence of harmful algal blooms is predicted to increase as the climate changes, increasing the risk of exposure to algal toxins for waterfowl and shorebirds. Previous studies have detected the presence of algal toxins (e.g., microcystins) in egg contents of waterbirds, suggesting algal toxins may interfere with embryonic development. While there is some evidence that exposure to algal toxins can alter cardiac development and function in other taxa (e.g., fish), no study has examined whether such effects occur in avian embryos. In this study, we used chicken (*Gallus gallus*) embryos to examine the developmental toxicity of two algal toxins: microcystin-LR and cylindrospermopsin. Specifically, we investigated whether *in ovo* exposure to these algal toxins interferes with avian embryonic growth, heart rate, and metabolic rate. Exposure to 60 ng/g egg mass cylindrospermopsin substantially impaired embryonic growth and metabolic rate, whereas microcystin had a limited effect on embryonic growth. Neither cylindrospermopsin nor microcystin altered embryonic heart rate. These results highlight the potential for harmful algal blooms to cause avian embryotoxicity.

2.08.P-Mo-078 Development and Validation of Analytical Methods for Measuring Diverse Cyanotoxins in Complex Matrices Using Automated Solid Phase Extraction, Isotope Dilution LC-MS/MS

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The analysis of cyanotoxins in various matrices is complicated by challenges in extraction, concentration, and quantification. Common cyanotoxins in the United States include microcystins, anatoxins, and cylindrospermopsins, which across their various congeners have considerable differences in hydrophilicity, complicating efforts to analyze for many toxins in a single extraction/analysis workflow. Various approaches have been developed to address this challenge, including optimization of extraction conditions for individual toxin classes, and the use of chemical derivatization techniques to convert target compounds to more readily

extracted products (e.g. the MMPB derivatization for microcystins). These approaches can increase the cost and time required for sample processing and analysis. Here are described efforts to develop and validate a multitoxin analytical method suitable for measurement of various microcystins, anatoxins, and cylindrospermopsin variants compatible with various matrices including tissues and soils/sediments. Following extraction, automated sample clean-up and concentration using online solid phase extraction is used to simplify sample preparation and reduce method detection limits. Isotopically labeled analogs of toxins are used to monitor and track method performance for a group of chemicals with diverse chemistry using a single workflow. Details and results of the method development and validation will be presented, as well as applications to matrices including fish tissues, macroinvertebrates, zooplankton, and benthic samples. The method detailed here would increase the degree of automation and simplify pretreatment steps thereby increasing throughput.

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 Analysis of the Sub-lethal Impacts of *Karenia brevis* at a High Concentration in Hard Clams (*Mercenaria campechiensis*)

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Karenia brevis, a toxin-producing dinoflagellate, is the most prevalent harmful algal bloom in the Gulf of Mexico. Shellfish, such as *Mercenaria campechiensis*, have high *K. brevis* resiliency leading to accumulation of brevetoxins within their tissues. Due to the retention of brevetoxins in shellfish, shellfish aquaculture farms are required to cease operations when *K. brevis* concentrations reach 5,000 cells/L causing high economic loss. Aquaculture farms can reopen after *K. brevis* concentrations decrease and *M. campechiensis* tissue reaches safe consumption levels through the detoxification process. Clam farmers have self-reported *M. campechiensis* taking substantially longer than previously observed to detoxify brevetoxins after exposure to high *K. brevis* concentrations. The current study aimed to analyze the accumulation and depuration of brevetoxins in *M. campechiensis* exposed to 100,000 cells/L for 2 weeks. The second aim looked to identify detoxification enzymes, and enzymes that are representative of stress. The focus of the second aim was to identify if high concentrations of *K. brevis* resulted in a slowed metabolic rate. The enzymes assessed were cholinesterase (ChE) and glutathione s-transferase (GST). This was done by exposing *M. campechiensis* to a continuous flow of 100,000 cells/L of *K. brevis* for two weeks, followed by a 47-day depuration. Total body burden of brevetoxin in the tissue was assessed using solid-phase extraction and ran on a LC-MS/MS. Standard ChE and GST activity assays were applied. There was an increase in brevetoxin concentration during the exposure with a rapid 75% decrease at the onset of the depuration followed by a gradual decline with detectable toxins for the duration of the experiment. GST activity had no noticeable or significant change. ChE activity slightly increased in the exposed clams during the exposure with a slight decrease in activity during the depuration, indicating high concentrations of *K. brevis* may induce stress in *M. campechiensis*. Findings from this study indicate that after exposing *M. campechiensis* to high concentrations of *K. brevis*, there was a rapid depuration of brevetoxin, however, low levels were maintained for periods up to 1.5 months. These findings indicate *M. campechiensis* health may be impacted by high concentrations of *K. brevis* leading to longer depuration periods and potentially longer closing periods for clam aquaculture farms.

2.08.T-02 Most Treatments to Control Freshwater Algal Blooms Are Not Effective: Meta-Analysis of Field Experiments

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Harmful algal blooms negatively impact freshwater, estuarine, and marine systems worldwide, including those used for drinking water, recreation, and aquaculture, through the production of toxic and nontoxic secondary

metabolites as well as hypoxic events that occur when algal blooms degrade. Consequently, water resource managers often utilize chemical, bacterial, physical, and/or plant-based treatments to control algal blooms and improve water quality. However, awareness of available treatments may be limited, and there is ambiguity among the effects of algal bloom treatments across studies. Such variation within the literature and lack of knowledge of other tested treatments leave uncertainty for water resource managers when deciding what treatments are best to control algal blooms and improve water quality. Our primary objective was to synthesize data from 39 published and unpublished studies that used one of 28 chemical, bacterial, physical, and/or plant-based treatments in field experiments on various water quality measurements, including phytoplankton pigments and cell density, cyanobacterial toxins (microcystin), and common off-flavors (i.e., taste and odor compounds; geosmin and 2-methylisoborneol). We hypothesized that treatments would improve water quality. Across all studies and treatment types (227 effect sizes), water quality improvements were observed when measured at the time of greatest decline following treatment or at the end of the experiment. However, these findings were primarily mediated by only four chemicals, namely copper sulfate, hydrogen peroxide, peracetic acid, and simazine. None of the bacterial, physical, or plant-based treatments were shown to significantly improve water quality by themselves. Results from this synthesis quantitatively showed that most treatments fail to improve water quality in the field and highlight the need for more research on existing and alternative treatments.

2.08.T-03 Microcoleus (Cyanobacteria) from Streams in California: Growth and Anatoxin-a Production in Laboratory Culture Conditions

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Anatoxin-a (ATX)-producing benthic cyanobacterial proliferations in running waters are an emerging global issue with adverse effects on aquatic ecosystems, water resources, and human health. Laboratory experiments with cyanobacterial strains are powerful tools to study their biology and environmental ranges at species level. For this study, we used six non-axenic unialgal *Microcoleus* strains recently isolated from streams and rivers in northern California with the aim to create growth curves and to evaluate the ATX production during the exponential growth and stationary phase. Cultures were incubated for 49 days in a 30 mL glass tubes with 20 mL BG11 medium at 21 C°, with a light irradiance of 100 $\mu\text{moles} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and a 12:12-hr light: dark cycle. Cell density was determined from triplicate cultures at 3–5-day intervals starting on day 5. The ATX variants and dry weight were measured by LC-MS/MS chromatography from duplicate cultures at 7-day intervals starting on day 5. Chlorophyll-*a* concentrations were measured from duplicate cultures at 7-day intervals starting on day 12 by filtration, freezing, and subsequent fluorometric determination. DNA and RNA were extracted from 0.5 g of each *Microcoleus* strain using the All-prep DNA/RNA kit from QIAGEN. Subsequently, the extracted DNA and RNA were stored at -80°C for further analysis (e.g., phylogeny and functional gene analyses), library preparation, and sequencing. *Microcoleus anatoxicus* (PTRS1) from the Russian River (isolated in 2015) and a strain from the Rock Creek within the Southern Cascade Mountain Range (isolated in 2020) produced dihydroanatoxin-a (dhATX) only. Two strains from the Klamath River and the Scott River watersheds (isolated in 2022) produced both ATX and dhATX. Two *Microcoleus* strains from the Eel River (isolated in 2022) were not toxic. Most strains reached stationary growth phase after 30 days of culturing and formed mat on the surface of the culturing liquid, except for one of the non-toxic strain which was still actively growing. Microscopically, the cell of all toxic strains showed signs of starvation (e.g., structural changes in granulation, pigmentation, and widened thylakoids) and produced volatile organic compounds during stationary phase in contrast to the non-toxic strains. Toxin, chlorophyll-*a*, and cell density results are discussed in light of trade-offs between biomass and energy-expensive toxin production observed in the experiment.

2.08.T-04 Multi-Year Investigation of Benthic Cyanobacteria Accumulations in Stormwater Ponds

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Extensive cyanobacteria-dominated growth and mats were observed during late summer of 2021 and 2022 within a stormwater retention pond network in northwest Florida. Weekly sampling was performed from late summer throughout fall to examine potential toxin-producing cyanobacteria, associated cyanotoxins, and other potentially bioactive cyanobacterial metabolites at several sites within the system and surrounding area. During 2021, the sampling periodicity corresponded to weekly oscillations in heavy rainfall from tropical systems and arid conditions. In 2022, the environmental conditions were nearly opposite with drought-like conditions persisting for the majority of the sampling campaign with only minor precipitation events during the final timepoints. Dynamic cyanobacterial communities were observed and multiple cyanotoxin classes were detected in whole water, SPATT, and mat/periphyton samples using targeted and non-targeted analysis that differed dramatically between years. The distinct patterns suggest ecohydrological controls over cyanobacteria-dominated growth, community structure, and toxin composition in stormwater management systems.

2.08.T-05 Development of In Situ, Near-Real-Time Cyanobacterial Monitoring Using Digital Microscopy and Artificial Intelligence

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The persistent appearances of cyanobacterial harmful algal blooms (cHABs) are a threat to freshwater system around the world. Of particular concern are cyanobacteria- and cyanotoxin-driven health effects, including liver toxicity, gastrointestinal distress, allergic response, and others. Therefore, research enabling timely monitoring capabilities and deployment of methods enabling real-time, accurate risk assessment is critical to protecting public health, enabling smart water management, and better understanding cHAB dynamics. Here, we present an affordable, accessible method using portable, digital microscopy and BloomOptix artificial intelligence (AI) to image, detect and quantify cyanobacterial colonies to a genus level, across 6 of the most common cyanobacterial genera: *Microcystis*, *Dolichospermum*, *Woronichinia*, *Aphanizomenon*, *Limnoraphis* and *Gloeotrichia*. During the 2022 pilot study, digital microscopes and AI access via mobile app was distributed across a 5-state user network of citizen scientists, regulators, public health officials, utilities and lake managers. To test the accessibility of the monitoring platform, users were given only 40 minutes of training on microscope use and app upload procedures. Upon the conclusion of the study, the monitoring network collected over 170 unique cHAB samples across 36 unique lakes, gathering 5000 microscopic images and generating cHAB diagnostic reports in 30 seconds or less, with 95% accuracy across all 6 genera. Here, we will present more detailed findings of this monitoring network study, including: the method's cell count validation against established lab methods; findings in cyanobacterial community changes across select lakes; user experience and proficiency in method use; plans for monitoring network scaling in 2023; and inclusion of data archiving and proactive alert functions.

2.08.T-06 Management Perceptions on Minimizing Harmful Algal Bloom Impacts

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Harmful algal blooms (HABs) are an environmental, economic, and public health hazard due to their ability to degrade freshwater ecosystems with fish kills, decrease recreation and tourism, and cause severe illness or death in mammals. Monitoring practices and understanding of publics' perceptions of HABs risk are not cohesive across federal, state, and local agencies. This study conducted interviews with various water managers and

researchers mainly across Ohio and New England. This provided perspectives of managers facing different HABs concerns from agriculture and drinking water supply in Ohio to wastewater management and recreation in New England. Interviews were transcribed and qualitatively coded through NVivo software to determine common themes. There was a perception of increased public awareness of HABs, due to increased monitoring in New England, and following the Toledo 2014 drinking water crisis in Ohio. There was also a primary emphasis across regions on reducing nutrient inputs to decrease HAB events across all freshwater bodies. Funding was a large concern for maintaining or increasing monitoring efforts in response to more frequent HAB events and longer HAB seasons. Finally, there was a strong concern for people to know that HABs are harmful to humans and should be more widely regarded and communicated as a risk. The research in this study provides perceptions of monitoring priorities and public understanding of HABs, demonstrates opportunities to improve current HAB monitoring efforts, and can be used to better inform those interviewed about potential ways to create a more holistic view of monitoring and HABs risk.

2.09.P-We Stormwater Runoff Impacts and Potential Solutions

2.09.P-We-045 Water Quality Impacts of Oil and Gas Produced Waters Versus Commercial Dust Suppressants Applied to Gravel Roads

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Particulate matter (PM) has been linked to adverse human and environmental health impacts, such as respiratory disease, decreased ecosystem productivity, and pollutant transport. Gravel roads, which comprise one-third of the road system in the United States, generate 35% of domestic PM emissions annually. Commercially available dust suppressants are applied to gravel roads to minimize PM releases, but these products can be cost-prohibitive. To reduce the cost of seasonal dust suppression, certain states permit the use of produced water from conventional oil and gas wells (OGPW), which are often offered as a free alternative to commercial products. OGPW generated in the Appalachian Basin has been shown to have elevated concentrations of salt, radioactivity, heavy metals, and organic micropollutants, but the risks to water quality from using OGPW as a dust suppressant are still unknown. This study compares the water quality of runoff from unpaved roads treated with two commercial dust suppressants (calcium chloride brine and soybean oil), three OGPWs, and synthetic rainwater. A 2-year, 24-hour, NRCS Type-II storm event was used to mobilize pollutants from treated roadbeds. Runoff samples were collected and analyzed for a suite of water quality criteria. The maximum analyte concentrations in the runoff were proportional to concentrations in the dust suppressants. The calcium chloride brine had the highest concentrations of most analytes, and roadbeds treated with this dust suppressant generated runoff that exceeded regulatory thresholds designed to protect human and environmental health. Runoff from roadbeds treated with soybean oil had the highest concentrations of organic compounds. Roadbeds treated with OGPWs generated runoff that was similar to but less concentrated than roadbeds treated with the calcium chloride brine except for radium and sodium. Radium added to the roadbed through application of OGPWs was mobilized in the runoff, likely via transport on clay particles. Sodium, the dominant cation in the OGPWs, was partially retained in the roadbed and could lead to clay dispersion, limiting produced waters' effectiveness as a dust suppressant.

2.09.P-We-046 Discrete and Passive Sampling of 6PPD-Q in Stormwater-Impacted Surface Waters

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Urban runoff mortality syndrome in coho salmon has been linked with 6PPD-quinone (6PPD-Q), the ozone transformation product of an antioxidant used in tires and rubber products. Since this discovery in 2021, research has focused on the mobilization, transport, and mechanisms of action for 6PPD-Q exposure. To expand

the knowledge regarding 6PPD-Q exposures, this study provides a comparison between discrete sampling verses in situ passive samplers in stormwater-impacted surface waters. Pilot sampling sites in Seattle, Washington were identified and prioritized based on urbanization gradients, traffic density, suspected tire use/storage locations, and known Coho Salmon habitat. The Thornton Creek watershed and sites on the Skagit river were sampled during the fall of 2022 and included grab samples and deployment of passive diffusive gradients in thin-films (DGT) samplers built with an HLB sorbent layer. As concentration pulses have been observed when stormwater runoff events mobilize 6PPD-Q, in situ passive sampling can be an advantageous technique to capture a time weighted average concentration that is likely missed via discrete sampling. Both discrete samples and DGT samplers were analyzed for 6PPD-Q by liquid chromatography tandem mass spectrometry (LC/MS/MS) and performance testing of the DGT samplers were done at the U.S. Geological Survey Organic Geochemistry Research Laboratory in Lawrence, KS. Results from the discrete samples and passive samplers will be used to inform future spatial national reconnaissance work to assess ecological risk.

2.09.P-We-047 Testing Removal of 6PPD-q and Coho Salmon Lethality by High Performance Bioretention Media Blends

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Urban stormwater can contain 6PPD-quinone (6PPD-q), a tire-derived chemical highly toxic to coho salmon and associated with Urban Runoff Mortality Syndrome (URMS). Lab studies indicate filtrating stormwater through bioretention soil media (BSM) consisting of 60% sand and 40% compost by volume (60/40 BSM) can eliminate toxicity for aquatic organisms. This same BSM unfortunately exports excess nitrogen, phosphorus, and some metals. 60/40 BSM is approved by Washington Department of Ecology (Ecology) for bioretention in Washington, but to address these pollutant exports, Ecology has added three high performance BSMs (HPBSMs) to its approved stormwater treatment technologies. The three HPBSM types all include a primary layer of volcanic sand, coconut coir, and carbon wood ash (biochar). One type adds components for polishing (activated alumina and iron) below the primary layer. The last type adds a 2-inch compost blanket on top to support robust plant growth. The latter two types meet all of Ecology's treatment criteria. Whether any of the three HPBSM types removes or attenuates 6PPD-q and/or reduces acute toxicity of stormwater to coho, and their performance at these functions relative to 60/40 BSM, has not been tested. In the Pacific Northwest, stormwater regulators have been incentivizing use of Low Impact Development (LID) and Washington State is increasing permit requirements for LID in urban areas. King County is planning to add bioretention to its stormwater manual as an option to meet water quality treatment objectives; however, we do not know how well the 60/40 BSM or HPBSM types treat for 6PPD-q and coho toxicity. As a first step, we are conducting bench-scale soil column tests to compare effectiveness of 60/40 BSM and the HPBSM types in lowering 6PPD-q concentrations and/or reducing stormwater toxicity to coho (URMS). Our study includes analysis of treated and untreated stormwater for 6PPD-q and for acute toxicity to juvenile coho. We are also tracking changes in common water quality parameters before and after filtration to inform how they may impact 6PPD-q concentrations and toxicity of effluents. In effluent tested for coho toxicity, we are also measuring total dissolved carbon and total suspended solids to evaluate whether they may affect toxicity, e.g., impeding 6PPD-q bioavailability. We will present our findings, including the extent of 6PPD-q reduction by each BSM and treatment performance of the HPBSM types relative to 60/40 BSM.

2.09.P-We-048 The Fate of 6PPD and 6PPD-Quinone in Air and Aquatic Conditions

Rory Mumford, Graham Crabtree and Pete Yeomans, Smithers

The antiozonant and antioxidant 6PPD (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine) and its transformation product 6PPD-quinone (6PPDQ) are believed to be derived from tire and road wear particles (TRWP) and washed into watercourses. Some publications have identified 6PPDquinone as a contributor to

Urban Runoff Mortality Syndrome (URMS) of a single specific fish species, Coho salmon. The current study was undertaken to further understand the behaviour of 6PPD and 6PPDQ once they have entered the aquatic environment. ¹⁴C-radiolabelled 6PPD and 6PPDQ were synthesised to aid the tracking of degradation products throughout the study. The hydrolytic behaviours of 6PPD and 6PPDQ were studied through the environmentally relevant pH range using the standard OECD 111 buffers. Sterile natural waters were also used to assess the influence of alkalinity, hardness, and trace element composition. A second element of this study was designed to generate data around the degradation of 6PPD and 6PPDQ in air. The radiolabelled compounds were applied as a thin film to the inner surface of glass vessels before sealing. A constant stream of nitrogen, air, or air containing modified levels of ozone was passed over the thin film. Vessels were either kept in the dark or irradiated using simulated sunlight to study the effects of oxygen and ozone in the presence of light on the degradation of 6PPD and 6PPDQ. Initial results and transformation products have been presented at previous SETAC meetings; this poster presentation provides more focus on the transformation pathways along with quantitation data from the complex data sets produced.

2.09.P-We-049 The Fate of 6PPD-Quinone in Soil and Water-Sediment Systems Using a ¹⁴C Radiotracer *Rory Mumford, Smithers*

The antiozonant and antioxidant 6PPD (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine) and its transformation product 6PPD-quinone (6PPDQ) are believed to be derived from tire and road wear particles (TRWP) and washed into watercourses. Some publications have identified 6PPDQ as a contributor to Urban Runoff Mortality Syndrome (URMS) of a single specific fish species, Coho salmon. The potential mobility of 6PPDQ in soils and sediments has been investigated using the OECD 106 test guideline (Adsorption - Desorption Using a Batch Equilibrium Method) and ¹⁴C-radiolabelled 6PPDQ. This study is used to indicate how likely a compound is to move through soils and potentially enter groundwater sources. Additionally, the transformation of 6PPDQ 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 6PPDQ in the key environmental compartments. This presentation focuses on the initial results, including identification and quantification of transformation products generated during these aforementioned studies.

2.09.P-We-050 Differential Acute Sensitivity to 6PPD-Quinone Among Aquatic Species and Regional Applicability

Stephanie B. Kennedy, David Feifarek and Julie Panko, (1)ToxStrategies, LLC

N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), the transformation product of the tire antiozonant, 6PPD, was recently identified as a causative chemical in recurrent coho salmon (*Oncorhynchus kisutch*) urban runoff mortality syndrome. Since this discovery, there has been a rapid increase in peer-reviewed literature evaluating 6PPD-quinone toxicity in aquatic organisms. In turn, it has become evident that *O. kisutch* are uniquely sensitive to 6PPD-quinone, and effect concentrations vary widely among other aquatic organisms. These data have potential to inform stormwater best management practices which are tailored to account for regional species variability. To better understand this, a review of all currently available literature reporting 6PPD-quinone-related effects in aquatic organisms was conducted. A comprehensive literature search was performed using multiple search engines and publications were collated for review. Using the systematic review software, DistillerSR (V2.43.0), literature underwent initial categorization based on terminology present within the title and abstract. The full text of all relevant literature was reviewed, and acute toxicity data were extracted (e.g., LC50s). Study reliability of each publication was evaluated using the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) framework to ensure a robust critical appraisal of the identified ecotoxicity data. The results are presented in the context of regional applicability and provide a landscape of the available hazard data which can be investigated further to answer additional research questions

or identify where knowledge gaps exist. Previous studies indicate that stormwater best management practices are effective at reducing effects of 6PPD-quinone and other roadway runoff pollutants in coho salmon and other aquatic species. Therefore, these results support the implementation of regional best management practices in areas where species sensitive to 6PPD-quinone occur.

2.09.T Stormwater Runoff Impacts and Potential Solutions

2.09.T-01 Predicted Aquatic Exposure Effects from a National Urban Stormwater Study

Paul Bradley, Kristin M Romanok, Kelly L. Smalling, Jason R Masoner, Dana Kolpin and Stephanie E Gordon, U.S. Geological Survey

A multi-agency study of 438 organic and 62 inorganic chemicals measured in urban stormwater during 50 total runoff events at 21 sites across the United States demonstrated that stormwater discharges can generate localized, aquatic exposures to extensive contaminant mixtures, including organics suspected to cause adverse aquatic-health effects. The aggregated risks to multiple aquatic trophic levels (fish, invertebrates, plants) of the stormwater mixture exposures, which were documented in the national study, were explored by calculating cumulative ratios of organic-contaminant *in vitro* exposure-activity cutoffs and health-benchmark-weighted cumulative toxicity quotients. Both risk assessment approaches indicated substantial (moderate to high) risk for acute adverse effects to aquatic organisms across multiple trophic levels (fish, macroinvertebrates, non-vascular/vascular plants) at or near stormwater discharge points across the United States. The results are interpreted as potential orders of magnitude underestimates of actual aquatic risk in stormwater control wetlands or in the immediate vicinity of such discharges to surface-water receptors, because the 438 organic-compound analytical space assessed in this study is orders of magnitude less than the 350,000 parent compounds estimated to be in current commercial use globally and the incalculable chemical-space of potential metabolites and degradates.

2.09.T-02 Microplastic Emissions via Stormwater Runoff

Matthew Ross¹, Alyssa Loutan¹, Danielle Molenaar¹, Tianna Groeneveld¹, Taylor Bujaczek¹, Victoria Irene Arnold² and Norma Ruecker², (1)MacEwan University, (2)The City of Calgary

Urban stormwater runoff is a source of pollutants and sediments to surface waters and is thought to be an important vector for the introduction of microplastics into the aquatic environment. However, there is a paucity of data available on the prevalence of microplastics in urban runoff, limiting assessment of its role in transporting microplastics and preventing any form of risk assessment or mitigation strategies to be implemented. This study investigates the concentration and characteristics of microplastics in urban stormwater runoff from the City of Calgary, Canada. Samples (n=75) were collected under both baseflow and rain event conditions from 15 sampling locations. Microplastics were found in all samples at concentrations ranging from 0.7 to 200.4 pieces L⁻¹ (mean=31.9 pieces L⁻¹), with microplastics <125 µm being the dominant size class. Total concentrations, dominant morphologies, and particle sizes differed between rain events and baseflow conditions and morphology, size, and color distribution differed amongst land use types. We estimate that individual outfalls discharged between 1.9 million and 9.6 billion microplastics to receiving waters per rain event. This study adds to the growing knowledge that urban stormwater runoff is a significant pathway by which microplastic particles and anthropogenic fibers enter aquatic environments and will provide a baseline for future monitoring and mitigation studies.

2.09.T-03 Particle Size-Based Evaluation of Effectiveness and Performance of Stormwater Management Systems to Limit Sediment Recontamination of Polycyclic Aromatic Hydrocarbons (PAHs) From Stormwater Runoff

Cesar Ivan Gomez-Avila, Balaji Anandha Rao, Tariq Hussain, Huayun Zhou, David Sackey and Danny D. Reible, Texas Tech University

Stormwater runoff is a major non-point source of water and sediment pollution. To mitigate this issue, Stormwater management systems (SMS) can help reduce both runoff and contaminant loads. Historically, stormwater assessment has focused on total contaminant loads to receiving waters, with little attention paid to the particle-size distribution of contaminants — a piece of critical information necessary to assess their fate in receiving water bodies. The current study focuses on better understanding the performance of different SMS to reduce particle-size-based pollutant loads and their implications for receiving water systems. Several SMS were sampled, including bioinfiltration systems, media filters, a hybrid bioinfiltration + media filter system, and a retention pond. Automatic samplers were deployed in each management system's inlet and outlet, collecting stormwater samples during rain events. Contaminant concentrations were then measured in the selected particle-size fractions and the dissolved phase for both the inlet and outlet of each system. Stormwater flow intensity, volume, and rain characteristics were also 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 solid association of the contaminants. The data analysis showed that most stormwater management systems effectively remove coarse particles (coarse silt and sand) that would readily settle in the near field of receiving water bodies. Contaminants with higher K_{ow} also showed better removals due to their association with these coarse solids. In contrast, dissolved contaminants and contaminants associated with fine solids were generally not effectively removed in most systems, except for the hybrid system. Some systems designed to remove dissolved contaminants showed uneven performance, which was attributed to inadequate maintenance. The performance of the various stormwater management systems was summarized and put in context with recontamination risks in the receiving water systems.

2.09.T-04 Effectiveness and Performance Evaluation of Stormwater Management Systems in Limiting Sediment Recontamination of Heavy Metals

Huayun Zhou, Balaji Anandha Rao, Cesar Ivan Gomez-Avila, Tariq Hussain and Danny D. Reible, Texas Tech University

Sediment recontamination caused by stormwater runoff is widely recognized and raising public awareness. To better manage the major recontamination and reduce the impact of contaminants on humans and the environment, stormwater management and best management practices (BMPs) are carried out to control the contaminants loading from the receiving water. Among the various choices of stormwater management facilities, it is important to evaluate the performance and effectiveness of the BMPs for better future management. When contaminants in stormwater are mostly non-point sources, they interact with particles when they are transported with stormwater runoff, they interact with particles. Therefore, it is important to know the distribution of contaminants with different particle size intervals, to better predict the contaminants reduction. Samples collected from Puget Sound Naval Shipyard in Bremerton, WA, Naval Base in San Diego, CA, and Reese Technological Center in Lubbock, TX, were filtered and removed the different sizes of particles in the water. Then 9 metals (Cr, Mn, Ni, Cu, Zn, As, Cd, Pb, and Hg), were analyzed using the inductively coupled plasma mass spectrometry (ICP-MS) and automated mercury system MERX-T, respectively, after hot plate digestion. The removal efficiency for different facilities was evaluated with different size intervals. Partition coefficients for individual metals were analyzed to help compare the difference between different BMPs and individual metals. The statistical analysis of the heavy metal removal and partition coefficients identifies different mechanisms for heavy metal removal strategies and could provide a reference for adaptive management in the future.

2.09.T-05 Nutrient, Metal, and Polycyclic Aromatic Hydrocarbon Fate and Transport in Intermittent Flow Stormwater Management: Sorbent Media Effects

Narges Esfandiar, Rominder PS Suri and Erica R. McKenzie, Temple University

Stormwater is known to contain many contaminants, including nutrients, metals, and hydrophobic compounds including polycyclic aromatic hydrocarbons (PAHs). However, the fate and transport of these compounds is not

well understood in intermittent-flow infiltration-based stormwater management approaches, especially in the presence of sorbent amendments. This vegetated column study was conducted over a 7-month period and evaluated a sandy loam reference and three sorbents – coconut coir fiber (CCF), blast furnace slag (BFS), and waste tire crumb rubber (WTCR) – for removal of metals, nutrients, and PAHs from the intermittent application of synthetic stormwater. The unamended and amended media were able to sorptively remove > 99% the six metals that were included in the influent (save Zn leaching from WTCR), however high intensity more frequent storms resulted in temporary partial breakthrough of select metals (Cu and Cr), though BSF prevented any breakthrough regardless of hydrology. PAHs generally exhibited a similar pattern and were removed > 99% in most cases, but greater breakthrough of low molecular weight compounds was observed during the high intensity/high frequency storms, though CCF maintained the lowest effluent PAH concentrations. For metals and PAHs that exhibited partial breakthrough during high intensity/high frequency storms, the effluent concentrations were reduced when low intensity, long antecedent dry period events re-occurred, suggesting that mass transfer limitations or geochemical differences were important for removal. For nutrients, among all media, BFS-amended media had high phosphate removal capacity (> 90%) under tested conditions. Nutrient removal varied widely by species and storm event characteristics, where nitrate breakthrough was comparatively rapid during low frequency events, but then removal increased during high frequency/high intensity events. Effluent ammonium concentrations were greater than influent, with the highest effluent concentrations occurring during high intensity/high frequency storms, suggesting that dissimilatory nitrate reduction to ammonium was occurring at these times. Phosphate breakthrough was observed, save the BSF-amended system. Overall, this study identifies how contaminant dynamics depend on media and hydrology and further work should be conducted to evaluate climate and climate change effects on the performance of bioretention systems.

2.09.T-06 Exploring the Acute Cardiometabolic Impact of 6PPD-Quinone on Juvenile Salmonids: A Comparative Analysis

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is a transformation product of the most widely used rubber tire antioxidant, 6PPD. Commonly found in stormwater runoff, this compound has been reported to cause acute lethality at roughly ≤ 1 ug/L in a variety of salmonid species including coho salmon, rainbow trout, and brook trout. However, additional studies have shown other salmonid species such as arctic char and bull trout to be insensitive, even at significantly greater concentrations (20 ug/L). Sensitive species show distinctive symptoms including gasping, spiraling, increased ventilation, and loss of equilibrium, suggesting a possible impact on cardiorespiratory physiology. In an effort to understand why related species respond differently to exposure, we investigated the acute cardiorespiratory effects of 6PPD-quinone to two salmonids of varying sensitivity: a sensitive species, rainbow trout, and a tolerant species, Arctic char. Fish were exposed to 1 ug/L or 10 ug/L 6PPD-quinone in respirometry chambers for 48h to assess temporal changes in resting oxygen consumption compared to unexposed controls. Following exposure, cardiac ultrasound was used to characterize cardiac function by analyzing changes in ejection velocity, stroke volume, ventricular and atrial contractile rates, and cardiac output. Furthermore, electrocardiography was used to determine changes in the heart's electrical activity, and blood gas analyses was used to analyze changes in 19 parameters of the blood. Data show that 6PPD-quinone exposure does not significantly impact resting oxygen consumption rates in either species. In just rainbow trout, an increase in passive ventricular filling and cardiac output and a decrease in end systolic volume and PR interval length were observed, providing evidence of sympathetic stimulation. Cardiorespiratory symptoms observed following rainbow trout exposure might partly be driven by a significant increase in methemoglobin, resulting in an impaired ability to oxygenate the tissues. Significant cardiovascular effects were not observed in Arctic char. This is the first study to analyze the toxicity of 6PPD-quinone to the

cardiorespiratory system of fishes of commercial, cultural, and ecological importance at environmentally relevant concentrations and provides information invaluable to a better understanding of the mechanism of 6PPD-quinone toxicity.

2.10.P-Th Use of Freshwater Mollusk Toxicity Data for Improved Conservation of Water and Sediment Quality

2.10.P-Th-033 Tracking the Fate of Aged and Pristine Polyester Microfibres in Freshwater Mussel *Megalonaias nervosa*

*Yaryna Kudla*¹, *Patricia Gillis*², *Karen A. Kidd*³ and *Ryan S Prosser*¹, (1)University of Guelph, (2)Environment and Climate Change Canada, (3)McMaster University

Plastic debris polluting our waterways has been a concern for decades. Recently increased attention has been placed on microplastics (MPs) in aquatic ecosystems. These small plastic particles (<5 mm) have been observed in marine and freshwater ecosystems globally. To date, freshwater studies have focused on the presence and/or concentration of MPs in surface waters. To assess their risk, there is a need to compare environmental concentrations of MPs to concentrations that cause adverse effects. Freshwater mussels are a group of filter-feeding organisms that have experienced a decline due to habitat destruction and poor water quality, and they are under-represented in MPs research. Microfibers are one of the most common MP detected in freshwater environments, due to textile shedding and discharge from wastewater treatment plants. In this study, 7-day exposures were conducted with polyester microfibers generated from a red fleece blanket at 150,000 MP/mL. This concentration was chosen to facilitate detection of fibers in digested tissues. Adult *Megalonaias nervosa* (washboard) mussels were exposed through spiked algae food stocks, and tissue dissections occurred at day 1, 3, 7 and 2 days after the last spiked exposure. A short depuration period was included to assess whether adult *M. nervosa* has the ability to quickly egest un-palatable particles. Digestive gland, gonad, foot, and hemolymph samples were collected at each time point to determine whether microfibers tend to concentrate in a particular area of the mussel anatomy. Since MPs are prone to biofouling when released in the environment, a test with both aged and pristine microfibers was conducted to assess whether biofilm coatings influence initial ingestion as well as tissue translocation once ingested. This study serves as a preliminary assessment of the fate of an MP commonly detected in Canadian surface waters. Furthermore, this study investigates the potential of microfibers to accumulate in an organism that is a critical component of freshwater ecosystems, yet imperiled due to numerous anthropogenic impacts.

2.10.P-Th-034 Evaluation of Ammonia Toxicity to Juvenile Fatmucket (*Lampsilis siliquoidea*) at Different pH Levels in Short-Term Chronic Exposures

Chris D. Ivey, *Ning Wang* and *James L. Kunz*, U.S. Geological Survey

Freshwater mussels are extremely sensitive to ammonia. In 2013, the U.S. EPA included mussel toxicity data to update water quality criteria for ammonia to protect mussels and other sensitive organisms. A previous survival study indicated that ammonia toxicity increased with increasing pH values in acute 96-h exposures. Little is known about the influence of pH on ammonia toxicity to mussels in chronic exposures. The objective of this study was to determine chronic toxicity of ammonia to juvenile fatmucket (*Lampsilis siliquoidea*) mussels under a range of pH conditions with growth and survival as endpoints. We tested ammonia concentrations (expressed as total ammonia nitrogen (TAN)) from 0.125 to 16 mg TAN/L in four different pH waters (7.0, 7.5, 8.0, 8.5) following standard methods for conducting short-term chronic 7-d tests with juvenile mussels. The four different pH levels in the dilution water were maintained with the addition of hydrochloric acid or sodium hydroxide. Tests were conducted at 23°C under static-renewal conditions with daily test solution renewal. Mean measured pHs were 7.3, 7.7, 8.1, and 8.6 in the four pH waters, respectively. Measured TAN were within 5% of nominal concentrations. Relative to the control, mean length, dry weight, and total biomass were significantly reduced in the medium high to high concentrations in all four different pH waters. Total biomass was the most

sensitive endpoint, with lowest observed effect concentrations (LOEC) from 0.26 to 4.1 mg TAN/L in the four different pH waters. The ammonia LOECs significantly increased with pH ($r^2 = 0.94$). Results demonstrate the effect of ammonia on survival and growth of mussels is influenced by pH in chronic exposures. Results also provide guideline values to monitor water quality in water and sediment toxicity testing with juvenile mussels.

2.10.P-Th-035 Evaluation of Sediment and Water Quality to Support Freshwater Mussels in the Conasauga River, Georgia

Molly Martin, Brian J Irwin, Peter D Hazelton, Robert Bringolf and Kelly Filer Robinson, University of Georgia

Freshwater mussels are some of the most imperiled taxa in the world. Historically, threats to mussel populations have been attributed to habitat degradation or loss from dams, pollution, invasive species, and siltation. These disturbances are often correlatively associated with declines, yet research has often lacked rigorous methods of testing for causative factors of enigmatic loss. We plan to implement a combination of field and laboratory exposure trials to assess the effects of multiple contaminant stressors on freshwater mussel survival and growth in the Conasauga River, located in northwest Georgia and southern Tennessee. We will conduct substrate-exposure studies in the laboratory using sediment collected throughout the watershed following established sediment toxicity test conditions. Sediments will be collected in the Conasauga at multiple sites representing a gradient of municipal and agricultural contamination and varying land use practices. Additionally, we will deploy juvenile mussels in silos at the same sites utilized for the toxicity trials to assess responses to waterborne contaminants *in situ*. Mussel silos are concrete structures that allow for containment and retrieval of mussels. We expect to find differences in survival and growth across the contamination gradient. This study will inform reintroduction efforts for freshwater mussels in the Conasauga River as well as focus stream conservation efforts in the basin. Additionally, data collected will further our understanding of the role sediment and waterborne contaminants play in the decline of freshwater mussels.

2.10.T Use of Freshwater Mollusk Toxicity Data for Improved Conservation of Water and Sediment Quality

2.10.T-01 Assessing Ammonia Toxicity of Texas Unionid Mussels

Ericah D Beason¹, Somerley J Swarm², Lee Jacob Gudgell³, Tara L Lanzer⁴, Clinton R Robertson⁵ and Astrid N Schwalb⁴, (1)South Carolina Department of Natural Resources, (2)U.S. Fish and Wildlife Service, (3)Guadalupe-Blanco River Authority, (4)Texas State University, (5)Texas Parks and Wildlife Department Co-presented by Somerley J. Swarm. Degradation to water quality poses a threat to freshwater mussels, including non-point source nutrient pollution from agriculture, and permitted point sources such as wastewater discharges. Early life history stages of unionid mussels are known to be highly sensitive to ammonia, yet the Texas Commission on Environmental Quality (TCEQ) wastewater effluent limits on ammonia follow pre-2013 United States Environmental Protection Agency (USEPA) ammonia guidelines that are known to be lethal to freshwater mussels. Data on ammonia toxicity for freshwater mussels in Texas should help inform revision to TCEQ's wastewater discharge criteria for ammonia and the development of the Guadalupe River Habitat Conservation Plan to limit impacts to freshwater mussels. Hence, the objective of this study was to determine median lethal concentrations (LC50s) for total ammonia nitrogen (TAN) with glochidia (24 – 48 hours) and newly released juveniles (96 hours exposure) of state-listed mussel species in Texas, including species proposed to be listed under the Endangered Species Act, following the American Society for Testing and Materials (ASTM International) Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels. Brooding mussels were collected from the Sabine and Guadalupe Rivers in Texas. The 24-hour LC50s for glochidia of *Fusconaia askewi*, *F. mitchelli*, *Lampsilis bergmanni*, *L. hydiana*, *L. teres*, *L. satura* and *Potamilus purpuratus* ranged between 27 and 83 mg/L of TAN (at pH 7 & 20°C). The 96-hour LC50s for juveniles of *L. bergmanni*, *L. hydiana*, *L. satura* and *P. purpuratus* ranged between 19 and 51 mg/L of TAN (at pH 7 & 20°C)

Additional trials are ongoing, but initial results indicate that EPA 2013 acute criterion maximum concentration (CMC) toxicity limits for TAN are likely protective of most Texas Unionid species. Extracting glochidia from short-term brooders with conglutinates was challenging and it was extremely difficult to extract a sufficient number of glochidia with high viability from different females. Consequently, during further testing for this project we will hold short-term brooding mussels until they naturally release their conglutinates and conduct toxicity tests with one individual at a time.

2.10.T-02 The Toxicity and Bioaccumulative Potential of the Anti-Malaria Pharmaceutical, Atovaquone to Freshwater Mussels and Chironomid Larvae

Patricia Gillis¹, Naomi L. Stock², Danielle Milani¹, Shirley Anne Smyth¹, Joseph Salerno¹, C. James Bennett¹, Erika Burton¹ and Steven Teslic¹, (1)Environment and Climate Change Canada, (2)Trent University

Atovaquone (CAS-95233-18-4) is a drug administered for the prevention and treatment of malaria and toxoplasmosis due to its broad-spectrum antiprotozoal properties. However, information was lacking on the toxicity and bioaccumulative potential of Atovaquone to non-target organisms as well as its fate in ecosystems that receive municipal wastewater effluents. In addition, no analytical methods were available to quantify Atovaquone in environmental matrices. Methods to analyze Atovaquone in water, biota, and sediment were developed using Deuterium-labelled Atovaquone and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Optimized solid phase extraction was required to quantify trace levels of Atovaquone in environmental water samples. Analysis of wastewater effluents and surface water from an urban river revealed that Atovaquone was below the detection limit (13 ng/L) in surface waters and 6 of 7 wastewater effluents examined, while the seventh effluent contained 15.5 ng/L. The toxicity of Atovaquone to first instar *Chironomus riparius* was assessed using 10-day exposures to Atovaquone-augmented reference (i.e. natural) sediments. Because no significant differences in chironomid survival among treatments (up to 450 µg Atovaquone/g sediment) were observed, an LC50 could not be calculated. Effects on *C. riparius* growth were observed, with an estimated 50% reduction at ~355 µg/g (i.e., Inhibition Concentration, IC₅₀). The toxicity of Atovaquone to mussels was determined using 14-day aqueous exposures and adult *Eurynia dilatata*. An LC50 of 83.7 µg/L (95% confidence intervals, 62.2-105.1) was derived for mussels. The bioaccumulative potential of Atovaquone was assessed using lab-exposed adult mussels. The uptake (28 d) and depuration (14 d) patterns of aqueous Atovaquone were investigated using two exposure concentrations, 10 and 100 µg/L (nominal) as per the USEPA Oyster Bioconcentration Factor protocol. While mussels accumulated Atovaquone at both exposure concentrations, the data were variable, likely due to poor solubility. The concentration of Atovaquone in mussels showed an average (Log₂) fold-increase of 1.9 in the 10 µg/L treatment and 3.1 in the 100 µg/L from day 1 to 28 of the exposure period. During the 14-day depuration period in clean water there was an average (Log₂) fold-reduction of 2.3 in both treatments, indicating that mussels eliminated 80% of accumulated Atovaquone from their tissues post-exposure.

2.10.T-03 Comparing the Response of Glochidia and Juveniles of Common and Federally Endangered Freshwater Mussels to Three Contaminants of Concern

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Freshwater mussels are in sharp decline and while many factors are thought to contribute to this, contaminants and degraded water quality are often cited as significant factors. The state of Michigan is home to several populations of the federally endangered *Epioblasma triquetra* (Snuffbox), and long-term monitoring by numerous agencies indicates prevalence of mercury (Hg) and per- and polyfluoroalkyl substances (PFAS) both within watersheds inhabited by Snuffbox and more widely across the Great Lakes Basin. Little is known about how these contaminants may impact mussel health and recruitment. In addition, freshwater salinization, resulting from a variety of anthropogenic land use changes including resource extraction and road salting, has

become a water quality issue in the region as it has globally. The most direct approach to assessing the risks of contaminants to federally threatened or endangered (TE) species like *E. triquetra* is to test them in the laboratory; however, there are often barriers to this approach like animal availability or permitting issues. Therefore, surrogate species such as *Lampsilis siliquoidea* (Fatmucket) which are more common and/or more amenable to culture in the laboratory are often tested in place of TE species. The extent to which the responses of common species of mussels like Fatmucket represent the responses of endangered species like the Snuffbox is poorly understood. Our objective was to compare the acute responses of Fatmucket and Snuffbox early life stages to Hg, the PFAS perfluorooctane sulfonate (PFOS), and NaCl (representing road salt), conducting 96- and 24-h bioassays with juveniles and glochidia, respectively. We observed similar sensitivities between the two species for juvenile responses, but Snuffbox glochidia were approximately 4- and 7-fold more sensitive than Fatmucket glochidia to PFOS and Hg, respectively. Conversely, Snuffbox glochidia were nearly 2-fold less sensitive to NaCl than Fatmucket; however, it should be noted that source population for snuffbox was from a site with background chloride concentration greater than 200 mg/L, which may have resulted in reduced Snuffbox sensitivity via adaptation. With similar juvenile responses but disparate effect levels for glochidia, it is evident more work is needed to ensure risks of environmental contaminants to early life stage endangered species are adequately assessed.

2.10.T-04 Developing a Reproductive Toxicity Test Method for Freshwater Mussels

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Current ASTM standard methods for toxicity testing with freshwater mussels only use survival and growth endpoints. The objective of this study aimed to develop a method for evaluating contaminant effects on mussel reproduction, specifically on the spawning process, fertilization, embryonic development of glochidia, and parasitism success, using a short-term brooder (Deertoe, *Truncilla truncata*) and a reference toxicant, potassium chloride (KCl). Field-collected adult male and female Deertoe were held separately in two flow-through diluters. The diluters supplied 6 KCl concentrations (50% serial dilution). Mussels were exposed individually in each of 4 replicate chambers per test concentration at 10°C for 2 weeks. Thereafter, temperature was raised to 15°C to trigger spawning. Male spawning was monitored through video cameras. Fertilization was performed by exposing females to a milt mixture from untreated males 3 times a day for 3 days. Following spawning, females were exposed to KCl for an additional 6 weeks during embryonic development. Afterwards, glochidia were collected to inoculate the host fish (Freshwater drum, *Aplodinotus grunniens*). All males spawned, mostly within 10 hours of the temperature increase. The onset of male spawning and spawning duration were not significantly different between the control and any treatments. Although 90% of females spawned, only 22% had viable glochidia at the end of the exposure. Many females released immature glochidia throughout the embryonic development period, and the quantity of brood varied greatly among females. Glochidia viability, at the end of adult exposures and in a 24-hour continuous exposure, as well as parasitism success and juvenile length post-metamorphosis were not significantly different between the control and any treatments. Our results indicate that the reproductive toxicity test was successfully completed with male Deertoe but not with females. We will refine our methods in the second year of the study to improve fertilization and embryonic development under control conditions.

2.10.T-05 Effects of Copper, Elevated CO₂ and their Combination on Growth, Calcification, Gene Expression and Acid-Base Balance in *Lymnaea stagnalis*

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Early life stages of the freshwater pond snail, *Lymnaea stagnalis*, are highly sensitive to copper with growth and calcification being reduced at low exposure concentrations. Although acidification resulting from elevated CO₂ is known to challenge calcification in aquatic animals, we are aware of no studies examining impacts of climate

change relevant CO₂ levels on freshwater mollusks. Further, few studies have addressed the effects of combined exposures to copper and acidification caused by elevated CO₂ even though these two stressors both challenge calcification and therefore are likely to exhibit synergistic effects. In agreement with earlier studies, we observed growth reduction in juvenile snails exposed to copper (10 µg l⁻¹). Contrary to expectations, elevated CO₂ (1900 µAtm) did not inhibit growth but, as predicted, resulted in slight reduction in calcification over time. Surprisingly, elevated CO₂ did not act synergistically with copper but rather appeared to alleviate copper effects. These effects were not explained by reduced copper accumulation in snails exposed to the combination of copper and CO₂. Experiments on adult snails revealed a respiratory acidosis in copper exposed snails, which was partially compensated by elevated hemolymph HCO₃⁻. Exposure to elevated CO₂ resulted in full compensation with normal hemolymph pH and elevated HCO₃⁻. It appears that exposure to elevated CO₂ stimulates compensatory elevation of hemolymph HCO₃⁻ which serves as substrate for calcification. In agreement with growth, calcification and acid-base balance data, expression of candidate genes involved with calcification and acid-base balance showed modest changes. However, copper exposed snails showed reduced expression of carbonic anhydrase 1 (CA1), an isoform likely involved in maintaining acid-base balance, an effect which was again alleviated by simultaneous exposure to copper and elevated CO₂. In conclusion, copper exerts potent effects on acid-base balance, growth, and calcification in *Lymnaea stagnalis*, whereas climate change relevant elevations of CO₂ resulted in only mild effects on calcification. Elevated CO₂ appears to protect against copper exposure, rather than act synergistically, in juvenile snails fed ad libitum.

2.10.T-06 Metal Accumulation, Fitness Effects, and Maternal Transfer in Non-Native Mysterysnails

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Originally from Asia, non-native mysterysnails have spread to waters of the United States and negatively impacted native aquatic species. In non-native waters, these species can readily accumulate metals from aquatic environments into their shells and soft tissues. The extent to which mysterysnails accumulate metals, the maternal transfer of metals associated with the ovoviviparous reproduction of these species, and the fitness consequences associated with this accumulation, however, are poorly documented. Therefore, the objectives of this study were to 1) quantify metal accumulation within the tissues and shells of mysterysnails collected from western Pennsylvania waterbodies, 2) determine the impact of metal accumulation on mysterysnail fitness, and 3) evaluate maternal transfer of metal contaminants. Non-native mysterysnails were collected from six locations across western Pennsylvania, including Presque Isle State Park, Lake Arthur, Pymatuning Reservoir, Lake Canadohta, Edinboro Lake, and Lake Pleasant. Mysterysnail fitness was then evaluated via behavioral assays to monitor activity, shell morphology, shell thickness, and shell/tissue weight. After a 24 h depuration period, adult snails were euthanized and dissected. Tissues (i.e., gonad, gut, mantle, foot, operculum), shells, and babies, when present, were weighed and acid digested for metal analysis along with metal and water samples from collection sites using an inductively coupled plasma mass spectrometer. Mysterysnails accumulated metals, including cadmium, copper, lead, nickel, and zinc into soft tissues from sediment, with very little accumulation in the shell. Increasing metal concentrations in the foot and operculum correlated to reduced snail activity, while increasing copper concentrations caused changes in shell morphology. Similarly, significant correlations in copper concentrations between mothers and offspring suggest maternal transfer, yet this offloading did not reduce maternal metal concentrations in adult females compared to adult males. Understanding accumulation of contaminants by non-native species provides insight into ecological effects of invasion and better informs population management efforts.

2.11.P-Mo Assessing Wildfire and Organic Stressor Effects on Aquatic Ecosystems

2.11.P-Mo-079 CVOCs Kinetics and Adsorption Studies on Selective Macrocyclic Adsorbents for Advanced Groundwater Bioremediation

Elham Abaie, Texas Tech University

Chlorinated volatile organic solvents (CVOCs) are often found in combination with 1,4-dioxane which has been used as a stabilizer. It would be preferred to separate these compounds since biodegradation of 1,4-dioxane follows an aerobic pathway while anaerobic conditions are needed for biodegrading CVOCs. Conventional adsorbents such as activated carbon and resins have high adsorption capacities for both 1,4-dioxane and CVOCs, limiting their use in separating them. In the current work, two macrocyclic adsorbents, β CD-TFN and Res-TFN, were examined for selective adsorption of chlorinated ethenes in the presence of 1,4-dioxane. Both sorbents exhibited rapid sorption of the CVOCs and minimal sorption of 1, 4 dioxane. Sorption isotherms were approximately linear although both linear and Freundlich models were used to fit the observations at groundwater relevant $\mu\text{g/L}$ concentrations. Res-TFN had a higher adsorption capacity for CVOCs than β CD-TFN (estimated $K_{\text{ds}} \sim 10000 \text{ L/kg}$ versus 1000 L/kg , respectively). The higher adsorption capacities of Res-TFN could be attributed to its higher hydrophobicity compared to β CD-TFN.

2.11.T Assessing Wildfire and Organic Stressor Effects on Aquatic Ecosystems

2.11.T-01 Post-Fire Sediment Deposition and Associated Geochemistry Threaten Water Quality

Elizabeth Tomaszewski, Johanna Blake, Michelle Hornberger and Sheila Murphy, U.S. Geological Survey

In April 2022, the Hermit's Peak and Calf Canyon fires burned the Gallinas Creek watershed, west of Las Vegas, New Mexico, impairing the community's water quality and potentially causing ecosystem damage. Post-fire, increased erosion during monsoons and other storm events in the watershed led to the deposition of sediment and burned material on stream banks. The depths of these deposits ranged from 10-24 cm and were a mixture of ash-rich, burned material and unburned fine-grained layers interbedded with sand. The Gallinas Creek watershed contains deposits of copper (Cu), uranium (U), and rare earth elements (REEs) and sediments transported downstream post-fire may potentially be enriched in these elements and others. Historical mining (Cu) and prospecting (U) activities may further influence metal mobility in the area, and the long-term effects and reactivity of these sediment deposits post-fire are unknown. To address these knowledge gaps, sediment deposits and water quality samples were collected from two sites 6 months and 1-year post-fire. To evaluate constituent mobility, laboratory extractions using deionized water and aqua regia were performed on distinct sediment layers at each site. Dissolved organic carbon, major and trace metals, alkalinity, and anions were analyzed in both surface water and leachates. Concentrations of all constituents varied between sites and with increasing depth along stream banks. For example, vanadium (V) ($16 \pm 0.36 \mu\text{g/L}$) was mobilized from sediment deposits with deionized water from one site, but not mobilized from sediments at other sites. Concentrations of REEs were quantifiable in deionized water extractions of sediment deposits; however, the highest REE concentrations ($3.8 \pm 0 \mu\text{g/L}$) were measured in unfiltered water samples. Elevated metal concentrations in deep sediment deposit layers and in particulate matter are a potential risk to the receiving waters given that future storm events are most likely to interact first with the lower bank material and particulates. This work examines the longer term and secondary effects that wildfires can have on water quality and highlights the need for further geochemical monitoring and studies post-fire.

2.11.T-02 Critical Drivers of Post-Wildfire Water Quality and Biological Impairment in the Gallinas Creek New Mexico Watershed

Michelle Hornberger, Sheila Murphy, Elizabeth Tomaszewski and Johanna Blake, U.S. Geological Survey

The 2022 Calf Canyon/Hermit's Peak Wildfire was the largest wildfire in New Mexico history, burning 95% of

the Gallinas Creek watershed. Heavy storms during the summer monsoon season mobilized ash, soil, and wildfire debris into Gallinas Creek and a drinking water reservoir for Las Vegas, New Mexico. The El Porvenir Mining District, a quartz-pegmatite vein, lies within the Gallinas Creek watershed and has a history of copper and uranium prospecting and mining. Here we use a conceptual framework (Driver—Factor—Stressor—Effect) to characterize the critical drivers influencing post-wildfire water quality in the Gallinas Creek Watershed. Critical drivers are categorical and include weather, fire, ecology, land cover, and soils. For the purposes of the conceptual framework, metal concentrations in post-fire bed sediment and water were determined as the primary “stressors”. Bioaccumulation and macroinvertebrate density were identified as the “effects”. Sediment and water samples were collected 6 months and 1-year post-fire from Gallinas Creek and several tributaries within the watershed and measured for a variety of constituents, including metals, nutrients and DOC. Metal concentrations in fine-grained (<63µm) bed sediment were spatially dispersed, with arsenic concentrations low at the most upstream site (Gallinas Creek at Oak Flat, $4.1 \pm 1.1 \mu\text{g/g}$), moderate downstream (Gallinas Creek near Montezuma, $5.3 \pm 0.3 \mu\text{g/g}$) and highest in the Beaver Creek sub-watershed (6-10 $\mu\text{g/g}$) which drains into Gallinas Creek. Cadmium and copper concentrations followed a similar spatial pattern concentrations where the highest concentrations occurred near legacy mining activities (e.g., Cu 100-443 $\mu\text{g/g}$). Biological effects associated with post-fire metal exposures were determined by assessing macroinvertebrate density and tissue residue concentrations at each site. While macroinvertebrates were mostly absent during the 6-month post-fire sampling effort, mayflies, chironomids, and stoneflies were present 1-year after the fire, indicating initial signs of recovery.

2.11.T-03 Extent and Pathways of Per- and Polyfluoroalkyl Substances (PFAS) Bioaccumulation and Biomagnification in Mid-Atlantic Stream Food Webs Impacted by Firefighting Foams

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Environmental contamination of per- and polyfluoroalkyl substances (PFAS) is a major problem at hundreds of sites with historical use of firefighting foams. PFAS are transported into aquatic systems via multiple pathways and remain highly mobile and persistent. There is growing evidence that some PFAS bioaccumulate, however, the extent and mechanisms of PFAS bioaccumulation and biomagnification in food webs are not well characterized nor understood. Thus, assessing the ecological and human health risks associated with the presence of PFAS in aquatic systems requires a better understanding of the extent, pathways and rates by which PFAS enter, are taken up by and transported through streams and aquatic food webs. We conducted a comparative field study to assess PFAS exposure, bioaccumulation and biomagnification in three streams in Willow Grove, PA that range in size, PFAS concentration, and PFAS transport pathway from a common source attributed to firefighting foams, plus an upstream regional background site. Water and sediment were sampled weekly for 4 weeks, ending with a single campaign sampling the full aquatic food web, incl. algae, vascular plants, insect and non-insect macroinvertebrates, amphibians, and 17 species of fishes. All samples were analyzed for 24 PFAS concentrations, biotic samples for stable isotopes, and water and sediment samples for relevant environmental chemistry. We observed distinct environmental PFAS concentration profiles across the different sites, consistent with PFAS source and transport via stormwater runoff vs. groundwater pathways. Temporal variability in environmental PFAS concentrations and profiles was high, as was within-site spatial variability in sediments, with implications for accurately quantifying uptake. Concentrations in all media are dominated by PFOS. We see clear PFAS bioaccumulation across trophic levels, with differences with respect to PFAS compound head group, chain length, and concentration. We also see clear trophic magnification but the relationships to head group and chain length is less predictable, with no apparent threshold behavior with trophic position or clear trends within organismal groups. The pending carbon isotope results may help to resolve the effects of trophic level and food web structure on biotic exposure route and biomagnification.

2.11.T-04 The Effect of Wildland Fire-fighting Chemicals on the Reproductive Success of an Aquatic Invertebrate

Holly J Puglis, Michael Iacchetta and Christina Mackey, U.S. Geological Survey

With wildfires increasing in frequency and intensity in the western United States, it is essential to understand how the chemicals used to suppress the spread of wildfire interact with the landscape. The effect on environmental health must be understood to use these chemicals safely and effectively in emergencies. Studies have recently been published on the lethality of fire-fighting chemicals currently used by the U.S. Forest Service; however, little is known about how these chemicals may impact the reproductive success of organisms affected by chemical intrusions. We investigated the reproductive success of a commonly tested cladoceran (*Ceriodaphnia dubia*). Specifically, female cladocerans were exposed to a concentration series of two current-use fire-fighting chemicals (Phos-Chek® 259-Fx and Phos-Chek® LC95A-R) at two durations (15 or 60 minutes) to determine effects on reproduction. There was no effect on reproduction of *C. dubia* in the Phos-Chek® LC95A-R exposures (up to 3,500 mg/L). There was a significant decrease in number of neonates produced in the 3,500 mg/L Phos-Chek® 259-Fx treatment compared to the control females, but duration of exposure was not significant (nor the interaction). Measures of reproduction are a hallmark indicator of the ecological impact of chemical products on biota because impaired reproduction has significant consequences for the structure of natural populations, as well as for the stability of the food web. Further investigation of aquatic biota found in streams native to areas with high occurrence of wildfire should be studied to determine the potential population level changes that may occur from fire-chemical intrusion.

2.11.T-05 Lipid Profiling in Aquatic Organisms: Does Field Exposure to Per- and Polyfluoroalkyl Substances (PFAS) Affect Oxylipin Stress Markers?

Eve Gilroy¹, Stacey A Robinson¹, Amy Rand² and Almira Khan¹, (1)Environment and Climate Change Canada, (2)Carleton University

The oxidized lipid (oxylipin) metabolome refers to the lipid metabolites derived from polyunsaturated fatty acids (PUFAs). Oxylipins have far-reaching roles as regulators of inflammation, vascular tone, cardiac function, and development. LC-MS/MS based tools to analyze the oxylipin metabolome are gaining popularity in pharmacological based studies of drug development and mechanisms of disease. However, little is known about how the oxylipin metabolome is impacted from exposure to environmental contaminants. The purpose of this study was to determine relationships between levels of per- and polyfluoroalkyl substances (PFAS), PUFAs, and oxylipins in *in situ* caged freshwater snails. An LC-MS/MS targeted metabolomic method was developed to include four PUFAs and 45 oxylipins derived from several PUFAs (e.g., the omega-6 PUFA arachidonic acid and the omega-3 PUFA docosahexaenoic acid), and quantified with 20 labelled oxylipin standards. Instrumental limits of detection ranged from 0.5-3 ng/g. To assess extraction efficiency, spike and recovery experiments with biotic tissue were carried out, yielding recovery values between 70 – 125%. Snails (n=25) were exposed to mixtures of PFAS by caging them downstream from a local airport, at locations where PFAS contamination has been reported. Samples were homogenized, extracted with acidified methanol and cleaned using HLB SPE prior to instrument analysis. Metabolite profiles were differentiated using a three component, partial least squares discriminant analysis model. We anticipate that the PFAS will significantly correlate with PUFAs and select downstream oxylipin metabolites. More advanced location-specific analyses on these freshwater organisms and insights into the altered biological mechanisms and toxicology are underway.

2.11.T-06 Concentration Dependency of PFOS Update by Benthic Algae

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PFOS (perfluorooctanesulfonic acid) is included on the United Nation's Stockholm Convention List of Persistent Organic Pollutants for its extreme persistence, bioaccumulation, and toxicity. Although PFOS has

been voluntarily phased out by US manufacturers, it is still produced internationally and can be detected in a range of organisms worldwide including mammals, fish, birds, and reptiles from the Great Lakes to Antarctica. A better understanding of how PFOS enters the aquatic food web at the energetic base is needed to characterize the compound's abilities to move and magnify through the food web. However, limited research has investigated PFAS bioaccumulation by primary producers. Here, we conducted a laboratory study to investigate PFOS incorporation by benthic algae and determine if the uptake process is concentration dependent. We exposed freshwater diatoms (*Mayamaea atomus*) to a log-series of concentrations consisting of 0, 0.01, 0.1, 1, 10, 100 $\mu\text{g/L}$ PFOS for 7 days (n=4 replicates per concentration). Diatoms were grown on plastic microscope slides and exposure chambers all received algal growth media. Slides were removed after 7 days, biofilm was scraped, and the tissue was air dried; 250-mL water samples were also collected from each chamber to confirm target concentrations. PFOS was extracted from the biofilm using methanol, formic acid, and sonication and samples were cleaned-up with ENVI-carb cartridges. We used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify PFOS concentrations in water and biofilm. PFOS concentration in the biofilm increased in an asymptotic fashion suggesting saturation of uptake. We also observed a decreasing bioconcentration factor as PFOS exposure concentration increased. BCF values ranged from 10 to 78. The results indicate that bioaccumulation of PFOS in freshwater diatoms is concentration dependent and that freshwater diatoms could provide a route for PFOS exposure in higher trophic levels of the aquatic food web.

2.12.P-Tu Freshwater Salinization: Causes, Effects and Working Towards Solutions

2.12.P-Tu-069 Measuring Intraspecific Variation in Tolerance to Road Salt for *Daphnia* Populations Sampled Along Spatial Gradients in Lake Chloride Levels

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Road salt levels are increasing in many temperate lakes across the Northern Hemisphere; urbanization and overapplication results in excessive road salt (i.e., chloride) run-off. Existing chloride $[\text{Cl}^-]$ concentration guidelines may not protect all aquatic food webs due to spatial variability in salt loadings, background water chemistry, and variable species responses. Local losses of primary consumers (e.g., *Daphnia*), leads to increased algal biomass and decreased fish abundances. This negatively impacts people across the Northern Hemisphere with losses in ecosystem services and functions (e.g., recreation, agriculture, drinking water). This study will contribute to filling a knowledge gap about spatial variability in a species of *Daphnia* (i.e., *D. galeata*) tolerance to chloride concentrations $[\text{Cl}^-]$. Our hypothesis is that intraspecific variation in *D. galeata*'s tolerance to $[\text{Cl}^-]$ is positively related to previous exposure levels in source lake systems. We tested *D. galeata*'s tolerance to $[\text{Cl}^-]$ using 48-hour mortality ("LC50") tests. Live *D. galeata* and grab water samples were collected during the summer of 2022 at six sites known to differ in conductivity levels: two sites were located along the urbanized shore of Lake Ontario and four sites were located in Lake Scugog – a rural lake 1 hour northeast of Toronto. A single iso-female clonal line was established per site with two generations reared under standardized conditions. Genetically identical *D. galeata* individuals of a standard age (<24 hours) were placed into 10 mL test tubes for 10 $[\text{Cl}^-]$ treatments (18-2700 mg/L^{-1}), each with three replicates, to determine survival after 48 hours based on movement of appendages and internal organs. Each replicate per treatment concentration was tested for the number of survivors out of 10 individuals. The results showed that survival declined with increasing chloride concentrations for all *D. galeata* collected from different regions of Lakes Ontario and Scugog; however, variation was found to exist in the overall trend (i.e., rate of decline) with an LC50 range of 900-1500 $[\text{Cl}^-]$ mg/L^{-1} across sites. Therefore, on a small scale, intraspecific variation was found to exist for areas of Lakes Ontario and Scugog. Future research could incorporate this data into a larger pool of data to better understand intraspecific variation in *Daphnia*'s chloride tolerance and better inform chloride guidelines.

2.12.P-Tu-070 Toxicity Study for Supporting Louisiana State Water Quality Standards for Chloride and Sulfate

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Louisiana's water quality standards (WQS) for chloride (Cl) and sulfate (SO₄) were established in the 1990s by Louisiana Department of Environmental Quality (LDEQ) using the arithmetic mean plus three standard deviations from ambient chemistry data of the nearest sampling station. The LDEQ is considering the development of toxicity-based WQS and proposed toxicity testing with native species in low-hardness conditions representing Louisiana inland waters (median hardness of ~40 mg/L as CaCO₃). In a 3-year study, we first reviewed existing Cl and SO₄ data in literature and found limited toxicity data from tests conducted in soft waters (<50 mg/L hardness) and met ASTM test acceptability criteria. To meet the minimum data requirement for 8 taxa in USEPA's Guidelines for deriving national water quality criteria (except for salmonids that are absent in Louisiana), we conducted acute toxicity tests with 7 species (6 taxa; including a federally threatened mussel) per toxicant in a soft test water (40 mg/L hardness) following ASTM standard methods. Acute EC50s were close to 1,000 mg Cl/L and 1,050 SO₄/L for the most sensitive species (mussel) and close to 6,000 mg Cl/L and 11,500 mg SO₄/L for the least sensitive species (midge). We further evaluated the hardness influence by conducting acute tests with 3 sensitive species in 4 waters with varying hardness (15, 30, 60, and 120 mg/L) and found that the Cl and SO₄ toxicity significantly decreased with increasing hardness, except for one species in a Cl test. We compiled the Cl and SO₄ toxicity-hardness datasets from the present and previous studies at a low hardness range (14-142 mg/L) to calculate an acute value-hardness slope. An insignificant species:ln(hardness) interaction was observed for both Cl and SO₄ datasets, and a pooled slope for the log(hardness):log(acute values) was calculated (0.406 for Cl; 0.656 for SO₄). Acute values at low hardness of <120 mg/L in our compiled datasets were then normalized to the hardness of 40 mg/L using the derived slopes and genus mean acute values (GMAVs) were calculated. We also conducted chronic toxicity tests in a 40-mg/L hardness water with a fish and 2 invertebrates to estimate acute-chronic ratios (ACRs) for chronic WQS development; ACRs were similar (~2-4) for the two invertebrates in the Cl or SO₄ exposures but was much higher (~30-40) for the fish. A total of 32 GMAVs for Cl and 18 GMAVs for SO₄ as well as ACRs have been provided to LDEQ to inform WQS development.

2.12.P-Tu-071 The Physicochemical Characterization of Natural Dissolved Organic Carbons (DOCs) and Their Physiological Effects in Pacific sanddab (*Citharichthys sordidus*) as a Function of Salinity

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Many flatfish species are partially euryhaline, such as the Pacific sanddab which spawn and feed in highly dynamic estuaries ranging from seawater to near freshwater. With the rapid increase in saltwater invasion of freshwater habitats, it is very likely that in these estuaries, flatfish will be exposed to increasing levels of dissolved organic carbon (DOC) of freshwater origin at a range of salinities. Diffusive water flux (a proxy for transcellular water permeability), oxygen consumption, ammonia excretion and urea-N excretion rates were measured as a function of salinity. As salinity fluctuations often coincide with changes in DOC concentration, two natural freshwater DOCs [Luther Marsh (allochthonous) and Lake Ontario (autochthonous)] were used to evaluate the interactive effects of salinity and DOC. In the absence of added DOC, an increase in salinity resulted in a reduction in diffusive water flux and ammonia excretion rates. The effects of DOC (10 mg C/L) on diffusive water flux, ammonia and urea excretion rates were both salinity-dependent and source-dependent, which may be due to physicochemical changes in the DOCs between salinities. The physicochemical differences between the two natural DOC sources, at each test salinity (7.5 ppt and 30 ppt) were optically characterized using absorbance and fluorescence measurements, revealing changes in their optical behavior. Our results demonstrate that marine flatfish alter gill function in the presence of DOC as a function of salinity which may be a result of the physicochemical changes in DOCs between salinities. (NSERC Discovery).

2.12.P-Tu-072 Evaluating Response of Several Freshwater Species to Major Ion Mixtures: Inferences Regarding Toxic Mechanisms, Exposure Metrics, and Integrative Assessment

Russell J. Erickson and David R. Mount, U.S. Environmental Protection Agency

Anthropogenic elevations of major geochemical ions in freshwater systems have varied sources and involve complex mixtures in which ion ratios can vary widely. Based on extensive testing of the effects of major ions on several diverse freshwater species (*Ceriodaphnia dubia*, *Pimephales promelas*, *Hyaella azteca*, *Chironomus dilutus*, *Lumbriculus variegatus*, *Lymnaea stagnalis*), the toxicity of such mixtures may be a function of several mechanisms which are expressed differentially depending on the combination of major ions present and on the receptor organism. Apparent mechanisms or associations include: osmotic impacts of total ions; non-specific toxicity correlated with total ions but not associated with osmotic effects; toxicity specific to Ca/Mg, K, and SO₄; and effects of high pH/alkalinity. Overall, the data indicate that total ion concentration is a useful correlate of response for many species and major ion mixtures, but that separate provisions are needed to assess effects of mixtures dominated by an ion with specific action. The implications of these findings for developing generalized exposure-response expressions that might support aquatic risk assessment and the development of regulatory guidelines are discussed. *This abstract does not necessarily reflect U.S. EPA policy.*

2.12.P-Tu-073 Evaluating Response of Several Freshwater Species to Major Ion Mixtures: Interspecies Comparisons Regarding Toxicity Mechanisms and Effects of Background Water

Russell J. Erickson and David R. Mount, U.S. Environmental Protection Agency

Risk assessments for anthropogenic elevations of major geochemical ions need to consider both the high variability of ion composition for exposures of concern and the wide diversity of ion regulation/effects among different organisms. Extensive testing of the toxicity of major ion salts and mixtures to several diverse freshwater species (*Ceriodaphnia dubia*, *Pimephales promelas*, *Hyaella azteca*, *Chironomus dilutus*, *Lumbriculus variegatus*, *Lymnaea stagnalis*) has indicated toxicity to be a function of several mechanisms that are differentially expressed depending on the relative amounts of the different ions and on the receptor organism. Apparent mechanisms or associations include: osmotic impacts of total ions; non-specific toxicity correlated with total ions but not associated with osmotic effects; toxicity specific to Ca/Mg, K, or SO₄; and effects of high pH/alkalinity. Toxicity can also depend on exposure water characteristics separate from any direct contribution to toxic action. This poster examines the apparent mechanisms operational in each species, relationships for effects across various ion mixtures, and similarities/differences in major ion toxicity across the species. *This abstract does not necessarily reflect U.S. EPA policy.*

2.12.P-Tu-074 Effect of NaCl on Feeding Behaviors of *D. magna* and *H. azteca*

Paige Elizabeth Kohler, Ellie E Casey and Amanda D Harwood, Alma College

While the acute toxicity of road salts has been historically understood for a variety of aquatic invertebrates, there is less research available on the effects of these compounds on ecosystem health and function. Research suggests that freshwater salinization can induce trophic cascades, altering the structure of the ecosystem. One way it may do this is by impacting organism behaviors. The objective of the current study was to evaluate the effects of road salts, specifically NaCl, on the ability of *Daphnia magna* to filter green microalgae (*Pseudokirchneriella subcapitata*) and *Hyaella azteca* to shred conditioned *Acer saccharum* leaves when exposed to sublethal concentrations of NaCl. Juvenile *D. magna* or adult *H. azteca* were exposed to various sublethal concentrations of NaCl and the proportion of algae removed, or leaf material broken down was determined post exposure. Preliminary data indicates that NaCl may have an impact on the feeding rates of both species. This indicates that when NaCl concentrations are elevated, namely during the winter months, the function of freshwater ecosystems may be vulnerable to additional stressors.

2.12.P-Tu-075 The Interactive Effects of Major Ions, Dissolved Organic Carbon and pH on the Electrical Responses of the Gill in Rainbow Trout (*Oncorhynchus mykiss*)

Carolyn Morris, Colin J. Brauner and Christopher M. Wood, University of British Columbia

Freshwater salinization through anthropogenic activity or seawater invasion leads to increases in major ion concentrations including Na^+ and Ca^{2+} cations and Cl^- anions. These elevations in ions disturb the ionoregulatory processes of freshwater organisms. Transepithelial potential (TEP), the electrical gradient across the gills between the animal and the external water, can be used as an index of ionoregulatory stress. The Multi-Ion Toxicity Model (MIT) predicts that a disturbance in TEP is indicative of salt toxicity. At circumneutral pH, dissolved organic carbon (DOC) has been found to mitigate ionoregulatory stress responses to low environmental pH in freshwater fish by lowering TEP. We investigated whether DOC and exposure to elevated major ions interact with TEP responses at circumneutral and low environmental pH in our test organism, the freshwater rainbow trout. The heterogeneity and complexity of natural DOCs make them challenging to study in a mechanistic investigation, therefore, model compounds of known chemical structure were used. These compounds were chosen based on the criteria that they structurally resemble or functionally behave like certain chemical moieties of humic or fulvic acids, major components of DOC. DOCs sourced from the natural environment often cause a decrease in TEP which is pH-dependent, while low pH alone increases TEP. Our results demonstrate that some model compounds likewise decrease TEP (e.g., sodium dodecyl sulfate, tannic acid and bovine serum albumin) and these responses are dependent on water pH. We show that increased ambient ion concentrations (Na^+ and Cl^- from NaCl , Ca^{2+} and Cl^- from CaCl_2) result in increases in TEP in a concentration-dependent manner and that these responses are altered by the presence of natural DOCs and model compounds. Furthermore, these effects are pH-dependent. Therefore, DOC is a water quality parameter that should be considered in conjunction with pH, for risk assessments of freshwater salinization, such as those employing the MIT model (NSERC Discovery).

2.12.P-Tu-076 A Critical Review of Laboratory Toxicity Data for Species Sensitivity Distributions of Chloride Salts in Freshwater

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The concentrations of major ions in freshwater systems have been increasing globally, making the ecological impacts of salinization a pressing concern. Rising ion concentrations from salts could pose a direct and indirect risk to freshwater organisms and irreversible impairment on the structure and function of food webs.

Additionally, concerns about data quality and reporting within the peer-reviewed literature for risk assessments have been raised (e.g., poor experimental design, improper controls); which may lead to ecological impacts if used to generate insufficiently protective guidelines. Therefore, high quality toxicity data are needed to inform risk assessors when making regulatory decisions regarding freshwater protection for chloride. To address the concerns surrounding the reliability and relevance of ecotoxicological literature, we conducted a critical review with the overall objective to assess the current state of laboratory toxicological data as it relates to chloride ions associated with freshwater salinization. Specifically, we sought to 1) evaluate the strength of methods and ecological relevance of aquatic toxicity studies for chloride salts on freshwater organisms via objective scoring rubrics; 2) use those studies deemed suitable for risk assessment to create Species Sensitivity Distributions (SSDs), where it is possible to estimate water quality protection thresholds; and 3) identify major knowledge gaps and make recommendations to address current and future toxicity data needs (e.g., specific species and exposure scenarios) related to freshwater salinization. A total of 281 papers were reviewed. Of these, 115 papers (with over 300 data points) met the inclusion criteria and were scored based on their study design and ecological relevance of endpoints. SSDs were created using the strongest chloride toxicity data to contrast with current water quality guidelines and criteria in Canada and the United States. This research contributes to the advancement of knowledge by providing data that is useful for ecological risk assessment and freshwater protection.

2.13.P-Tu Novel Methods and Approaches for Assessing Effluents and Ambient Water Toxicity

2.13.P-Tu-077 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*). Acute freshwater toxicity test methods include the use of several cladocerans (*Daphnia magna*, *Daphnia pulex*, and *C. dubia*) and freshwater fish (*P. promelas*, *Cyprinella leedsi*, *Oncorhynchus mykiss*, and *Salvelinus fontinalis*). 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- to 10-day short-term chronic test method to determine mortality and growth effects of three reference toxicants (zinc sulfate, ammonia chloride, and diazinon) on *N. triangulifer*. Standardization of *N. triangulifer* culturing conditions including reformulated water 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. The standardization of the acute and short-term chronic WET test methods and culture criteria for *N. triangulifer* will provide a new tool for use in detecting both known and unknown chemical and biological contaminants in wastewater and in ambient water quality testing for aquatic life protection. The progress and results of culture and test method development will be presented.

2.13.P-Tu-078 Effects of Aging on Acute Toxicity of Candidate Fluorine-Free AFFF Replacement Formulations

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The Department of Defense (DoD) is actively engaged in research to identify suitable replacements in response to human health and environmental concerns arising from the historic use of aqueous film-forming foam (AFFF) formulations containing per and polyfluorinated alkyl substances (PFAS). The Strategic Environmental Research and Development Program (SERDP) is supporting several efforts to determine environmental bioavailability and toxicity of selected candidate replacement formulations in relation to traditional PFAS containing AFFF. This presentation summarizes work conducted as a component of a larger effort in collaboration with NOAA and NIST and is focused on evaluating the effects of aging aqueous exposures of candidate formulations on the acute toxicity of two candidate replacement formulations (Avio F3 Green and Bio-Ex Ecopol A) in relation to a C-6 PFAS based formulation (Buckeye Platinum Plus). Each of the candidate formulations were evaluated in acute (48-hour survival) toxicity tests with the freshwater pelagic zooplankton (*Ceriodaphnia dubia*). Formulations were prepared as serial dilutions; exposure media was aged for 4 weeks, 3 weeks, 2 weeks, 1 week and compared against a freshly prepared dilution series (no aging). Dilutions were prepared with filtered moderate hard reconstituted water. Aging of dilutions series occurred

under test conditions (i.e., temperature = 25 °C, 16:8 light dark regime under full spectrum fluorescent light, no aeration, and covered to prevent evaporation). For all three formulations evaluated, aging substantially reduced toxicity after 2+ weeks. *C. dubia* exposed to 29.6 mg/L Avio F3 were initially acutely toxic but toxicity reduced by greater than 2X after 2 weeks aging, where no acute lethality was observed. Similarly, nominal 100 mg/L Ecopol exposures were initially toxic but no toxicity was observed after only 1 week of aging. Results from the acute toxicity tests will be presented along with results of non-targeted chemical analysis of the exposure waters. Toxicity data derived from this project will be used to support selection of candidate replacement formulations meeting current DoD performance requirements.

2.13.P-Tu-080 Application of an Effect-based Method, the Water Cytotoxicity Test, for Water Quality Monitoring in Alberta, Canada

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For over twenty years, the Alberta Centre for Toxicology has utilized effect-based methods (EBMs) as a complementary tool to routine and trace water quality monitoring of Alberta, Canada waters. The water cytotoxicity testing program uses an in-house developed EBM, the water cytotoxicity test, which is based on the measurement of basal cytotoxicity in HepG2 cells after exposure to a whole (non-concentrated) water sample. Because cytotoxicity is the effect overlying all cellular toxicity pathways, it is a useful measure for screening purposes and a clear indicator of the presence of bioavailable components of toxicological concern. The output of the water cytotoxicity test is the water quality index (WQI), a unique quantitative measure derived from the area under the curve (AUC) generated by six dilutions of the whole water sample respective to the AUC of the negative control, as measured in HepG2 cells by a real-time cell analysis (RTCA) platform. Because it is derived from the dose-response measured over the entire 72 h exposure period, the WQI reflects concentration and time effects, and both low and high toxicity water samples can generate a WQI to use as a comparative metric. As many traditional environmental monitoring bioassays require the measured inhibitory response to reach 50% before a comparative value can be generated, this is a clear benefit of our EBM. Results from a three-year (2017-2020) monitoring program of Alberta surface waters and water treatment plants (drinking, wastewater) in areas impacted by the 2016 Horse River Wildfire demonstrate the application of the water cytotoxicity test to water quality monitoring. The water samples with the highest measured cytotoxicity were collected from rivers in the 2017 open water season, the year immediately following the wildfire. Many samples contained trace amounts of targeted substances but still presented high cytotoxicity, suggesting the contribution of both targeted and untargeted substances to the measured response (mixture-effects). The water cytotoxicity test is a quality controlled, reliable, and robust screening tool that can be applied as a complementary approach to routine chemical analysis. Although the water cytotoxicity test was initially envisioned as a tool to screen for cytotoxic substances of potential human health relevance, it is important to note that the application of the EBM is not limited as such and could be modified to potentially inform ecological health monitoring.

2.13.P-Tu-081 Embryotoxicity of Chlorpyrifos on Gastrulation, Segmentation, and Hatching in *Clarias gariepinus* (Burchell, 1822)

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This study was aimed at investigating the effect of chlorpyrifos on the embryonic development of *Clarias gariepinus*. Freshly fertilized eggs of *C. gariepinus* were exposed to 0.01µg/l, 0.1µg/l, 1.0µg/l, and 10µg/l of chlorpyrifos through embryogenesis in a static renewal bioassay. The effect of chlorpyrifos was investigated on three of the seven broad stages of embryogenesis: gastrulation, segmentation, and hatching. At the gastrulation stage, germ rings, caudal edge, and cephalic edge were observed across the treatment and control groups. Results from the segmentation stage showed complete somite blocks across the treatment groups and control. At hatching, it was observed that the optic primordial, myotomal muscle, yolk sac, notochord, and mouth gape were fully formed at 24hrs post-hatching in the control and treatments except at the highest concentration

(10µg/l), where hatching was not observed. Thus, there was no significant aberration during the period of embryogenesis across the treatments. Results of the hatchability test showed that at the least concentration of chlorpyrifos (0.01µg/l), about 60% of the thirty (30) fertilized eggs hatched, 0.1µg/l treatment hatched about 40% of the thirty (30) fertilized eggs, and 1.0µg/l treatment group hatched about 20% of the thirty (30) fertilized eggs. Although, embryonic exposure of chlorpyrifos at reduced concentrations did not have any significant effects except at 10µg/l, the mere presence of pesticides calls for continuous monitoring of water quality to check the effects of toxicants on developing eggs and larvae of fish and other fauna in the aquatic environment.

2.13.P-Tu-082 Chlorantraniliprole and Cyantraniliprole Toxicity to the Standard Surrogate Species *Daphnia magna* and a Native Cladoceran *Simocephalus vetulus*

Stephanie Eckard and John D. Stark, Washington State University

Insecticide resistance has led to the creation of more novel pesticides with different toxic modes of action. As new pesticides are produced, the US EPA screens them for toxicity to various organisms before they can be registered to sell in the United States. The cladoceran *Daphnia magna* is a commonly used surrogate species representing all aquatic invertebrates. However, the use of surrogate species can be problematic if the species is nonnative to the region and less sensitive than the organisms it represents. Differences in susceptibility to pesticides among both aquatic and terrestrial species have been reported, suggesting that the current method of screening is not protective of all species that may be exposed to pesticides. The objective of this study is to investigate the acute and chronic toxicity of two emerging ryanodine-receptor modulator pesticides, Chlorantraniliprole and Cyantraniliprole, to two species of daphnia: the standard surrogate species *D. magna* and a native species *Simocephalus vetulus* collected and cultured from a local pond in Puyallup, WA. Based on preliminary tests, we hypothesize that the native *S. vetulus* will be more sensitive to these pesticides than the surrogate species *D. magna*. Better understanding of differing species susceptibility to pesticides can guide regulators to improve their screening methods and prevent the registration and/or use of pesticides that unintentionally harm the environment.

2.14.P-Tu General: Aquatic Toxicology, Ecology and Stress Response

2.14.P-Tu-084 Red Drum Hematological and Biochemical Health Relative to Pharmaceutical Exposure Across Florida Estuaries

Shakira Trabelsi¹, Jennifer S Rehage¹, Andy Distrubell¹ and Christopher R Malinowski², (1)Florida International University, (2)Ocean First Institute

Studies have shown valuable correlations between environmental perturbations and hematological analyses. Hematological and biochemical analyses can show signs of toxicity, physiological stress, and organ and tissue damage. Pharmaceutically active compounds and their metabolites have been recognized as an important class of emerging contaminants in aquatic environments, found at concentrations that can elicit effects in fish. In Florida, their presence and risk are unknown with products such as antibiotics and antidepressants having increasing evidence of adverse sublethal effects. We hypothesize that pharmaceutical exposure has the potential to cause deteriorated health in exposed wild fish which can be assessed through hematological, biochemical, and protein electrophoretic analyses in comparison to pharmaceutical exposure concentrations. In this study, nine different estuaries located along Florida's coasts were sampled for Red Drum (*Sciaenops ocellatus*), an economically important sport fish. Red Drum are an excellent indicator species for pharmaceutical toxicity due to its continuous presence along the shores of Florida's coast, benthic foraging in sediment, low mobility, and high site fidelity which makes them ideal for reflecting their local environmental conditions. The blood and plasma of 101 different fish was assessed through hematological, biochemical, and electrophoretic analyses as well as for 95 common pharmaceuticals including antibiotics, psychoactive, and cardiovascular medication.

We focus on establishing reference intervals, evaluating the hematological and biochemical health of an economically important and valuable sport fish, and understanding the risk of pharmaceuticals to fishes. Data currently in review.

2.14.P-Tu-086 The Role of Hydrogen Peroxide as Seed Priming for Spring Barley Plant (*Hordeum Vulgare* L.) Resistance to Different Abiotic Stressors

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This paper reports the observations and evaluation of possible outcomes and mechanisms of cross-adaptation of spring barley (*Hordeum vulgare* L.) to different stressors following ozone (O₃), UV-B (ultra-violet radiation) and heavy metals (Cu and Cd). After H₂O₂ pretreatment with seed were exposed to these stressors, the following parameters were assessed: shoot and root growth (dry shoot biomass DW, fresh weight FW and height) photosynthetic pigment (Chlorophyll A and B, carotenoid), Oxidative stress (O₂ . – accumulation, malondialdehyde (MDA)), and antioxidant enzymes (superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT)) activity. The exposure of pre-primed seedlings (hardened seeds with H₂O₂) to various stressors displayed an improved resistance to stressors. All data is expressed as a result of triplicate experiments and data were subjected to a one-way analysis of variance (ANOVA), and the mean differences were compared (P < 0.05). Enhanced CAT activity, reduction of O₂ accumulation, and lipid peroxidation were the most possible reasons of cross-adaptation to this redox-active heavy metal, which caused very high oxidative stress in non-hardened barley seedlings. H₂O₂ hardening was the only factor that caused cross-adaptation to cadmium (Cd). The research shows the key reasons for the adaptation of the plants with Cu to stimulate the activity of CAT and inhibit oxidative stress; whereas cross adaptation to Cd is assessed by processes which are non-connected by oxidative stress but as antioxidative protective. The antioxidant enzyme activities and physiological parameters showed the same responses to oxidative stress condition.

2.14.P-Tu-087 Are Pollutants Breaking Reproductive Barriers and Facilitating Fish Hybridization? - A Case Study

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Fish hybridization has been reported in freshwater ecosystems around the globe, creating growing interest in its eco-evolutionary causes and consequences. Although common over evolutionary time scales, hybridization is suggested to have become more frequent in populations subjected to anthropogenic disturbance (e.g., introduction of nonnative fish species, physical modification of habitats). However, not much attention has been paid to the influence of degraded water quality and chemical pollution on the occurrence of hybridization. Here, we explore the relationship between water quality and chemistry and the incidence of hybridization among swordtail (*Xiphophorus*) fish lineages in Mexico. To look at patterns of contamination along a hybrid gradient, we measured various parameters of water quality (e.g., pH, hardness, ammonia, dissolved organic carbon), along with a large suite of organic and non-organic chemical pollutants (e.g., pesticides, pharmaceuticals, trace metals) in locations inhabited by *X. malinche* and/or *X. birchmanni*, and hybrids. These measurements indicate that numerous anthropogenic compounds are elevated in the sites where hybrids are found, and that much of the observed variation in water chemistry can be captured by the first two axis by principal component analysis (72.5% of the variation for parameters of water quality and metals analyzed together, and 93.8% of the variation for organic contaminants). To determine which of these chemical changes might be most relevant to the occurrence of hybridization, we then assessed the current state of knowledge regarding contaminant effects on mate selection. By discussing what is known and identifying knowledge gaps, we generate hypotheses relevant to how degraded water quality and chemistry may be facilitating hybridization in this system.

2.14.P-Tu-088 Characterizing the Distributions and Bioavailability of Per- and Polyfluoroalkyl Substances on the Savannah River Site, A National Environmental Research Park

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The Anthropocene is characterized by radical global changes that includes the near ubiquity of environmental contaminants across terrestrial and aquatic systems. Per- and polyfluoroalkyl substances (PFAS) are a family of anthropogenic chemicals widely used since the 1940s for commercial and industrial purposes to create heat, grease, and water-resistant products. Subsequent to their broad-scale use, studies have reported that exposure to PFAS is associated with a spectrum of consequences to vertebrate health including alterations to immune, metabolic, and reproductive function. Given the potential health risks to people and wildlife, there is a growing concern for these environmental contaminants because PFAS are highly mobile, recalcitrant, and ubiquitous. Aqueous Film Forming Foam (AFFF) are used in firefighting training and operations on military bases and airports and are thought to be a major contributor to PFAS contamination in the environment. The South Carolina Department of Health & Environmental Control (SC DHEC) in January 2020 released the first report on the issues and concerns of PFAS in South Carolina, and specifically indicated that AFFF had been used at the Savannah River Site (SRS). However, the extent, duration, and frequency of those activities are not known, and raise critical questions as to their overall distribution and bioavailability of these contaminants. The overarching goal of the study is to provide an initial characterization of the spatial distribution of abiotic and biotic contamination levels of PFASs on the SRS. In addition, the study will investigate potential trophic biomagnification of PFASs in identified hot spots and identify the bioaccumulative congeners in biotic samples.

2.14.P-Tu-089 Effects of Warming and Hyposalinity On The Blue Mussel (*Mytilus edulis*) : A Multi-Scale Approach

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Coastal and marine ecosystems are currently facing many challenges related to environmental change. Changes in seawater temperature and salinity, related to global warming and disruption of the global water cycle, can have significant consequences on these ecosystems, including water quality, biodiversity, and the health of marine organisms. Therefore, it is necessary to identify and understand the biological effects of these environmental changes on marine organisms to assess their ability to tolerate future conditions associated with climate change. The present study aimed to assess the effects of short-term warming and hyposalinity of seawater, reflecting the large variations found in coastal environments that are likely to intensify with climate change. Chronic exposures were conducted under laboratory conditions using blue mussels (*Mytilus edulis*) as a biological model. Mussels from a farm were exposed for 9 days to two warming conditions (+3.5°C and +6°C relative to control) and two hyposalinity conditions (-6 PSU and -12 PSU relative to control). At the molecular level, biomarkers of oxidative stress (catalase, superoxide dismutase, glutathione-S-transferase activities, and lipid hydroperoxides) and neurotoxicity (acetylcholine esterase activity) were analyzed in gills, mantle and digestive gland. At the individual level, the physiological status of mussels was assessed by measuring condition index, clearance rate and lipid content.

2.14.P-Tu-090 Binning Data to Support Derivation of Aquatic Life Ambient Water Quality Benchmarks for Data-Limited Perfluorinated Sulfonic and Carboxylic Acids

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Although numerous per- and polyfluoroalkyl substances (PFAS) are present in the aquatic environment, empirical toxicity data remain limited for a majority of these substances. Empirical toxicity datasets are relatively robust for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in freshwater environments, and US EPA recently derived aquatic life criteria for these substances using the traditional

toxicity test-based derivation approach presented in EPA's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*. However, there remains the need to address potential risk to aquatic communities associated with the numerous other PFAS for which data are more limited. EPA is exploring the use of alternative approaches for the development of acute protective benchmarks for data-limited chemicals, with focus on a selected group of data-limited PFAS. Here we discuss applying a published approach for binning the toxicity data of related chemicals to support derivation of protective values for selected data-limited perfluorinated sulfonic acids (PFSAs) and perfluorinated carboxylic acids (PFCAs). Specifically, available empirical toxicity data for multiple PFSAs and PFCAs were compiled into separate groupings. Data within these groupings were then normalized across constituent substances using a common species and the normalized data were used to develop sensitivity distributions for PFSAs and PFCAs. These sensitivity distributions, each comprised of chemical data for multiple constituent substances, provide more robust datasets that can be used to support the calculation of protective values for the groups and for the individual constituent compounds. Deriving benchmarks using such alternative approaches is consistent with EPA guidance and allows the agency to provide information to states and tribes regarding protective values for aquatic life for data-limited chemicals. Additionally, development of these 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 application of New Approach Methods (NAMs).

2.14.P-Tu-091 Impact of Dissolved Organic Carbon and Divalent Cations on Per- and Polyfluoroalkyl Substances Bioaccumulation in Freshwater Algae

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Per- and polyfluoroalkyl substances (PFAS) have caused global concern due to persistence, toxicity, bioaccumulation exhibited by some compounds. Our previous studies suggested that key physicochemical factors affect PFAS bioaccumulation in freshwater benthic macroinvertebrates, but PFAS interactions with algae are less studied. Freshwater algae, a primary producer, constitutes the base of the food web in freshwater ecosystem, and changes in their population level can cause rapid and serious effects on higher trophic levels in the freshwater environment. Previous studies demonstrated that PFAS inhibit algae growth, reduce enzymes activity, down-regulate metabolic genes, and negatively impact photosynthesis. However, information is limited on PFAS uptake and bioaccumulation in freshwater algae and will be addressed in this study. As a complementary study, the impact of dissolved organic carbon and dissolved divalent cations will be evaluated to study their influences on PFAS bioaccumulation in freshwater algae. Mixed 14 PFAS (8 perfluoroalkyl carboxylic acids, 3 perfluoroalkyl sulfonic acids and 3 fluorotelomer sulfonic acids) will be spiked into culture water to achieve a nominal same concentration for each individual analyte. Mixed algae (cultured in-house, originated from a local pond) will be exposed for a set duration under environmentally relevant conditions. For dissolved organic carbon study, three treatment conditions will be tested with dissolved organic carbon concentrations at 0.5, 5 and 50 mg-C/L; for dissolved divalent cations study, three treatment conditions will be tested with 0.2 mM Ca²⁺ and 0.2 mM Mg²⁺, 7.5 mM Ca²⁺ and 0.2 mM Mg²⁺, 0.2 mM Ca²⁺ and 7.5 mM Mg²⁺, which is consistent with one of previous macroinvertebrates studies. It is hypothesized that increasing dissolved organic carbon will decrease bioconcentration factors, and the decrease extent will be PFAS chain length dependent. While the elevated divalent cations will increase PFAS bioaccumulation in algae, Ca²⁺ will cause a greater increase in PFAS bioaccumulation compared to Mg²⁺. Understanding the impact of dissolved organic carbon and divalent cations on PFAS bioaccumulation in freshwater algae is crucial to understand the lowest trophic level bioaccumulation processes that affect the whole freshwater aquatic ecosystem dynamics.

2.14.P-Tu-092 Thyroid and Sex Hormone Disrupting Effects of DEHTP in Embryo-Larval and Adult Male Zebrafish (*Danio rerio*)

Yunchul Ihn¹, Yoojin Cho¹, Yura Lee¹, Hyesun Seok¹, Jin-su Oh², Hyo-Bang Moon² and Kyungho Choi¹, (1)Seoul National University, (2)Hanyang University

Di(2-ethylhexyl) terephthalate (DEHTP) has been increasingly used to replace di(2-ethylhexyl) phthalate (DEHP). DEHTP or its metabolites have therefore been widely detected in the environment and human urine samples, often at high concentrations. However, little is known about its endocrine disrupting potential. In this study, its thyroid and sex steroid hormone disrupting potentials were investigated on embryo-larval zebrafish and adult male zebrafish. Fertilized zebrafish eggs were exposed to DEHTP (0, 4, 20, 270 ug/L) for 5 and 7 days to evaluate alterations of thyroid hormones and transcriptional changes of thyroid regulating genes. In addition, adult male zebrafish were exposed to DEHTP (0, 2, 30, 190 ug/L) for 21 days, and evaluated for thyroid and sex steroid hormone levels, as well as related transcriptional changes. The 5 and 7-day exposure of zebrafish to DEHTP resulted in significant increases in whole-body thyroid hormone levels. Several genes involved in hypothalamus-pituitary-thyroid (HPT) axis were down-regulated in 5 dpf larvae, but no apparent changes were detected in 7 dpf larvae. In adult male zebrafish, decreased triiodothyronine (T3) levels with increased thyroid stimulating hormone (TSH) levels were observed. Significant up-regulation of *dio1* and *dio3a* genes, along with downregulation of *dio2* may explain decreased T3 levels. Furthermore, in adult male zebrafish, decreased estradiol (E2) and 11-ketotestosterone (11-KT), along with increased testosterone (T) levels, were observed. Downregulation of *vtg1* and *vtg2* genes and upregulation of *shbg* support sex hormone changes. Altered expression of steroidogenic genes in the gonads, also supports these hormonal changes. While making a direct comparison with other studies may be challenging due to variations in exposure periods and conditions, the concentrations that resulted in the alteration of thyroid and sex hormones in this study were comparable or slightly lower than those observed in DEHP exposure. The current observations show that this terephthalate plasticizer could alter hormonal balances at different life stages of zebrafish, and hence may influence its normal development and reproduction. This work was supported by National Research Foundation of Korea through "Science and engineering basic research projects (NRF-2020R1A2C3011269)".

2.14.P-Tu-093 Pre and Post Remediation and Restoration Analysis of Benthic Macroinvertebrate Size Spectra in the Upper Arkansas River, Colorado

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The relationship between body mass and abundance of organisms within a system is defined as size spectra. This pattern illustrates how energy flows through an ecosystem by indicating that there are fewer large individuals compared to small ones. Body mass-abundance distributions are relatively consistent across natural communities and habitats, with evidence suggesting that anthropogenic disturbances, such as stream degradation through mining, alter the slope of the size spectra. Some researchers have proposed that body mass-abundance relationships are useful indicators of ecological function and may provide a better understanding of the energetics within benthic communities. Size spectra has previously been used as a biomonitoring tool within degraded freshwater streams but remains understudied because of limited pre-treatment data and post-restoration monitoring. Here, we analyze the size spectra of benthic macroinvertebrate communities from the Upper Arkansas River before and after water quality remediation and habitat restoration. We take into account the streams' significant history, including the initial discharge of metal concentrations from mining activities in the watershed, the listing of the stream as an USEPA Superfund Site, the remediation of metal contamination, and large scale habitat restoration initiatives. For this project, we utilized benthic samples previously collected over a 30-year period from a reference site and an impacted site, AR1 and AR3, respectively. These samples of macroinvertebrates were then sorted down to their family taxa, photographed using a microscope camera, and exported for measurements. Using R studio, the measurements were compiled for the size spectra analysis. We hypothesized that the size spectra slope would be steeper at the impacted site, AR3, compared to the reference site, AR1, over the course of this study. Furthermore, it was predicted that water quality remediation and habitat

restoration would result in an improvement on the size spectra of the impacted site, AR3. Our results show that macroinvertebrate size spectra approached reference conditions soon after improvements in water quality and habitat were observed, supporting the use of this novel approach for ecological assessments. This study is the first to document the application of size spectra to assess responses to remediation and restoration efforts in freshwater streams.

2.14.P-Tu-094 Evaluations of Ziram Toxicity to Non-Target Invertebrate and Fish Species

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The grass carp, *Ctenopharyngodon idella*, is one of the four invasive carp species established in North America. The other invasive carp are bighead carp (*Hypophthalmichthys nobilis*), black carp (*Mylopharyngodon piceus*), and silver carp (*Hypophthalmichthys molitrix*). Grass carp are originally from eastern Asia, with a native range from northern Vietnam to the Amur River on the Siberia-China border. In the United States, grass carp have been recorded in 45 states with breeding populations in several major basins including the Missouri and Mississippi rivers. A need to identify new chemicals that can be used for 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 broad-spectrum pesticide belonging to the dimethyl-dithiocarbamate class of agricultural fungicides. Few studies have measured the toxicity of Ziram to fish or freshwater invertebrates. However, many of these studies were based on nominal concentrations as no water chemistry was performed. A series of gavage experiments indicated that Ziram was nontoxic via the oral route. However, Ziram exposure through intraperitoneal injection resulted in acute mortality at 150 mg/kg. These results show that Ziram is acutely toxic to grass carp, however, additional research is required to formulate a successful novel grass carp toxicant that can be used to target invasive carp species while minimizing effects on non-target fish species. The objective of this study was to evaluate the potential acute toxicity of Ziram in an aqueous solution to suite of non-target aquatic invertebrates and fish. We exposed six species, 3 invertebrates and 3 fish, to a series of aqueous Ziram concentrations for 96-hours. In the invertebrate bioassays, 96-h LC₅₀ 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. 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.

2.14.P-Tu-095 Assessment of the Effects of Cadmium, Samarium and Gadolinium on the Blue Mussel (*Mytilus edulis*): A Biochemical and Lipidomic Approach

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Rare earth elements are a group of 17 metallic elements. Their electronic configuration gives them particular properties which make their use in the composition of many products of our daily life essential. Their worldwide popularity disrupts natural biogeochemical cycles and enrichments in lanthanum (La), gadolinium (Gd) and samarium (Sm) are already observed in rivers. This study was carried out to assess the bioaccumulation and potential effects of 2 rare earth elements, Gd and Sm, in comparison with cadmium (Cd), on a marine sentinel species: the blue mussel (*Mytilus edulis*), combining biochemical marker analyses and a more global lipidomic approach. Laboratory exposures were carried out at doses of 50 and 500 µg/L in tanks of 13 liters of artificial seawater. Three tanks per element – Cd, Sm, Gd – each containing 13 mussels, were maintained in parallel with 3 other tanks without contamination. After an 8-day exposure, during which the water and contamination were renewed every 2 days, the mussels were dissected and a battery of biomarkers were performed on digestive gland, gills and mantle. Superoxide dismutase (SOD), catalase (CAT), and glutathion S-transferase (GST) activities served to monitor the oxidative stress. The neurotoxicity was analysed

through the acetylcholinesterase activity (AChE). Acid Phosphatase (AcP) measures were used as a biomarker for immunotoxicity in haemolymph. Moreover, lipidomic analyses were performed using flow injection analysis coupled to high resolution mass spectrometry (FIA-HRMS) on lipid extracts from the digestive glands of mussels. Finally, the metal bioaccumulation was measured on exposed organisms by ICP-MS. All the metals were bioaccumulated by the mussels. Concerning the effects, Sm led to oxidative stress – induction of GST – in both gills and digestive glands. No significant effect was observed on the other biomarkers, i.e. phosphatase acid, CAT and AChE. Concerning lipidomics, no significant unsupervised discrimination between the conditions of exposure was highlighted by PCA. But, the exposures to Cd, Sm and Gd led to a total of 31 compounds up- or downregulated compared to the controls in the supervised analysis, using PLS-DA. Among them, one compound was upregulated by the mussels exposed to the 3 different metals and it would correspond to PIP3, which is involved in cell growth and survival. To conclude, biochemical and lipidomic approaches are complementary tools in Environmental Risk Assessment.

2.14.P-Tu-096 Impacts of Chronic Nickel Exposure on the Growth of Native Crayfish (*Faxonius virilis*)

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Previous research has documented that mineral extraction and processing activities may negatively impact crayfish communities through the chronic release of potentially toxic metals and other compounds into aquatic environments. However, data on chronic and sublethal impacts of metals on crayfish are extremely limited. Metals are known to impact crayfish survival, fecundity, hatching success in the short-term but understanding of effects on growth and consumption under longer-term exposure conditions is limited. Energetic modeling is a powerful tool for estimating long-term impacts on population and these endpoints are essential for effectively parameterizing these models. The goal of this study was to better understand the chronic sublethal toxicity of nickel on the growth and food consumption of the crayfish species, *Faxonius virilis*. This crayfish-metal combination is representative of potential real-world scenarios at legacy mining sites. Young-of-year crayfish were exposed to 31–500 ppb nickel for 28 days. We found that concentrations as low as 62.5 ppb nickel significantly reduce crayfish growth. Analysis of consumption data is still underway. These data will be incorporated into a bioenergetics model to help predict how long-term low-level metal exposures may impact crayfish populations and associated aquatic communities.

2.14.P-Tu-097 Long-term Trends of Pyrethroid-Driven Toxicity in California Watersheds

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The Stream Pollution Trends Monitoring Program (SPoT) provides critical information about sediment contaminants and toxicity at 90 sites across diverse watersheds in California. As a statewide program under California's Surface Water Ambient Monitoring Program (SWAMP), SPoT has identified long-term trends for various contaminants since 2008. One of the most notable findings of this program is pyrethroid trends and their relationships to land use and toxicity. Sediment samples were collected annually between 2008 and 2022 and analyzed for several contaminants including pyrethroid insecticides. Standard toxicity tests were conducted using the amphipod, *Hyalella azteca*, and the midge, *Chironomus dilutus*. Pyrethroid concentrations were carbon-corrected, toxic units (TUs) were calculated, and trends were analyzed using Mann-Kendall tests. To identify linkages between contaminants, toxicity and land use, watershed delineations and land cover data extractions were conducted for drainage areas using automated scripts based on digital elevation models. Statewide, median concentrations of deltamethrin as well as sum of all pyrethroid TUs were increasing. Bifenthrin concentrations were increasing at 24% of the sites and permethrin was decreasing at 23% of the sites. Concentrations of most pyrethroid compounds were significantly correlated with urban land use in the watersheds. An average of 20% of the sites were toxic between 2008 and 2022, and amphipod survival was identified as the most sensitive endpoint. Amphipod survival and growth responses, as well as the midge growth

response, had significant negative correlations with urban land use, indicating elevated toxicity with increasing urban land use. Eighty-five percent of sediment samples with summed carbon-corrected pyrethroid TUs greater than five showed significant amphipod toxicity, and 69% of samples toxic to amphipods had greater than one pyrethroid TU. Among the pyrethroids measured in the sediments, bifenthrin contributed the most to summed TUs. These long-term monitoring results inform state agencies about the health of watersheds across CA to facilitate management actions.

2.14.P-Tu-098 Bioaccumulation of Rare Earth Elements Nd, Pr, and Y, Individually and in Mixtures to *Daphnia magna*

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Neodymium (Nd), Praseodymium (Pr), and Yttrium (Y) are three rare earth elements (REEs) that occur in the mineral ore bastnaesite, the primary ore of Canada's first REE mine at Nechalacho, NWT. These elements are being increasingly used in modern technologies including green energy equipment (e.g., wind turbines & electric vehicle batteries) and as such, there is an increased concern for the potential environmental risk associated with anthropogenic contamination. There is very little data available for individual REEs and even less available on mixtures. The objective of this study was to investigate the bioaccumulation of Nd, Pr, and Y in *Daphnia magna*, alone and as ternary mixtures. Mixture exposures were designed using a toxic unit (TU) approach, based on converting the EC₅₀ concentrations from previously conducted acute toxicity tests to TUs and applying a matrix isobologram approach. Daphnids were initially exposed to sublethal concentrations for 24-h in an artificial soft water medium with a hardness of 50 mg CaCO₃/L and a pH of 6.8 with no added bicarbonate. For example, at 0.2 TUs of each element (36 µg/L Nd, 133 µg/L Pr & 190 µg/L Y), similar levels of REE accumulation were observed whether it was in single metal exposures or in mixture exposures indicating that accumulation was not influenced by other REEs. Bioaccumulation tests were also conducted using environmentally relevant conditions found in Thor Lake, NWT (the first fish bearing lake downstream of Nechalacho). This research is supported via an NSERC Alliance Grant with additional funding from Environment and Climate Change Canada, Stantec Inc and Cheetah Resources.

2.14.P-Tu-099 Don't Blame the Nano: Nano Ink Toxicity to *Daphnia pulex*

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The novel physical and chemical properties of nanomaterials drive their use in consumer products. Nano-gold and nano-silver inks are used in the manufacturing of a multitude of everyday consumer products, for example electronics and sensors. The release of both nano inks is possible through several point sources in the life cycle of the nano-enabled products. The goal of this study was to determine the toxicity of nano-gold and nano-silver formulations for inkjet printers to *Daphnia pulex* used in the manufacturing of novel nano-enabled sensors. *Daphnia pulex* was selected since it is a well-studied model species along and its previously developed oxidative stress gene panel. Acute toxicity (48-hour) of the whole nano-gold ink formulation and nano-silver ink formulation were determined. However, it is critical to consider that for the nano-gold ink (experimentally determined 20% nano-gold by mass), the LC₅₀ value is close to that of the major solvent reported in the manufacturing SDS (LC ~ 0.08 mg/L) suggesting the solvent, not the nano material, is the main contributor to toxicity for in *Daphnia pulex*. Reproduction and oxidative stress were investigated in *Daphnia pulex* as chronic toxicity end points. The 25% inhibition concentration (IC₂₅) was determined using total formulation mass of the nano-gold and nano-silver ink. The production of neonates was not statistically different from either the control or other concentrations except for the highest concentration for both nano-gold and nano-silver inks. However, the *Daphnia pulex* showed statistically significant oxidative stress in several of the targeted genes. At least for these nano-gold and nano-silver inks, ingredients in the formulation other than the nanomaterials appear most responsible for the observed aquatic toxicity. The addition of other solvents and chemicals need to be studied before toxicity is assigned to the nanomaterial.

2.14.P-Tu-101 Grazing Effects of an Obligate Herbivore Fish Species on Periphyton Biomass in a Stream Mesocosm Study

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We evaluated the effect of a native, free-roaming, herbivorous fish (Central Stoneroller, *Campostoma anomalum*, an obligate herbivore) on periphyton in a stream mesocosm set-up. Including free-roaming fish in mesocosm tests adds realism to the experimental conditions. We hypothesized that the presence of fish would affect periphyton dry weight (BP), ash free dry mass (AFDM), % organic matter (%OM, i.e., AFDM as a percentage of BP) and the inorganic sediment fraction (BP-AFDM). Six indoor stream mesocosms were prepared with and without grazing fish (three replicates each). The fish were approximately 85 mm long, had a mean weight of 6.24 g, and were obtained from a local small stream. After a 24-hr acclimation period inside the facility, the replicates with fish each received six non-sexed individuals. Survival over the experiment averaged 83%. Periphyton in the six mesocosms were sampled weekly over a 46-day test. Irradiance, water quality, and water sources were the same among streams and sampling events. The periphyton responses were analyzed conditional on an interaction between time and fish treatment. There was no significant difference in periphyton dry weight, AFDM, or in its inorganic content. However, periphyton in streams with fish had a significantly higher fraction of organic matter (%OM). This resulted from relatively lower inorganic sediment and higher AFDM in the streams with fish. Periphytic BP, AFDM, and inorganic fraction all increased with time in both treatments, but in streams with fish, the increase lagged until an observed change occurred between days 32 and 39. The time by treatment interaction was not significant. Although, the presence of grazing fish did not affect periphyton biomass, they did affect its structure by increasing its organic fraction. We suspect this could be due to fish swimming or grazing behavior, which may act to prevent settling or retention of inorganic sediment in the periphytic matrix.

2.14.P-Tu-102 Exposure to Di-(2-propylheptyl)-phthalate (DHP) Causes Thyroid Disruption in Zebrafish

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Di-(2-propylheptyl)-phthalate (DHP) is a phthalate plasticizer commonly used in consumer products such as vinyl flooring, plastics, and carpet backing. DHP is often used as a replacement for conventional high-molecular weight phthalates such as DEHP and DINP, which have been linked to various adverse health effects. Some studies suggest that DHP may pose potential health risks similar to those of DEHP and DINP, but only limited information is available. The objective of this study is to evaluate whether DHP disrupts the thyroid endocrine system and function in zebrafish (*Danio rerio*). For this purpose, zebrafish embryos were exposed to different concentrations of DHP (0.001, 0.01, 0.1, 1 mg/L) from 4 hours post fertilization (hpf) to 168 hpf. The whole-body content of thyroid hormones were examined along with transcription of genes involved in the hypothalamic-pituitary-thyroid (HPT) axis. As a result, the levels of both thyroxine (T4) and triiodothyronine (T3), as well as thyroid-stimulating hormone (TSH), exhibited a significant decrease, indicating that the thyroid hormone homeostasis in zebrafish larvae was disrupted by DHP. Transcription of genes (*tshβ*, *nis*, *tg*, *ttr*, *dio1*, *ugt1a*, *sult1st5*) related to hypothalamic-pituitary-thyroid (HPT) axis were also disrupted. These changes observed in zebrafish larvae, which were exposed to DHP for 168 dpf, are not consistent with the changes seen in DEHP-exposed larvae reported elsewhere. These differences suggest potential differences in the mechanisms by which DEHP and DHP induce disruptions in thyroid hormone levels. In conclusion, exposure to DHP leads to alterations in whole-body thyroid hormone levels in zebrafish larvae, as well as changes in the expression of key genes related to the HPT axis. This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through "Core Technology Development Project for Environmental Diseases Prevention and Management", funded by Korea Ministry of Environment (MOE) (2022003310006). This work

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2.14.P-Tu-104 Ecotoxicity of Water-Soluble Synthetic Film

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Water-soluble synthetic film and polymer (WSSP) are used in industrial, food, household and biomedical fields, but their impact in the aquatic environment and effects to aquatic organisms are still unclear. So far, we carried out acute and extended acute toxicity tests using *Daphnia magna* and juvenile Medaka (*Oryzias latipes*) for two grades of water-soluble films, Hi-Selon™ (manufactured by Mitsubishi Chemical Corporation), which is typical WSSP used for the packaging of liquid detergents. The result showed the ecotoxicity of Hi-Selon™ were low and 5 kinds of additives in those films also showed same results in each toxicity test. Furthermore, the combined exposure testing of Hi-Selon™ and the chemical (zinc) were also carried out, and it was observed that the toxicity of zinc tends to be mitigated. In this presentation, the results of acute toxicity tests using *Daphnia magna* for different types of polyvinyl alcohol, and reproduction tests using *Ceriodaphnia dubia* for two types of Hi-Selon™ will be reported. Also, the co-exposure test results of Hi-Selon™ with pyrene, a hydrophobic chlorinated organic compound, using the *Daphnia magna* acute toxicity test and the extended acute toxicity test will be discussed. An overview of those results are---Neither the acute toxicities of different types of polyvinyl alcohols nor the reproduction toxicities of two Hi-Selon™ products were observed. And Hi-Selon™ seemed to also mitigate the toxicity of pyrene suggesting Hi-Selon™ somehow interacts both hydrophilic and hydrophobic substances.

2.14.P-Tu-106 Spatial and Temporal Variability of Per- and Polyfluoroalkyl Substances (PFAS) in Environmental Media and Biota Along an AFFF-Impacted Stream Gradient

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Per- and polyfluoroalkyl substances (PFAS) are a group of persistent, bioaccumulative, and toxic compounds widely used as ingredients in fire-suppressing aqueous film-forming foams (AFFFs), resulting in widespread environmental contamination. This study sought to characterize the spatial and temporal variability of PFAS in surface water, sediment, and fish from a forested stream within Joint Base Andrews, MD, a site with current and historic AFFF use. We sampled four locations along a stream gradient twice per week for five weeks during spring and summer, 2021. The primary PFAS identified were perfluorooctane sulfonate (PFOS) followed by perfluorohexane sulfonate (PFHxS) in environmental media and perfluorohexane sulfonamide (FHxSA) in biota. We observed significant seasonal variability in surface water, with elevated concentrations during the summer for all compounds except perfluorooctanesulfonamide (PFOSA). There was a significant negative relationship between surface water concentrations and dissolved oxygen and a significant positive relationship with water temperature. We hypothesize that higher observed concentrations in summer were due, in part, to low flow rates. Sediment concentrations were more enriched with PFOS and perfluorooctanoic acid (PFOA) in locations directly adjacent to known sources of local PFAS contamination. PFAS were highly variable in concentration and composition across fish species, with some compounds accumulating more readily in individuals of lower trophic levels, suggesting that microhabitat and diet may play an important role in bioaccumulation. Calculated log bioaccumulation factors ranged from -0.57 L/kg to 3.98 L/kg for perfluoroalkyl carboxylates (PFCA) and 0.61 L/kg to 3.42 L/kg for perfluoroalkane sulfonates (PFSA). The spatial and temporal variability of PFAS in environmental media and biota necessitates further investigation into external climatic factors like precipitation and hydrologic dynamics on the fate and distribution of PFAS in AFFF-impacted systems to better understand the effect of environmental variability on bioaccumulation in aquatic receptors and potential human health risks. Further, more research on how physiological and ecological

differences among species influence PFAS uptake may be useful in refining PFAS bioaccumulation models and subsequent risk estimation.

2.14.P-Tu-109 Gaining Insight on the Effects Anticoagulant Rodenticides have on Aquatic Species by Establishing Lethality and Sub-lethality Curves for Coho Salmon (*Oncorhynchus kisutch*)

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Invasive species are detrimental to many ecosystems. Rats have been especially problematic on islands that previously served as naval bases. Eradication efforts by the USDA involve aerial broadcast of food pellets containing anticoagulant rodenticides. 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 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 fish species after pellet applications on rat infested islands. The Aleutian Islands of Alaska are under consideration for rat eradication, which include spawning and rearing habitat for Pacific salmon. There is a large information gap for the aquatic toxicology of brodifacoum and diphacinone, with no information for salmonid species. To address this gap, we are using coho salmon (*Oncorhynchus kisutch*) to generate lethal and sublethal dose-response curves for estimating median effects concentrations of brodifacoum and diphacinone. 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.

2.14.P-Tu-110 Acute Toxicity of 4,4'-DDE, Bifenthrin, and Fipronil to Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) Using Whole Body Residues

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Historically, Californian salmonid populations have experienced decline due to habitat alteration, subsistence and commercial fishing, and other anthropogenic activities. Climate change, pollution, waterway modification, and other current stressors may contribute to sustained declines, as well as recent projections of extirpation for many of these regional populations in the next 50 years. Pesticides have been detected throughout salmon rearing and migration areas of the Sacramento-San Joaquin River Delta (the Delta). Many toxicological studies performed with fish and other aquatic species use aqueous concentration as a metric for dose. This method assumes that external concentration corresponds to the exposure at the target site. Confounding variables that affect the bioavailability of the compound, such as characteristics of the habitat and chemical of interest, alter the relationship between external concentration and what an organism is exposed to. Such complex factors limit the relevance of applying results from these studies to larger ecosystems. Moreover, utilization of field water pesticide concentrations may underestimate pesticide toxicity in risk evaluation because they only represent what may be available in that system at the time of collection. Quantifying toxic response using body residue is a more appropriate dose metric because it more accurately represents exposure in the organism. As an alternative to aqueous concentration, this work established a relationship between toxic response and internal body residue in Chinook Salmon (*Oncorhynchus tshawytscha*) by determining the median lethal residues (LR₅₀) for one legacy and two current-use pesticides that have been previously detected in field Delta salmon: 4,4'-dichlorodiphenyldichloroethylene (4,4'-DDE), bifenthrin, and fipronil. Chinook Salmon were exposed to 4,4'-DDE, bifenthrin, or fipronil for 96 h, subsequently euthanized, and analyzed for pesticide content. Pesticide body residues were related to acute toxicity as the lethal residue at 50% survival (LR₅₀). The calculated LR₅₀ for bifenthrin, parent fipronil, and the sum of fipronil and its toxic metabolites were 0.61, 7.4, and 9.0 nmol/g wet weight, respectively. Mortality was not observed in 4,4',-DDE exposures (LR₅₀ > 116 nmol/g wet weight).

2.14.P-Tu-111 Wetland Spiders as Monitors of Contaminant Export from Industrially Impacted Wetlands

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Aquatic insects commonly exhibit complex life cycles with both terrestrial and aquatic phases. Terrestrial adults lay eggs that hatch into aquatic larvae that feed and grow for from a few months to several years before emerging as flying adults. The aquatic larvae of many species have been shown to accumulate both essential and nonessential trace elements to toxic levels when exposed to diverse pollutant sources. Emergence of aquatic insects from wetlands provide a food source to terrestrial predators living along stream or wetland margins increasing productivity near the wetland. This linkage of aquatic to terrestrial food webs also presents the potential of exporting contaminants accumulated in the aquatic larvae from the contaminated wetland or stream to terrestrial food webs. Evaluating impacts of biovectors exporting specific contaminants from aquatic into terrestrial food webs is not simple to assess, and mechanisms driving this export remain poorly known. Tetragnathid spiders construct webs horizontally above the water's surface and capture diverse taxa of smaller emerging insects. Their diet can be dominated by emerging aquatic insects. Thus, contaminant accumulation in tetragnathid spiders can be an indicator of contaminant flux from the wetland. However, some contaminants can be shed during insect emergence, and/or not accumulate in spiders. Spiders were collected from four components of a constructed water treatment wetland, an industrial sedimentation basin, a coal combustion residue basin, and a reference pond on the Savannah River Site, SC, USA. Because of the diverse contaminant sources within these systems, 20 trace elements were analyzed in *Tetragnatha elongata* and bottom sediments. Concentrations of nine elements (Mg, Al, Be, V, Cr, Co, Ni, Mo, and Pb) in spider bodies did not differ among the seven wetlands despite concentrations in sediment differing among sites. Impact of industrial activity on contaminant accumulation was evident by lowest concentrations of Fe, Cu, and Zn accumulating in spiders from the non-industrial reference wetland. Concentrations of As, Se, Sr, Ag, and Tl accumulated to highest concentrations in the coal combustion residue contaminated wetland. Elements varied in their propensity to accumulate in spiders, but increased accumulation in wetland spiders was found for some elements, particularly when exposed to substantial coal combustion residue contamination.

2.14.P-Tu-112 Ecotoxicological Studies Indicate That Sublethal and Lethal Processes Limit Insect-Mediated Contaminant Flux

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Merolimnic insects can accumulate and transport considerable amounts of aquatic contaminants to terrestrial systems. The rate of contaminant biotransport, termed *insect-mediated contaminant flux* (IMCF), depends on emergent insect biomass and contaminant accumulation, both functions of environmental concentration. We developed a mathematical model of IMCF and apply it to three ecotoxicological studies obtained through the US Environmental Protection Agency's ECOTOX database to determine at which concentration maximum IMCF occurs. Model results demonstrate that the maximum IMCF depends on competing rates of biomass loss and contaminant accumulation and does not necessarily occur at the highest insect or environmental contaminant concentration. In addition, modeling results suggest that sublethal contaminant effects (e.g., decreased growth) on insect biomass can be an important and potentially underappreciated control on IMCF.

2.14.P-Tu-114 Development of a Response Spectrum Framework for Bifenthrin and Fipronil Using Internal Body Residues in Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*)

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With the general decline of salmonid populations in the Californian Central Valley, there is a growing need to

improve our understanding of the effects that stressors in the system impose on such populations. Urban and agricultural activity, both historical and current, within the Sacramento-San Joaquin River Delta (the Delta) watershed have provided sources of pesticide inputs to the system. Pesticide exposure has been shown to induce several effects on aquatic organisms other than direct mortality, such as reduced growth, impaired olfactory function, and altered swim performance. Such effects could lead to overall reduced fitness and make fish more susceptible to predation or alter likelihood of migration success. Thus, pesticide pollution in the Delta has been identified as a potential stressor to salmonid populations that utilize the system during outmigration to sea. Traditionally, aqueous concentration is used in toxicological studies to evaluate the effects of pesticides to aquatic organisms. This approach assumes that aqueous concentration is a valid surrogate for dose to the organism. Bioaccumulation of pesticide compounds is commonly observed in fish tissues, indicating that external concentration may not accurately represent exposure. Internal residue is seldom used to evaluate effects from pesticide exposure. This work establishes the residue-response relationships for two current-use pesticides that have been previously detected in the Delta: bifenthrin and fipronil. Juvenile Chinook Salmon were exposed to bifenthrin or fipronil via aqueous media for 4 or 10 days to evaluate lethal and sublethal effects, respectively. A response spectrum framework has been created to demonstrate the internal residue identified for an individual compound that results in an associated effect observed from lab exposures using Chinook Salmon. The endpoints evaluated in this work were mortality, growth rate, olfactory response, swim performance, and cardio-respiratory performance. Ultimately, the goal of the work is to provide a tool for comparing residues from field collected samples to evaluate the risk of pesticide exposure and to inform regulatory decisions in the Delta. Samples of Chinook Salmon have been previously collected from the Delta and will be used with the response spectra to provide insight to population health in recent years.

2.14.P-Tu-115 Characterizing Fluoxetine Metabolism in Four Fish Species Using Michaelis-Menten Enzyme Kinetics

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Discharge of pharmaceuticals into the environment is of emergent concern due to the unknown long-term effects on aquatic organisms. Wastewater treatment plants are not effective in completely removing pharmaceuticals, allowing them to be discharged into aquatic systems. To investigate the potential effects of fluoxetine, a common antidepressant, on aquatic species, the potential of four freshwater fish species to metabolize this drug was investigated. Livers from redbreast sunfish, bluegill, striped jumprock and bluehead chub were collected, homogenized, and centrifuged to collect the microsomes. These microsomes were used in enzyme assays to determine the in vitro hepatic metabolism of fluoxetine by cytochrome P450 (CYP) enzymes. Formation of fluoxetine's main metabolite in humans, norfluoxetine, was measured at different substrate concentrations using HPLC with fluorescence detection. Species differences of the calculated V_{max} and K_m values were compared and the bluegill enzymes were found to be the most efficient at binding fluoxetine, with the lowest average K_m value and the highest average V_{max}/K_m value. Fish liver microsomes were orders of magnitude less efficient than rat microsomes in metabolizing fluoxetine, which was expected, as fluoxetine was developed for use in mammalian systems. To obtain a suggestion of which CYP isoforms might be responsible for the low rate of biotransformation in fish, the microsomes were also tested for metabolism of model substrates for mammalian CYP2 and CYP3 isoforms. These results suggest that CYP3 isoforms in fish may be more active than CYP2 isoforms. Future work should focus on more specific details of CYP isoforms responsible for fluoxetine metabolism in freshwater fish and if these isoforms are inducible, which would support their use as potential biomarkers for increased pharmaceutical pollution in aquatic ecosystems.

2.14.P-Tu-116 Food Web Structures in Lake Superior Revealed by Stable Isotopes Help to Trace Bioaccumulation Pathways of PCBs

Libia Hazra, Azmat Naseem, Enid Partika, Judith Perlinger and Noel Urban, Michigan Technological University

Polychlorinated biphenyl (PCB) compounds are the persistent organic contaminant (POPs) found in highest concentrations in Lake Superior fishes. Fish consumption is a health risk, especially for local communities such as the Keweenaw Bay Indian Community (KBIC), since these fishes are a staple part of their diet. PCBs enter Lake Superior primarily through air-water exchange and enter food webs through the base of the food web (plankton) as well as through benthic macroinvertebrates. The degree of bioaccumulation in the fish species consumed by humans depends on the structure of the food web. Existing data for Lake Superior show that PCB concentrations in fish vary spatially, and thus the risk to humans varies with the location of fish harvest. We hypothesize that this variation in contaminant concentrations is due to spatially variable food web structures across the lake. To test this hypothesis, we analyzed stable isotopes in three fish species (lean lake trout, lake whitefish, cisco) from a region with high PCB concentrations in lake trout and from a region with low PCB concentrations. Values of $\delta^{13}\text{C}$ were used to infer profundal vs. pelagic components in the diet, and $\delta^{15}\text{N}$ was used to assign trophic positions. We use the results to evaluate whether length of food chain or extent of benthic feeding in food webs explain the spatial disparity in PCB concentrations in top predators.

2.14.P-Tu-118 An Evaluation of Aquatic Receptor Sensitivities to Novel Fluorine-Free Firefighting Foam Versus Short Chain PFAS AFFF Products

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Per- and Polyfluoroalkyl substances (PFAS) have surged to the forefront of environmental research due to their persistence in the environment and wide-spread use of PFAS-containing aqueous film forming foam (AFFF) in industrial and commercial practices as well as by the Department of Defense (DoD) for fire suppression needs. Candidate AFFF-alternatives [fluorine-free (FF) foam] products and new generation short-chain PFAS AFFF are emerging to address the gap created by the phasing out of PFAS AFFF. This research aims to fill ecotoxicology data gaps for several ecological receptors, including algae, macroinvertebrates, fish, birds, and reptiles. Collectively, these data will inform decisions regarding potential environmental concerns associated with replacement firefighting foams to prevent further compounding the global concern of prior legacy AFFF application. This presentation focuses on the aquatic toxicity testing portion of the project. Acute toxicity tests were performed on green algae, invertebrates (midge), and fish (fatheads). Definitive acute toxicity data for the FF products for algal toxicity are 0.69 – 259.59 mg/L and the comparative short-chain PFAS AFFF was near the mid-point within this range (136.08 mg/L). The definitive acute toxicity data for the FF products for chironomids are 24.51 – 1,036.8 mg/L and the comparative short-chain PFAS AFFF was near the higher end of this range in toxicity (885.2 mg/L). This data suggest two of the FF products are moderately toxic to chironomids. Preliminary chironomid chronic toxicity suggests emergence is the most sensitive endpoint. Definitive acute toxicity data for the FF products for fathead minnows are 2.31 – 813.12 mg/L with the short-chain PFAS AFFF serving as the high end of this range. Preliminary fish chronic toxicity data should be available by October 2023. Applying the alternatives assessment criteria developed by the U.S. Environmental Protection Agency, two of the seven PFAS-free foams tested appear to fall within moderate-very high hazard for all three test species. This appears to be toxicity specific and not an indirect effect related to the chemical oxygen demand of surfactants within the foams. These data are undergoing further synthesis and comparison while definitive chronic toxicity testing is expected to be conducted in the near term. Finally, a Species Sensitivity Distributions (SSDs) using aquatic LC50 data was computed, resulting in a hazard ranking of products tested.

2.14.P-Tu-120 Unintended Consequences of Stream Restoration: Iron Ecotoxicity in Regenerative Stream-water Conveyance Systems

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Visible iron flocculate and high iron concentrations are naturally occurring conditions in Anne Arundel County (AAC), Maryland streams and sediments given the underlying iron ore geology. It has been hypothesized that

restoration designs known as Regenerative Stream-water Conveyance (RSC) systems may increase dissolved iron concentrations and flocculate. Hence, there is concern that RSC systems may lead to unintended, negative consequences. Benthic habitat quality may be reduced by the coating and imbedding of iron into stream substrates and sediments as well as increasing indirect and direct toxicity, but this is poorly understood. Altered macroinvertebrate and microbial activity may impact ecosystem functions such as the decomposition of organic matter. The goal of this study was to elucidate the toxicological and functional impacts of iron in RSC systems and reference streams in AAC via water quality monitoring and field experiments. The water quality and iron conditions of RSC systems versus reference streams were characterized monthly via monitoring. *In situ* macroinvertebrate enclosures and leaf packs were deployed to evaluate the toxicological response of caddisfly larvae and to assess the decomposition of organic matter, respectively, under various iron conditions. We hypothesized that iron concentrations and toxicity would be higher in RSC systems while leaf pack decomposition would likely be lower. Most of the study stream's average iron concentrations exceeded the EPA chronic criterion, and iron concentrations were on average higher in RSC systems as compared to reference streams. Survival of hydropsychid caddisflies exposed *in situ* via enclosures was notably decreased in systems of higher iron concentrations, but this effect was not significant. While restoration status did not have a significant effect on decomposition the effect of iron conditions was significant. We hypothesize that iron conditions will also have an effect on abundance and diversity of macroinvertebrates colonies retrieved from the exposed leaf packs. Potentially exacerbated iron conditions as an unintended consequence of RSC restoration may have important implications for future restoration programs and goals particularly in AAC, such as ecosystem health. Research that applies field toxicity experimentation and survey methods to address concerns regarding RSC implementation and subsequent iron conditions can have important management applications.

2.14.P-Tu-121 Changes in Carbon and Nitrogen Stable Isotopes and Fatty Acid Biomarkers in Various Life Stages of the Laboratory Mayfly (*Neocloeon triangulifer*)

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Aquatic emergent insects serve as an important prey items in both aquatic and terrestrial food webs. The mayfly species *Neocloeon triangulifer* has been recently developed as a model invertebrate test organism, however, much is still unknown about the differences that exist across their various life stages. In this study, we aimed to understand if changes in carbon and nitrogen stable isotopes and fatty acid biomarkers occurred during mayfly metamorphosis and during egg deposition. We measured carbon and nitrogen stable isotopes as well as $\omega 3$ and $\omega 6$ fatty acids in laboratory-reared mayflies (*Neocloeon triangulifer*) in larvae, subimago and imago life stages collected from six different laboratories with existing cultures in the US and Canada. Due to inter-lab differences in diatom diets, isotopic values in mayflies were compared to their laboratory-specific diatom values. No major changes in $\delta^{13}\text{C}$ or $\omega 3$ and $\omega 6$ fatty acid values were observed between life stages, however, $\delta^{15}\text{N}$ and the %N, as well as %C varied between life stages. Pre- and post- oviposition mayflies saw no significant differences in $\omega 3$ and $\omega 6$ fatty acids, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, or %C, however, there was a significant decrease in %N. In addition, inter-lab differences occurred with isotopic values, but not fatty acid values, with the exception of eicosapentanoic acid (20:5 $\omega 3$). This work adds to the growing literature base on laboratory mayflies and their energetic dynamics. When considering aquatic emergent insects for use in food web studies, the different life stages may not have an impact on stable isotopes or fatty acid biomarkers, however, life stage and oviposition status in adults will need to be taken into consideration.

2.14.P-Tu-122 Evaluating Microcystin in Water and Fish Tissue from Four Reservoirs in the Georgia Piedmont, USA

Jacob Smith, Krista Capps, Cory Struthers, Xiaoyu Xu, Peter D Hazelton, Michelle Ritchie and Alex Strauss, (1)University of Georgia

Harmful algal blooms (HABs) are among the greatest inland water quality threats to aquatic wildlife, fish, and humans. Certain cyanobacteria in HABs produce cyanotoxins, such as microcystins, which are highly toxic to

terrestrial and aquatic organisms. The primary routes of microcystin exposure are through dermal contact with, or consumption of contaminated water, and consumption of accumulated toxins in aquatic animal tissue. The accumulated microcystin levels in fish tissue can vary across species and may depend on fish diet; however, little evidence exists for biomagnification of the toxin. To investigate the species and locations that pose a greater risk for microcystin accumulation, this study will utilize toxin screening techniques (i.e., enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC)) on fish and water samples collected from four reservoirs around Athens, GA, across a six-month timespan. Given the initial data already collected, we also plan to evaluate the time points of greatest microcystin levels, possible trophic associations between fish species, and the potential risks to human health from recreation and fish consumption within these reservoirs.

2.14.P-Tu-123 Effect of Ion-Exchanger to Inorganic Chemicals Using *Daphnia magna*

Tatsuo Abe, Tsuruoka College

Various inorganic chemicals exist in environment, their come from natural source and artificial source. Inorganic chemicals include metal, metal ion, metal salts, and nano materials. Some inorganic chemicals have toxicity like heavy metals. Ion exchanger is very useful for adsorption, removal and selectively separation. Inorganic ion exchanger and ion exchange resin were used for ion exchanger. Cesium nitrate (CsNO_3) and Cesium chloride (CsCl) were used for inorganic chemicals. *Daphnia magna* acute immobilization test was used for this study. The test method was based on OECD Test Guideline 202. Each 100 mL test solution in beaker was prepared in M4 medium. 20 neonates (daphnia within 24 hours old) placed each concentration and control, each beaker observed after 24 and 48 hours to obtain a 50% effective concentration (EC_{50}). Dowex 50Wx8 100-200 mesh (FUJIFILM Wako Pure Chemical, Japan) and IXE-300 (Toagosei co., LTD) were used for ion exchanger. Inorganic chemicals were prepared as concentrated solution in M4 medium. The test conducted with control, humic acid control and five concentrations. EC_{50} for IXE-300 was lower than EC_{50} for Dowex 50Wx8. Further study will be need about ion exchanger combined inorganic compounds.

2.14.P-Tu-126 An Evaluation of the Toxicity of Chemical Mixtures in Aquatic Environment; Integration Of Ostracodtoxkit Ftm Bioassay Toxicity, Hepatotoxicity and Oxidative Stress in Albino Rats

Arinze Ik. Ari Onwurah¹, Obinna A Oje², Emeka Ogbuene¹ and Ikechukwu N.E. Onwurah¹, (1)University of Nigeria, (2) Alex Ekwueme Federal University

Long range transport of heavy metals has been reported to play significant roles in the concentrations of metals in sediments of water bodies, through transported/diffusion of gaseous and particulate contaminants in the troposphere, which is followed by either dry or wet deposition. In Port Harcourt, River State, Nigeria with myriads of oil industry and petrochemical related activities, air pollutants which were reported to contain Fe, Ni, Cd and Cr and included polycyclic aromatic hydrocarbons (PAHs) formed obvious deposit as black soot. The impact of such chemical mixtures in aquatic ecosystems could be diverse due to interaction or by other habitat degradation. The present study utilised Ostracodtoxkit F tm bioassay as qualitative whole- water toxicity assay (QWTA), coupled with two risk factors, namely protein oxidation products (assayed as carbonyl compounds) and hepatotoxicity in rats' hepatocytes of albino rats intubated with contaminated "pool water" as a model polluted aquatic ecosystem water. Results show that all the pools were in fairly good sanitary conditions based on the mean bacterial counts ($2.33 \pm 0.57/100$ ml), while all were slightly acidic and with normal temperature range. The toxicity indices of the 5 pool water samples designated SP 1 and SP 5 are respectively 20.8 and 49.0 units, while that of SP 2 and (SP 3) are the same (42.5 units). "No-observed-effect-toxicity" (NOET) was observed for the control indoor pool water sample - SP 4. Results of protein oxidation in the rats and hepatotoxicity of their hepatocytes suggest that oxidative stress-related risks may be involved.

2.14.P-Tu-127 Congruency of Environmental DNA (eDNA) Metabarcoding Approach to Conventional Fish Communities Assessments in Low Order Streams

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Environmental DNA (eDNA) may be captured through collection of bulk environmental samples and analyzed using DNA barcoding approaches to detect biological species without organism capture or sighting. Fish community biodiversity assessment is possible through eDNA metabarcoding, using universal primers and next-generation sequencing technology. However, limitations imposed by the taxonomic coverage of existing universal primer sets and their corresponding databases remains a significant challenge for community detection. In this study we evaluate the congruence between eDNA generated fish community identification using metabarcoding (genetic markers: 12S rRNA and COI mitochondrial gene fragments) and fish community assessment collection data in low order streams using standard electrofishing techniques. Water samples were filtered for eDNA collection on site prior to fish surveys to limit site disturbance. During the processing of the electrofishing collections, fin clips were taken from individuals of each species to develop a mock community to ground-truth the metabarcoding results, also compared to electrofishing collections. The fish community profile generated through metabarcoding was evaluated against conventional fish survey results to identify false positive and false negative detections and frequency of congruence between technologies. Using a combination of both 12S and COI markers we observed 76% congruence with the conventional fish collections. The lack of species sequence coverage of known species in these water highlighted the need to resolve genomic misidentifications and resolution blind spots emphasizes the importance of validating eDNA metabarcoding generated data through parallel experiments using conventional techniques as well as through historical and local knowledge, and experience.

2.14.P-Tu-128 An Evaluation of Alternative Approaches to Fish Testing to Meet the Needs for the National Pollutant Discharge Elimination System (NPDES) Permitting Program: An Industry Perspective

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The Clean Water Act prohibits facilities from discharging pollutants into U.S. waters unless they have a NPDES permit. Although the mission of the program remains consistent across all states, toxicity data requirements can be different based on state-specific guidance. Ecolab is a strong advocate of the 3R principles. To minimize vertebrate animal testing under the NPDES program, Ecolab has developed internal guidance to leverage alternative data to estimate a product's overall aquatic toxicity using three approaches (ingredient data, invertebrate data, similar product data). Earlier analyses of Ecolab products have shown that ingredient data used to estimate product toxicity correlates well with product testing results. To further our research, we evaluated aquatic toxicity with a new dataset and by comparing fish data to invertebrate data. Over the past five years, 56 aquatic toxicity tests in fish were conducted in support of the NPDES program. Approximately 6,360 fish were used in these tests. We evaluated the products with test data against two alternative methods. The first method relies on invertebrate test data. Of the 53 products with fish data, 45 products also had aquatic invertebrate toxicity data. 35 of the 45 products reported invertebrate toxicity values either more sensitive or equivalent to fish toxicity data. Only 2 of the 10 products with lower LC50 fish values resulted in a more severe Globally Harmonized System (GHS) hazard classification. The remaining 8 products had both fish and invertebrate LC50 values that would classify the product in the same GHS hazard classification. Therefore, invertebrate data indicates a product's toxicity with 95% accuracy. The second method relies on ingredient data to estimate the GHS aquatic classifications. Based on this method, 33 of the 45 products either had classifications estimated to be more conservative or aligned with the product test data. Ecolab also attempts to leverage existing aquatic toxicity data generated on similar product formulations to meet data requirements. Ecolab has had success with some states accepting our alternative approaches for toxicity data, but differences

in states' acceptability of these methods remains a challenge to industry. In the future, we hope that this combination of alternative approaches in evaluating product toxicity will reduce the need for vertebrate toxicity testing while still providing the regulatory agencies with high quality assessments.

2.14.P-Tu-129 Using a Probabilistic Approach in an Ecological Risk Assessment PFAS Food-Web Based Model

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The determination of PFAS bioaccumulation is crucial to understanding hazards associated with PFAS at contaminated sites, however, tissue sample analysis can be complicated and expensive. As a result, models that link toxicity and bioaccumulation are required. In the past year, a food-web based model was developed that allows the user to use measured concentrations of PFAS in abiotic media to estimate PFAS concentrations at different trophic levels in aquatic food webs. While the model provides a useful tool for risk estimation, it takes a deterministic approach. However, probabilistic risk assessment provides a means for characterizing uncertainty and variability, allowing the assessment of uncertainty in relation to environmental management decisions. Thus, the goal of this research is to develop the model into a probabilistic model. To achieve this, standard deviations of PFAS bioaccumulation factors were incorporated into the model from available literature. Following that, Crystal Ball was enabled in the model that allows the user to run several simulations and obtain PFAS tissue concentration distributions at different trophic levels. These probability distributions are crucial for understanding the uncertainty associated with risk estimates. Moreover, the model can be used to identify the key drivers of variability in PFAS tissue predictions. Median perfluorooctanoic acid (PFOA) concentration values in sediment and surface water from available literature were used in the model to determine the main sources of variability in PFOA tissue predictions. The results showed that the major source of variability for PFOA in fish came from biomagnification factor of fish. The model was also updated to incorporate calculations to determine the contribution of PFAS from sediment and water to PFAS in fish, bird, and mammal tissue. The results, based on median PFAS concentrations in sediment and water from available literature for short and long chained PFAS, showed that, on average, water was the primary source of PFAS for short chained compounds, whereas sediment played a significant role in contributing to long chained PFAS. Factors such as dissolved organic carbon and salinity can be incorporated in future model updates as these have shown to influence PFAS bioavailability. The enhanced probabilistic model can aid in improved risk characterization, communication of uncertainty, and informed risk management efforts.

2.14.P-Tu-130 Freshwater Insect-Mediated Polychlorinated Biphenyl Transfer from Freshwater and Terrestrial Ecosystems

Peter Wilson Blum and Justin N. Murdock, Tennessee Technological University

Discharged coolant water in streams and military activity have left a legacy of polychlorinated biphenyl (PCB) contamination near Tullahoma, Tennessee. Affected streams drain into a reservoir, creating a mosaic of PCB-contaminated sediment through the military installation, which is home to rare and endangered consumers that utilize freshwater insects as a food source. Emergent adult freshwater insects can transport bioaccumulated PCBs to insectivorous terrestrial consumers, such as endangered gray bats on the base. The transport of PCBs to terrestrial food webs is likely influenced by the route of bioaccumulation in immature insects, PCB flux from bodies of water, and possibly by insect stoichiometry. A better understanding of factors that control PCB transport could provide better management of species toxicity exposure and better focus limited resources. To assess the movement of PCBs within and between aquatic and terrestrial systems, we examined bioaccumulation pathways within stream food webs using stable isotopes, insect-derived PCB flux using floating emergence traps, and nutrient stoichiometry on regulating PCB transfer through the aquatic food web and into terrestrial consumers. We predict differences in insect-derived PCB flux between stream and reservoir sites, a positive correlation between higher insect trophic level and PCB concentration, and an inverse

correlation between insect carbon:phosphorus ratio and PCB concentrations. This research will provide information on how PCBs move into aquatic organisms and across ecosystem boundaries and how species diversity and nutrient availability may influence transport rates across trophic levels.

2.14.P-Tu-131 Mercury Contamination of Wolf Spiders from Northwest Greenland

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Widespread emissions of mercury (Hg) from anthropogenic sources coupled with a global atmospheric cycle have resulted in the contamination of all freshwater systems with Hg at concentrations above pre-industrial levels, including in remote regions like the Arctic. The degree of contamination varies between aquatic systems due to a variety of landscape and physicochemical factors that influence the transport and methylation of Hg. Therefore, individual systems must be monitored to assess the level of contamination in biota. Spiders have been used as sentinels of Hg contamination in temperate regions, but few studies have been conducted above the Arctic circle. We collected 1460 wolf spiders (*Pardosa glacialis*) from eight sites in the High Arctic of northwest Greenland (centered around 76.5° N, 68.8° W). For each spider, we measured spider leg length (tibia + patella length) as a proxy for body size. Spiders from each of the eight sites were composited by size (resulting in 4-7 composite samples per site) and analyzed for total Hg using direct Hg analysis. Total Hg concentrations ranged from 230 ng/g dry weight (dw) to 1100 ng/g dw. Using ANCOVA, we detected significant main effects of spider size ($p < 0.001$) and site ($p < 0.001$) indicating that total Hg concentrations were positively correlated with spider size and that total Hg concentrations varied by as much as 4.8x between sites. We did not detect a significant size x site interaction ($p = 0.72$), indicating that the relationship between body size and tissue Hg concentration in *Pardosa glacialis* was consistent across sampling sites. The present study suggests that total Hg concentrations of spiders are elevated and highly variable across northwest Greenland.

2.14.P-Tu-132 Current Contaminant Concentrations of Blue Crab (*Callinectes sapidus*) from New York State Waters

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Blue crabs (*Callinectes sapidus*) are an abundant and popular species in the Hudson River, New York Harbor, and surrounding waters. As a benthic omnivore, these crabs are a potential source of contaminant intake for consumers higher on the food chain, including humans. Current New York State Department of Health advisories call for limited consumption of blue crab due to contaminants including polychlorinated biphenyls (PCBs) and cadmium (Cd). The last comprehensive assessment of contaminants in blue crab in New York (2004-2005) showed lower concentrations post remedial actions for Cd, but not enough of a decline to remove the advisories. This new two-year study sampled crabs from New York State in a spatially structured sampling design. Sampling occurred during 2021 and 2022 around the migratory period of August–October when blue crabs are found throughout the New York Harbor and the Hudson River Estuary. Hepatopancreas and leg muscle tissues were used to assess contaminant concentrations since both are common for human consumption and have different properties affecting contaminant accumulation. Contaminants including PCBs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), and Cd were analyzed to reassess current health consumption advisories. Additionally, analytes including mercury (Hg), per- and polyfluoroalkyl substances (PFAS), and polybrominated diphenyl ethers (PBDEs) were chosen to evaluate current contaminant concentrations in blue crab. The results of this study will be used to inform future health consumption advisories, assess spatial/temporal trends, evaluate remedial actions, and give a snapshot of the current concentrations of a large suite of contaminants in this ecosystem. The first year of results focuses on blue crabs

sampled from the Hudson River Estuary. Among the salient results, Cd concentrations in the river were not found to be declining as previously observed.

2.14.V-004 - A Neotropical Fish Exposed to Zinc: Increasing Temperature Modifies Effects on Plasma Parameters

Vanessa Bezerra and Juliana Delatim Simonato Rocha, State University of Londrina, Brazil

Climate change is causing temperature increase not only in the atmosphere but also in aquatic ecosystems, posing risks to aquatic fauna. This problem can be exacerbated by environmental contamination, however, the effects of these different stressors in combination is still poorly understood. Zinc is an essential metal, but it can be toxic in concentrations above the necessary, causing concern as an environmental contaminant in aquatic ecosystems. Thus, the objective of this work was to evaluate whether the increase in temperature modifies the sublethal effects caused by zinc in hematological and plasma parameters of the Neotropical freshwater fish *Prochilodus lineatus*. Juveniles of *P. lineatus* ($n = 32$, 14.4 ± 5.3 g, 10.8 ± 1.2 cm, mean \pm SD) were purchased from a local fish farm and acclimated to laboratory conditions for at least 7 days. After acclimation, a 72-h exposure test was performed. The animals were divided into 4 groups containing 8 individuals each: a control group (CTR) containing water at room temperature (22.7 °C); a group with water at 27 °C (T27); a group exposed to increased temperature and 2 mg L⁻¹ of zinc (T27+Zn); and a group exposed to zinc at room temperature (Zn group). After the exposure, the animals were anesthetized for blood sampling and then euthanized by cervical transection. Part of the blood was used to evaluate hemoglobin concentration, hematocrit, and the number of red blood cells. The remaining part was centrifuged to obtain the plasma, which was used to assess glucose, total proteins, sodium, and potassium concentrations. The groups did not differ regarding the hematological parameters and plasma protein concentration. However, the Zn group presented an increase in glucose and sodium concentrations, and only the T27+Zn group showed increased potassium concentration. These results show that exposure to zinc causes an increase in sodium uptake and a stress response, as indicated by the hyperglycemia. However, increasing temperature prevented these effects and led to a different ionic imbalance, related to potassium. We can conclude that in *P. lineatus* increasing temperature changes the effects caused by zinc in plasma parameters. More studies evaluating other osmo-ionic biomarkers are needed to understand the mechanisms by which these responses occur.

2.15.P-Th Late Breaking Science: Aquatic Toxicology, Ecology and Stress Response

2.15.P-Th-185 Understanding the Impacts of Multiple Road-Related Contaminants on *Daphnia magna* Survival and Transcriptome

Elli Hung, Rachel Giles and Chelsea Rochman, University of Toronto

Toxicity experiments are informative tools to understand biological responses of organisms to anthropogenic contaminants. Anthropogenic contaminants such as salt, polycyclic aromatic hydrocarbons (PAHs), tire wear particles, and heavy metals are associated with roads, and enter aquatic ecosystems as runoff during rainfall or snow melt events. This mixture of contaminants can interfere with the physiological processes of organisms, such as gene expression, which can lead to effects at higher levels of biological organization (i.e., ecosystems). Although a significant amount of research has been conducted on individual anthropogenic contaminants, assessing the impact of these contaminants in combination has not been well studied. *Daphnia magna* are an ideal organism to investigate genomic responses to multiple stressors because of their frequent use in standard toxicity tests and ecological relevance to the Laurentian Great Lakes. In addition, the *Daphnia magna* genome has been extensively characterized allowing for robust transcriptomic analysis. The purpose of this project was to investigate the effects of a combination of road-related contaminants on the survival and gene expression of *Daphnia magna*. *Daphnia* were exposed to fifteen combinations of salt, copper, tire wear particle leachate, and pyrene as part of a two-day, acute toxicity test. A subset of surviving organisms from this test were subsequently preserved to extract RNA from and perform RNA-seq for transcriptomic analysis. Preliminary

analysis shows that different combinations of stressors have varying impacts on daphnia survival and that copper, at all concentrations, led to 100% mortality. Transcriptomic analysis will allow for exploration of the molecular mechanisms impacting survival and how these mechanisms change in response to different contaminants in combination and at various concentrations. Overall, this project will help us to better understand how multiple stressors interact with each other to impact aquatic biota, bring environmental relevance into toxicity tests, and ultimately inform protection and monitoring of aquatic systems in the Laurentian Great Lakes.

2.15.P-Th-186 Rapid Spread of Omicron Sub-Lineage as Evidence by Wastewater Surveillance

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The search for better tools for interpreting and understanding wastewater surveillance has continued since the beginning of the COVID-19 pandemic. SARS-CoV-2 has continued to mutate, thus complicating the interpretation of surveillance results. We assessed the role of Omicron variants (BA.1, BA.2 and BA.5) associated with wastewater-derived SARS-CoV-2 RNA trends by estimating the effective reproduction number R_{eff} using an epidemic model that integrates explicitly the SARS-CoV-2 N2 gene concentration detected in wastewater through rt-qPCR quantitative analysis. The model inferred COVID-19 cases based on wastewater data and compared them with the ones reported by clinical surveillance. The variant of the SARS-CoV-2 associated with the wastewater-derived SARS-CoV-2 RNA was monitored through wastewater whole-genome sequencing. Three major waves between January and September 2022 were associated with Omicron subvariants (BA.1, BA.2, and BA.5). This work showed that disease trend and forecast can be done with effective reproduction number. The effective reproduction number plot is simple and easy to understand.

2.15.P-Th-187 Relationship between Nitrate Level and Physicochemical Parameters of the Eleven Point River, Arkansas

Karimot Shobowale and Jennifer Bouldin, Arkansas State University

Agricultural activities are the mainstay of food availability. Several developments have been geared toward enhancing agricultural production to ensure meeting the food demand of the ever-growing human population. Increased use of fertilizers and industrial animal production are on the rise. Unfortunately, these activities pose a threat of pollution in the aquatic environment. Nutrient pollution is a major concern because they emerge from non-point sources. The Eleven Point River is scenic and supports recreational activities throughout the year. It emerges in Missouri and flows through Arkansas. Animal production is prevalent within the watershed located in Arkansas. To evaluate the impact of land use on the Eleven Point Watershed there is an ongoing three-year weekly monitoring of the watershed's physicochemical parameters and nutrient levels. Principal component analysis was used to determine the interplay between nitrate level and other physicochemical parameters measured over the span of eighteen months.

2.15.P-Th-188 Use of Riparian Predators to Study PFAS Bioaccumulation and Terrestrial Export from Farm Ponds

Abigail Corrine Valachovic, Tyler D. Hoskins, Youn Jeong Choi, Robert Wesley Flynn, Linda S. Lee and Marisol S. Sepulveda, Purdue University

Per- and polyfluoroalkyl substances (PFAS) are highly persistent, bioaccumulative contaminants that pose risks to human and wildlife health. Municipal biosolids, which are commonly spread on agricultural fields, can contain substantial levels of PFAS and therefore could contribute to human and wildlife exposure. To date no studies have quantified PFAS bioaccumulation and/or trophic transfer from farm ponds receiving biosolid runoff. Further, there is little understanding on the *in vivo* transformation of PFAS precursors in organisms, although available data suggest that there could be major differences in metabolic capabilities between vertebrates and invertebrates. Riparian predators like tree swallows (*Tachycineta bicolor*) and tetragnathid

spiders feed almost exclusively on emergent aquatic insects and have been demonstrated to serve as excellent passive samplers of bioaccumulative xenobiotics with aquatic sources. We sampled swallow eggs and nestlings and spider carcasses at two sites, one receiving biosolid runoff and one no-biosolid reference site. The primary objective of our study was to evaluate how patterns of PFAS accumulation differ between vertebrate and invertebrate species feeding at comparable trophic levels. We predict that, while total loads will be similar between spiders and birds, spiders will bioaccumulate substantial levels of PFAS precursors, whereas birds will contain primarily terminal degradation products. Our data provide insight on how land application of biosolids could contribute to wildlife exposure to PFAS.

2.15.P-Th-189 Mercury and Methylmercury Trophic Transfer in an Industrially Contaminated Stream

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Mercury (Hg) is a globally important contaminant which is of particular concern in stream systems. East Fork Poplar Creek (EFPC) is a point-source contaminated stream on the U.S. Department of Energy's Oak Ridge Reservation. Extensive studies have been conducted in EFPC over the past three decades to understand mercury science, for example, the genetic basis for Hg methylation, methodologies for sampling, relationships with water chemistry, and bioaccumulation. Because mercury concentrations in water and fish have regulatory implications, total Hg in water and fish in EFPC have been reported for decades despite the recognition that Hg, especially in methylated form is predominantly accumulated in fish via diet rather than aqueous exposure. Total Hg is often used as a suitable surrogate for MeHg due to reduced cost and analysis time, though the relationship of total Hg and MeHg biomagnification in food webs is not well described in EFPC. In 2015, fish and macroinvertebrates were collected during Spring and Fall to determine patterns of biomagnification for total Hg and MeHg through the food web in EFPC. This relationship can elucidate approaches to remediation efforts, as reducing total Hg input alone may not be adequate for reducing concentrations in fish if methylation is still occurring. Biomagnification of Hg was calculated as trophic magnification factors (TMF) using regression slopes of $\log_{10}10$ Hg concentrations and trophic level. Trophic magnification factors of less than 1 implies trophic dilution and greater than 1 implies trophic magnification. Results show that the two most upstream sites show trophic dilution of total Hg and the lower 3 sites being slightly above 1, implying minimal magnification. Methyl mercury TMFs were ~2, indicating significant trophic magnification, which is an expected result given the high efficiency of transfer. Trophic magnification factors in a reference stream, Hinds Creek, are >1.5 for both total and methyl Hg. The lack of evidence for trophic magnification of total Hg in EFPC fish suggests that remediation efforts focused on reducing total mercury concentrations in water may not result in achieving the desired fish tissue concentrations. Remediation strategies at industrially contaminated streams such as EFPC may need to include the use of best management practices to manage mercury methylation and trophic transfer in addition to reducing total Hg concentrations to mitigate risks of Hg and MeHg exposure.

2.15.P-Th-190 An Attempt to Assess the Ecological Risk of UV Filters in Japanese Subtropical Waters Using Native Coral Species

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UV filters are widely used in sunscreens and personal care products as an essential component to protect the skin from UV radiation. After use, they are either washed away through household drains during activities such as bathing, or directly discharged into environmental waters such as seawater. As the production and consumption of UV filters increases worldwide, the ecological risks they pose, particularly to coral reefs, have attracted scientific and environmental policy attention. Japanese subtropical waters are connected to the world's most biodiverse area, the Coral Triangle, by the Kuroshio Current. As a result, Japan may also see an increase in regulatory efforts regarding UV filters, taking into account the policies of other countries. However, there are few examples of ecotoxicity testing and risk assessment tailored to the ecological realities of Japan. This study focuses on assessing the potential risks of UV filters to coral growth and coral reef sustainability in Japanese

subtropical waters under realistic environmental conditions. In the first step of the study, ecotoxicity test protocols for native coral species found in Japanese coastal areas, such as *Acropora digitifera*, will be developed, and applied to conduct ecotoxicity tests on several types of UV scattering and UV absorbing agents. At the same time, sampling will be carried out in coral reef areas to determine current environmental concentrations. In a second step, environmental exposure models will be used to investigate the effectiveness of potential measures to reduce ecological risks to corals. The presentation will include an overview of the study, an ecotoxicity test protocol developed for *A. digitifera*, and preliminary results of ecotoxicity tests with UV scatterers using the developed protocol.

2.15.P-Th-191 Algal Community Dynamics and Bloom Formation in Tributaries Influenced by Navigational Controls on a Managed River Ecosystem

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Increases in harmful algal bloom frequency and severity in marine and freshwater ecosystems are well documented. Blooms in riverine ecosystems present questions and challenges different than their lentic freshwater counterparts. Subsequent to a 750-mile-long bloom in the Ohio River in 2015, high frequency sensors were deployed on lock structures of the Robert C. Byrd and Greenup Dams to better understand the algal dynamics in the managed river system, and specifically the 61.8 mile Greenup Pool. In addition, transect sampling was conducted through the pool's entirety at 22 locations to profile variability in the pool and relate pool conditions to the lock sites. These data showed low overall productivity in the pool with turbidity and velocity found to be significant variables limiting the algal community in the managed river ecosystem. Data collected over a 4-year period indicate the turbidity of the river is sufficient to limit photosynthetic activity greater than 90% of the time. Factors limiting productivity in the mainstem of the river are under further investigation, as well as the potential contribution of tributaries to mainstem algal blooms. Managing mainstem flow to maintain navigational pool was hypothesized to limit tributary inflow and amplify backwater conditions facilitating algal growth in the tributaries. Current investigations include acoustic doppler velocity profile characterization in 4th to 6th order tributaries of the Ohio River, as well as, benthic and water column algal sampling, zooplankton sampling and water chemistry analysis. Preliminary data evaluations indicated zooplankton plays a larger than expected role in the ecosystem and a challenging relationship exists between algal community development and flow. Tributary flow characteristics are substantially influenced by mainstem discharge. Results of these investigations are likely broadly applicable to other managed river systems.

2.15.P-Th-192 Artificial Intelligence, Machine Learning, and Deep Learning to Expedite Data Acquisition for Aquatic Biomonitoring of Contaminated Streams

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Biomonitoring to determine the health of an aquatic system is a labor-intensive process requiring sampling, sorting, and identification by an expert taxonomist. The Biological Monitoring and Abatement Program (BMAP) at Oak Ridge National Laboratory (ORNL) regularly samples aquatic macroinvertebrates as part of the ongoing efforts to remediate the mercury contamination of aquatic systems on the reservation. Recent advances in artificial intelligence (AI) have led to technologies that incorporate AI to classify images for scientific advancement. Machine learning and deep learning techniques are beginning to be used for identification and enumeration of biological organisms, thereby reducing the time and effort associated with typical biomonitoring techniques. A high throughput prototype machine, the Small Aquatic Organism (SAO) optical imaging system, utilizes computer vision and support vector machine (SVM) learning to classify and enumerate aquatic organisms. In this study, we use 28 different families of aquatic macroinvertebrates to compare the identification accuracy of the SAO to the identification accuracy of a deep learning convolutional neural

network (CNN) to determine if this technology could be advantageous to BMAP. Expertly identified families of macroinvertebrates were run through the SAO to build an image library as well as imaged under a stereomicroscope to produce ideal images for a CNN model. Macroinvertebrates were placed on a mesh to hold them in place for imaging. Color stereoinmager achieved 88% accuracy, while grayscale stereoinmager and the SAO achieved 76% and 61% accuracy respectively. Grad-CAM heat mapping code in Python showed that the mesh background was a large part of the image being used to classify each family, so these were re-imaged without mesh or background so only the organism is being used for classification. AI has great potential to revolutionize the biomonitoring process and assist with BMAP by dramatically reducing the labor and cost of traditional methods and thus should be a focus of future biomonitoring studies.

2.15.P-Th-193 Understanding Pharmaceutical Exposure and the Potential For Effects in Marine Biota: A Survey Of Bonefish (*Albula vulpes*) Across the Caribbean Basin

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Recently, pharmaceutical contaminants and their presence in biota have received increasing attention in aquatic and marine systems. Most research to date has focused on smaller scale assessments of freshwater and riverine systems, relying mainly on assays of water samples, but studies in marine ecosystems and of exposed biota are sparse. This study investigates the extent to which bonefish (*Albula vulpes*), which supports important recreational and artisanal fisheries, are exposed to pharmaceutical contaminants throughout their range in the Caribbean Basin. We sampled 74 bonefish from five regions (Lower and Upper Florida Keys, Belize and Mexico, The Bahamas, and Puerto Rico) and analyzed them for 102 commonly prescribed pharmaceuticals. We assessed the influence of sampling region on the pharmaceutical assemblage, number of pharmaceuticals, and risk of pharmacological effects. To quantify risk of pharmacological effect, we compared measured concentrations to a threshold of 1/3rd the human therapeutic plasma concentration (H_TPC) for each pharmaceutical. Every bonefish had at least one pharmaceutical, with an average of 4.9 and a maximum of 16 pharmaceuticals in an individual bonefish, and a total of 49 different pharmaceuticals were detected. At least one pharmaceutical was detected in exceedance of the 1/3rd H_TPC threshold in 39% of bonefish, with an average of 0.6 and maximum of 11 pharmaceuticals exceeding in an individual bonefish. The number of detected pharmaceuticals (i.e., pharmaceutical exposure) differed across regions, but the risk of pharmacological effects did not, with some regions experiencing lower degrees of exposure having higher instances of concentrations exceeding the 1/3rd H_TPC threshold. These findings suggest that pharmaceutical detections and concentration may be independent, emphasizing the need to monitor risk to biota regardless of exposure diversity. Our results further establish the widespread presence of pharmaceuticals in marine systems at concentrations capable of pharmacological effects and underscore the need to assess both exposure and risk to biota.

2.15.P-Th-194 Novel in vivo Model to Assess the Molecular Mechanisms of Iron and Other Essential Metal Homeostasis in Zebrafish (*Danio rerio*)

Theanuga Chandrapalan and *Raymond Kwong*, York University

Freshwater environments across North America have been affected by urbanization and accompanying metal pollution, however, the understanding of the implications of these changes and the regulatory parameters set to protect aquatic life lags. In cases of aquatic metal pollution, aquatic animals face a unique set of challenges arising from metal-metal interactions at routes of uptake and this can alter the bioaccumulation and toxicity of metals. To understand the molecular mechanisms underlying these processes, we utilized CRISPR-Cas9 technology to knockout an apical metal transport protein, the divalent metal transporter 1 (DMT1). DMT1 has

been proposed to be the major transporter for iron but it can also transport several other metals, including essential (zinc, manganese, cobalt, copper, and nickel) and non-essential (cadmium and lead). As such, the DMT1 mutants provide a valuable model to determine the *in vivo* importance of this transporter in both iron uptake and the regulation of other metals in the body. The significance of this protein in growth and maintenance of whole body metal homeostasis will be compared between wild type and mutants throughout development. Underlying changes in proteome will be compared to dissect any involvement of other metal transporters in compensation for the loss of DMT1. The results of our study will provide information on the homeostatic processes involved in iron regulation and its interaction with other metals. Additionally, this mutant line can serve as a novel model to elucidate the molecular mechanisms governing metal homeostasis during exposure to a metal mixture.

2.15.P-Th-195 Global Occurrence and Aquatic Hazards of Benzodiazepines in Influent Wastewater, Effluent Discharges, and Surface Waters

Hyeongyoung Choi and Bryan W. Brooks, Baylor University

Benzodiazepines are a group of psychoactive pharmaceuticals that are used to treat a variety of symptoms such as anxiety, epilepsy, and insomnia. Due to the widespread usage of this class of medicines, benzodiazepines have been frequently reported in aquatic systems. Because previous research has identified potential impacts on aquatic organisms, here we performed a critical review of the refereed literature to better understand the prevalence of benzodiazepines in aquatic matrices and their associated water quality hazards. Forty-five benzodiazepines were included in this review, of which 20 compounds have been detected in 5 different aquatic matrices ranging across 6 continents. Environmental exposure distributions were created for compounds that had sufficient data ($n > 5$) and probabilistic environmental hazard assessments were conducted using therapeutic hazard values (THV) of each compound. The most monitoring data was found in Europe with 1414 data points while Asia and North America were a distant second and third with 426 and 145 data points, respectively. Data gaps were identified in Antarctica, South America, Oceania, and Africa where there were 0, 10, 19 and 53 data points in these large geographic regions, respectively. Highest concentrations of benzodiazepines were detected in effluent samples. Bromazepam was detected at ~24 mg/L from pharmaceutical plant effluent at an unknown location. Surface water was another hotspot for benzodiazepines; for example, diazepam and temazepam have been detected at over 10 $\mu\text{g/L}$ in Odo-Iya Alaro River, Nigeria. Oxazepam was the most frequently detected benzodiazepine in aquatic matrices, though its highest THV exceedance was 2.066% in European influent waters. Temazepam was the fourth most detected benzodiazepine, but the highest THV exceedance among all compounds was observed for temazepam at 67.684% in African surface water and 12.389% in effluent across all geographic areas. This review highlights important water quality hazards among global regions and aquatic matrices, which can inform future research efforts to understand benzodiazepines in the environment.

2.15.P-Th-196 Investigation into the Differential Uptake and Bioaccumulation of Microplastics in *Daphnia magna*

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Daphnia magna (daphnia) are used to study the bioaccumulation of microplastics (MP) because they are a food source for higher trophic levels, and consequently, they can magnify the transfer of MP to larger organisms. Our aim is to determine the impact of MP on daphnia and freshwater ecosystems, by studying the effects of polyethylene (PE) in the presence of food (algae). In the environment, daphnia are exposed to food and different sizes of MP, however, the interaction between MP ingestion and food intake in daphnia is unknown. To evaluate the bioaccumulation of MP in daphnia, adults (16-18 day olds) were starved for 24h and then acutely exposed to low concentrations (0.001-0.02mg/mL) of 43-53, 63-75, and 90-106 μm fluorescent PE microbeads for 48h. The MP were isolated by digestion and filtration, and afterwards, they were counted with a fluorescent microscope. The results indicated that daphnia can differentially uptake MP based on size. Research is currently

underway to evaluate the impact of feeding on the uptake of different sizes of MPs during chronic exposure to varying food and MP concentrations. We are also examining how their interactions affect the biokinetics (i.e., bioaccumulation, filtration, and depuration) of MPs and the physiological stress responses in daphnia. We hypothesize that the daphnia will not be able to distinguish between the food and MP, and that the reduction in food will impact the fitness of the daphnia.

2.15.P-Th-197 Chronic Toxicity of Sodium Chloride and Sodium Sulfate to Different Freshwater Organisms in a Low Hardness Water

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Chloride (Cl) and sulfate (SO₄) are a common surface water contaminant originating from sources such as municipal wastewater treatment plant effluent, fertilizers in runoff during overland flow events, and discharges associated with energy and mineral extraction. The United States Environmental Protection Agency's current national water quality criteria (WQC) for Cl and state water quality standards (WQS) for SO₄ have been developed based on toxicity data from laboratory tests conducted in hard and moderately hard waters. However, the toxicity of Cl and SO₄ are likely influenced by water hardness. Many ecoregions in the Southeastern United States have median hardness values in a soft water range (0 to 60 mg/L as CaCO₃) where the toxic effect of these ions may be greater. In an ongoing study, acute sodium chloride (NaCl) and sodium sulfate (Na₂SO₄) toxicity tests were conducted in a hardness water of 40 mg/L as CaCO₃ with 7 freshwater species of different taxa, and 3 species (a fish, an invertebrate, and an acutely sensitive species) were selected for chronic testing to estimate acute-to-chronic ratios for the derivation of chronic water quality guideline values in low hardness water. Chronic tests were conducted following ASTM standard methods with Fathead Minnow (*Pimephales promelas*; 34-d exposure), unionid mussel (*Lampsilis siliquoidea*; 28-d exposure), amphipod (*Hyalella azteca*; 28-d exposure), and/or cladoceran (*Ceriodaphnia dubia*; 7-d exposure). Preliminary results from survival endpoint showed that LC20s from the NaCl exposures ranged from 65 to >800 mg Cl/L, with an order of species sensitivity: *H. azteca*, *P. promelas*, and *L. siliquoidea*, and LC20s from Na₂SO₄ exposures ranged from 137 to 1048 mg SO₄/L, with an order of species sensitivity: *P. promelas*, *C. dubia*, *L. siliquoidea*. Growth measurements and data analyses for sublethal endpoints are ongoing. The results of this study can be used to develop or update WQC, WQS, and other environmental guidelines for Cl and SO₄ to protect freshwater organisms in soft waters.

2.15.P-Th-198 Sunlight Exposure of Oil on Water Creates Genotoxic Photoproducts in the Aqueous-Phase

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Oil spilled in aquatic systems is known to have acute toxicity, which is enhanced by exposure to sunlight. However, the genotoxicity of oil photoproducts from solar exposure has not been assessed. Ames tests was utilized to assess genotoxic effects of the photoproducts of three different oils. The oils were placed over water and exposed to simulated sunlight with an intensity near that observed in the Northern Gulf of Mexico. The irradiation was conducted for times ranging from 6 hours to 48 hours, and a corresponding dark control was prepared for each time. The aqueous phase was collected and filtered with 0.2 µm glass membrane to remove any residual oil droplets. Two strains of *S. Typhimurium* (TA98 and TA100) were used to detect frameshift and base-pair substitution mutations, respectively. Testing for each treatment was completed in quadruplicate, and positive and negative controls were included. After two days of incubation, the samples were removed, and the number of colonies on each plate were counted. Mutagenicity increased as the oil was exposed to longer periods of irradiation that led to an augmentation in photoproducts. Both mutations, base pair and frameshift, were observed from the aqueous phase collected after irradiation of the three oils. The collected photoproducts after irradiation of one oil (Maya crude oil) induced the most pronounced frameshift mutation for *S. Typhimurium* (TA98 strain) after 48 h of irradiation. In the case of a second oil (NIST residual fuel oil), the collected

photoproducts exhibited the highest base pair mutation for *S. Typhimurium* (TA100 strain) following a 48 h irradiation period. Both of these oils significantly elevated the genotoxic response for both mutation types after simulated sunlight exposure. A third oil (Alaskan crude oil) did not exhibit discernible mutagenic effects with irradiation times as long as 48 h, although acute toxicity may have inhibited genotoxicity detection. These results demonstrate that sunlight exposure of oil spilled in aquatic systems can create photoproducts that have genotoxic activity, and such processes vary by oil. The impacts of oil spilled in sunlit regions therefore pose longer term threats to ecosystems than those observed for acute toxicity only. These impacts have not been well studied, hence their importance is not currently known nor used in predicting ecological damage from aquatic oil spills.

2.15.P-Th-199 Toxicity Assessment and Real-Time Metabolic Trait Responses of Juvenile *Macrobrachium rosenbergii* to Ammonia Exposure at Different Salinities

Cameron Michael Emadi, Fabio Dos Santos Neto, Breana Smithers, Miguel Acevedo and Edward Mager, University of North Texas

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, making it versatile for cultivation at typical salinity values of brackish groundwater desalination concentrate, thereby offering an opportunity to employ byproducts that would otherwise be disposed of. High density aquaculture results in the potential for harmful nitrogenous waste accumulation in the form of ammonia, which is a toxic compound, affecting 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 to brackish water. This study aims to determine the 24 and 48-hour ammonia LC50 values across three different salinities (1, 5, and 10 ppt). Informed by these LC50 concentrations, the acute real-time effects of ammonia exposure on the routine metabolic rate of juvenile *M. rosenbergii* will be investigated using static intermittent respirometry. This research will assist in developing a sentinel respirometry system that can detect changes in water quality in real-time by monitoring the metabolic rate of the prawns. Additionally, gathering data on the survival and metabolic costs linked with acute ammonia stress in different salinities will greatly inform effective aquaculture management of *M. rosenbergii*.

2.15.P-Th-200 Ancestral BPA Exposure Alters Susceptibility to Reproductive Defects Induced by Novel Adulthood PFOS Exposure

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During the past decade there has been a conscious effort to minimize exposure to BPA in plastics and resins, however, BPA's ability to establish epimutations in the germline of previous generation, has marred this effort. These epimutation signatures are transferred to next generations through germ cells, resulting in inherited early developmental, liver, and reproductive disorders. A chemical of emerging concern, Perfluorooctanesulfonic acid (PFOS), has become a novel global contaminant due to its use in oleophobic and hydrophobic items, and its exposure mirrors the health impacts promoted by BPA. While research has established the impacts of these chemicals individually, there is sparse information on the role of ancestral BPA exposure on the sensitivity to novel PFOS exposure. To understand the effects of this emerging chemical on organisms with prior history of ancestral exposure, medaka (*Oryzias latipes*) with prior history of BPA exposure were used. The BPA lineage, where F0 generation was exposed to 10 ug/L BPA during early development and the control lineage had no BPA exposure. These fish were raised without exposure to F4 generation. Reproductively active F4 fish from each lineage were exposed to four concentrations of PFOS (0.002 mg/L, 0.02 mg/L, 0.2 mg/L, and 2.0 mg/L) for 21 days. During exposure, eggs were collected daily to assess fecundity and fertility. Following exposure, the gonads of fish were collected and weighed, RNA was isolated and used for RNA sequencing. Preliminary

fertility and quantitative results suggested that males with prior history of ancestral BPA exposure were more susceptible to PFOS. RNA sequencing data revealed distinct sex-specific differentially expressed genes. The results also suggested that organisms with a history of ancestral BPA exposure are more susceptible to second hit with environmental contaminants. Therefore, exploring ancestral BPA exposure as a variable in physiological and toxicology research could be essential for establishing more accurate regulatory guidelines.

2.15.P-Th-201 Assessment of Nanoparticle Toxicity to Cyanobacteria

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Freshwater cyanobacterial harmful algal blooms (cHABs) are a wide-spread environmental problem and there is the need to explore innovative technologies for environmentally benign and cost-effective cHABs mitigation. We have developed a novel RNA interference-based gene silencing technology for species-specific control of cHABs-forming cyanobacteria using nanoparticle (NP) as the delivery vehicle for gene silencing nucleic acids. The aim of the present laboratory study was to determine and compare the toxicity of NPs to representative cyanobacterial species belonging to 7 genera. Pure cultures of *Microcystis aeruginosa* strains LE3, UTEX 2385 and UTEX 2386, *Lyngbya sp.* CCAP 1446/10, *Synechocystis sp.* PCC 6803, *Dolichospermum circinale* CS-541/06, *Planktothrix agardhii* 1810, *Aphanizomenon sp.* NZ, and *Microcoleus autumnalis* CAWBG635 were treated with three types of NPs prepared in-house or synthesized by a commercial manufacturer: Carbon Nanodots (CNDs), Carbon Nanotubes (CNTs), and Cell Penetrating Peptides (CPPs) at two concentrations (low and high, high = 5 x low) relevant to practical application rates in the gene silencing technology. The initial cell density of all cultures measured as optical density at 680 nm (OD₆₈₀) was ca. 0.3, corresponding to an early exponential growth phase. Following 48 hours of exposure to these NPs, five endpoints, i.e., OD₇₅₀, OD₆₈₀, fluorescence at 450 nm (Ft₄₅₀) or 620 nm (Ft₆₂₀), and quantum yield (QY), were measured to determine NP effects on cell density/turbidity (OD₇₅₀), chlorophyll a content (OD₆₈₀ and Ft₄₅₀), intensity of the photosynthetic pigment phycocyanin (Ft₆₂₀), and photosynthesis efficiency (QY). Measurements of NP-treated cyanobacteria were compared to untreated controls to evaluate the potential toxicity of each NP. Our results indicate that while all the NPs showed adverse effects on at least one cyanobacterial species or *M. aeruginosa* strain, NP toxicity was strain/species- and concentration-dependent. CNTs were the most toxic overall as they significantly decreased the turbidity of all the cultures by 22-95% at the low concentration. The differential acute toxicity of tested NPs (CNTs > CNDs > CPPs) to 7 representative species/genera demonstrated in this study implies that we should choose an appropriate NP to minimize side effects on non-target, non-toxin-producing or beneficial cyanobacterial species as part of the technologies involving the use of NPs for cHABs mitigation.

2.15.P-Th-202 Identifying the Synergistic Impact of PFAS and Nanoplastics on Brain and Liver Functionality of the Common Food Fish Species, Spotted Seatrout (*Cynoscion nebulosus*)

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Per and poly fluoro alkyl substances (PFAS) and micro/nanoplastics are some of the most common environmental contaminants observed in natural waters. While considerable research has been conducted on the effects of PFAS on freshwater fish, little is known about the potential for adverse impacts on marine species. Likewise, much attention has been paid to the potential impacts of microplastics, but we know significantly less about the effects of exposure to nano-sized plastics. Further, most of the research on the aquatic toxicity of PFAS has focused on PFOA and PFOS, with less work on the newer PFAS member molecules. Still less is known about the potential synergistic interactions of PFAS and nanoplastics, particularly on non-model marine species. To address these issues, we conducted a 7-day exposure of adult spotted seatrout to four treatments: control, GenX (a new PFAS compound), fluorescing nanoplastic particles, and GenX + fluorescing nanoplastic particles. The GenX was dosed at 50 uM, and the nanoplastics at 50

ug/L. The exposure was conducted as a static renewal exposure in quadruplicate exposure tanks with four adult seatrout per tank. At the end of seven days, half of the fish were removed and sampled for later analysis (brain, plasma, muscle, liver). The remaining fish were placed in fresh, uncontaminated water for seven days to assess depuration and recovery. We hypothesized that the nanoplastics would bind with the GenX and thereby increase the uptake and accumulation rates of GenX alone. We examined the expression of PPAR α and PPAR γ pathways via qPCR in liver samples, and measured nanoplastic and GenX concentrations in brain tissue. Our initial results support our hypothesis and provide evidence that the combination of GenX and nanoplastics produces greater effects than either treatment alone. Examining the potential impact of either contaminant in isolation may underestimate the true potential for adverse environmental effects on marine species.

2.15.P-Th-203 Hepatic Molecular Markers in Fathead Minnows (*Pimephales promelas*) in Relation to Thermal Oil Refinery Effluent Exposure

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A small stream in Illinois is home to the State-threatened Bigeye Chub (*Hybopsis amblops*), but also receives thermal effluents from a refinery. The combination of refinery effluent and thermal stress may impact fish health resulting in decreased recruitment. The present study constitutes a component of a larger project which included measurements of additional health responses. The objective of this study was to evaluate molecular responses from Fathead Minnows (*Pimephales promelas*) in relation to temperature and water type. We hypothesized that regardless of temperature, *P. promelas* exposed to effluent (EFF) water would have increased expression of hepatic detoxifying enzymes (cytochrome P4501A, *cyp4501a* and glutathione-s-transferase, *gst*) and oxidative stress genes (catalase, *cat* and superoxide dismutase, *sod*) in relation to controls. Male adult *P. promelas* were exposed to either EFF, upstream (UPS), downstream (DNS) or aged water (controls) in two separate trailers one maintained at 20°C and a second one at 30°C. Fish were lethally sampled on days 30 and 60 to remove livers, which were immediately preserved for gene expression work. RNA was extracted and relative gene expression was determined via two-step real-time quantitative polymerase chain reactions (RT-qPCR) using β -actin as the reference gene. Regardless of sampling time, there were no main effects of water treatment on any of the genes relative to controls. Models with weak evidence of treatment effects ($p \leq 0.1$) on gene expression were further evaluated and indicated that *P. promelas* exposed to the EFF and DNS water treatments had significantly increased *cyp1a* hepatic gene expression relative to the control group. On day 60, there was weak evidence that fish exposed to the EFF water treatments had a down-regulation of *cat*. Independent of water treatment, hepatic *gst* expression was decreased in fish held under high temperature. Overall, this study showed no evidence of oxidative stress and some evidence of *P. promelas* exposed to the EFF having increased hepatic detoxification. This is consistent with water chemistry results showing non-detectable polyaromatic hydrocarbons present in the waters tested. In addition, our results suggest temperature alone can impact gene expression.

2.15.P-Th-204 Hepatic Molecular Markers in Wild-Caught Spottfin Shiners (*Cyprinella spiloptera*) in Relation to Thermal Oil Refinery Effluent Exposure

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A small stream in Illinois is home to the State-threatened Bigeye Chub (*Hybopsis amblops*), but also receives thermal effluents from a refinery. The combination of refinery effluent and thermal stress may impact fish health resulting in decreased recruitment. The present study constitutes a component of a larger project which included measurements of additional health responses. The primary objective of this component of the larger study was to determine the health of fish sampled closest to the thermal effluent (EFF) in relation to upstream

(UPS, serving as control) and downstream (DNS) sites. The Spotfin Shiner (*Cyprinella spiloptera*) was used as the surrogate species for *H. amblops* as it was found at all sites. We hypothesized that *C. spiloptera* sampled from the EFF site would have increased expression of hepatic detoxifying enzymes (cytochrome P4501A, *cyp4501a* and glutathione s-transferase, *gst*) and oxidative stress genes (catalase, *cat* and superoxide dismutase, *sod*) in relation to UPS. Fish were seined from the creek in September and October 2022 and lethally sampled to remove livers which were immediately preserved for gene expression work. RNA was extracted and relative gene expression was determined via two-step real-time quantitative polymerase chain reactions (RT-qPCR) using β -actin as the reference gene. During the September sampling, there were no differences in the expression of any of the genes across sites. However, during the October sampling, *gst* expression was significantly upregulated in *C. spiloptera* collected at the EFF site compared to UPS. Overall, this study showed no evidence of oxidative stress and some evidence of *C. spiloptera* from the EFF having increased hepatic detoxification; however the inconsistency across sampling times as well as the lack of an upregulation of *cyp1a* suggests no recent exposure to *cyp1a* organic ligands. This is consistent with water chemistry results showing no polycyclic aromatic hydrocarbons from water samples collected from the EFF site.

2.15.P-Th-205 Using UPLC-HRMS and Metabolomic Analysis to Investigate the Toxicity of Road Runoff Water-Derived Contaminants in Juvenile Salmonid Species

Miranda Elizabeth Jackson, Chloe Fender, Lara Rivera, Adrienne La Joie, Makenzie Williamson, Stacey L Harper and Manuel Garcia-Jaramillo, 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 (PAH) 9,10-Anthraquinone (AQ). To determine sublethal concentrations, juvenile *Oncorhynchus* species chinook (*O. tshawytscha*), coho (*O. kisutch*), and rainbow trout (*O. mykiss*) salmonid species were exposed to a range of concentrations of 0 – 10 $\mu\text{g/L}$ 6PPD-q for 24-hours. Acute exposure to coho and rainbow trout resulted in mouth gaping and surface swimming PSM symptoms leading to mortality at 6PPD-q concentrations of 0.01 $\mu\text{g/L}$ and 10 $\mu\text{g/L}$, respectively. Chinook salmon did not experience any phenotypic effects from the tested exposure concentrations. 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. 6PPD-q and AQ quantification in water tanks were validated and measured within a 15% margin of error compared to nominal concentrations. To better understand the mechanisms of toxicity of 6PPD-q and the differences observed among the three *Oncorhynchus* species, fish were exposed to established sublethal concentrations of 1 $\mu\text{g/L}$ 6PPD-q and 10 $\mu\text{g/L}$ AQ separately and in combination over a five-day period. Non-targeted mass spectrometry-based metabolomics analysis was performed on brain samples from exposed and non-exposed fish, and over 20,000 features were detected in positive mode. Using an in-house metabolite library, 47 metabolite features were identified, and their relative abundances assessed. No statistically significant differences were found among the treatment groups for these metabolites within each of the species. Further analysis of the non-target MS data will be performed for additional metabolite annotations. The present study will help to elucidate the effects of these prioritized contaminants on salmonid health and will improve our understanding of the differences in toxicity observed among salmonid species.

2.15.P-Th-206 Microtox Bioluminescence Assay Sensitivity to Metals Toxicity in Full Strength Marine Water: Implications for Monitoring Anthropogenic Impacts in the Marine Environment

Fiona Quin Zabel, Katharine Kinter, Dana Sackett and Lance T Yonkos, University of Maryland

The Microtox *in vitro* bioluminescence assay with *Aliivibrio fischeri* has been proposed for near real-time

monitoring of toxicity in marine waters receiving deep ocean metal mining effluent. However, testing of background marine water samples (35 ppt) against the 2% NaCl diluent provided for the Microtox assay consistently yields 20 – 40% increases in microbial metabolic activity and thus light emission relative to the control. This increase in bioluminescence, likely associated with the change in osmotic pressure, serves to mute detection of subtle toxic effects. Because the 2% NaCl diluent proved inadequate for investigating low-level toxicity in marine samples, we tested a synthetic seawater (SSW) diluent (35 ppt; pH 7.5) for use with marine samples. The SSW diluent consistently yielded 10 – 20% increases in light emission following the 15-min exposure period, matching those of field-sourced marine samples. In contrast, the 2% NaCl diluent consistently yielded approximately 20% reductions in light emission after the incubation period, explaining the cumulative observed difference of 30 – 40%. Metals toxicity (CuSO_4 and ZnSO_4) was investigated by testing dilution series produced in each diluent. Resulting dose-response curves and calculated EC50s indicate near identical sensitivity of the Microtox assay to CuSO_4 at the two salinities, but significantly reduced sensitivity to ZnSO_4 at the higher marine salinity. Results suggest the assay may be useful for investigating metal-associated toxicity in marine waters, but additional research is necessary to determine effects thresholds to various metals individually and in mixtures for comparison to fish and invertebrate species of interest. Preliminary results with test-mining effluent also suggest that optimizing diluent characteristics to marine conditions (appropriate for *Aliivibrio fischeri*) allows for low-level metals toxicity detection in marine samples.

2.15.P-Th-207 Fin-Extracted Cortisol as a Stress Biomarker in Fish Exposed to Thermal Oil Refinery Effluent

Nathan T. Mak¹, Matt Hamilton¹, Tyler D. Hoskins¹, Marisol S. Sepulveda¹, Michael Chanov², Ken S Cummings² and Marty E Sneen², (1)Purdue University, (2)EA Engineering, Science, and Technology, Inc. At a refinery site in Illinois, concerns around thermal effluent release into a small creek and potential effects on State-threatened Bigeye Chub (*Hybopsis amblops*) prompted a parallel laboratory bioassay and field study aimed at determining whether exposure is associated with increased sublethal stress and ultimately, increased incidence of external anomalies. Cortisol is a hormone commonly used as an indicator of stress. Although most often assessed in plasma, recent studies have indicated that cortisol from fins can be a better measure of long-term stress. We hypothesized that both increased water temperature and exposure to the refinery effluent (EFF) would result in increased cortisol in fins. We conducted two studies, utilizing the EFF site and sites upstream (UPS) and downstream (DNS) from it: 1) a bioassay with Fathead Minnows (FHM, *Pimephales promelas*) exposed to water from the sites plus a control group of fish kept in aged tap water, at both 20°C and 30°C, with sampling on days 30 and 60; and 2) field study using the Spotfin Shiner (SS, *Cyprinella spiloptera*) sampled from the same sites in September and October 2022. For the field study, UPS served as control. Fish were lethally sampled to remove fins which were cleaned to wash off external sources of cortisol prior to hormone extraction in methanol. Dried cortisol samples were then reconstituted in an Enzyme-Linked ImmunoSorbent Assay (ELISA) to determine cortisol content. We found that cortisol was increased in FHM held at 30°C relative to those held at 20°C on day 30 of the bioassay, but not on day 60. On day 60, FHM exposed to EFF at 20°C had increased fin cortisol relative to controls at 20°C. There were no relationships found between fin cortisol and site for the SS field study. Results from the FHM study suggest that the fish experienced transitory stress when exposed to a higher temperature in the first 30 days of the bioassay but acclimated to the 30°C treatment by day 60. Importantly, FHM exposed to effluent water at a temperature within their thermal preference (20°C) did not appear to acclimate to this stressor, which was only evident at the end of the study. In sum, although we could tease out the influence of high temperature and chemical exposure on cortisol levels in a captive bioassay, these relationships were not observed in the field. Additional field studies would need to be conducted to determine the potential impacts of thermal effluents on fish health in this stream.

2.15.P-Th-208 In situ Degradation of Macroplastics: Tracking Fragmentation Rate and Dissolved Organic Carbon Leachate

Kathleen Mayer, Tyler Allen, Megan Gaesser, Beija Gore and Austin D. Gray, Virginia Tech

Plastic pollution represents one of the greatest environmental challenges of our time. It is estimated that ~80% of all plastic debris entering coastal waters originates from inland river systems. Plastic debris transported along river networks stems from stormwater runoff, mismanaged municipal waste, and accidental and intentional littering into low-order streams. Despite stream networks playing a crucial role in the transport of plastics, there is little information available regarding their influence on microplastic pollution in these systems, as well as their influence on carbon cycling since plastics represent an allochthonous carbon source in inland waters. Although plastics are formulated as materials resistant to degradation, they are subject to various degradation processes (ultraviolet, mechanical, and chemical) that can produce microplastics. To date, there is limited data regarding *in situ* plastic fragmentation in freshwater systems. To understand the process of plastic degradation in stream environments, macroplastic items (low-density polyethylene, polyethylene terephthalate, polystyrene, high-density polyethylene) along with natural items (cellulose, viscose cellulose) were set in stream sediments within two different habitats (urban vs. forested) with items collected at 0.5, 1, 3, 6, and 8 months to date. Biofilm formation and plastic fragmentation were observed in as little as two weeks. Items recovered at six months underwent laboratory leaching studies to understand if degraded plastic, in turn, releases dissolved organic carbon (DOC). The production of microplastics from the macroplastic items occurred at a rate ranging from 3.88 to 7.84 particles per month, with polystyrene reported as the polymer with the highest fragmentation rate. Fragmentation rates for each polymer were higher in urban streams versus forested. Our DOC assessment found that plastics leached DOC at rates ranging from 5.02 to 8.93 μmol of carbon per day. Our findings demonstrate that microplastic production due to plastic degradation in stream environments occurs along a relatively short time scale compared to previous assessments in salt marshes (4-8 weeks), with unknown implications for freshwater biota. The leaching of DOC from degraded plastics highlights a relatively unexplored research area that warrants further investigation. Are plastics an allochthonous carbon subsidy that influences ecosystem processes in freshwater habitats?

2.15.P-Th-209 Incorporating Industrial and Climatic Covariates into Analyses of Fish Health Indicators Measured in a Stream in Canada's Oil Sands Region

Tim Arciszewski¹, Erin Ussery² and Mark Ervin McMaster², (1)Government of Alberta, (2)Environment and Climate Change Canada

The influence of industrial mining and *in situ* extraction of bitumen on the environment is widely established. Keeping these two factors in mind allows us to understand specifics of the disturbances. First, mines are typically found in areas with the thinnest overburden and are therefore, the areas most likely to be affected by natural exposure to bituminous sands. Second, atmospheric deposition of contaminants from multiple facilities occurs throughout the region. The purpose of this work was to examine the potential influence of both industrial and natural factors on the health lake chub (*Couesius plumbeus*) collected at two sites (Upper and Lower) in the Ells River in 2013 to 2015 and again in 2018. The Upper site represents a reference or baseline site not exposed to contaminants, while the Lower site may receive increased sources of contaminants associated with the refining of bitumen due to development and anthropogenic influences. During the recrudescence period for the spring spawning lake chub, 20 adult male and 20 adult female fish were collected at each site. To examine the potential effects of industrial influence, several approaches were used. First, the typical environmental effects monitoring (EEM) approach was used to identify statistical differences both spatially and temporally using ordinary least squares (OLS) and generalized linear models (GLM), and were compared to the use of regression diagnostics. Second, the effect of including various sets of covariates on the temporal analyses was examined and the Elastic Net regularized regression (EN). The results of the variable selection study suggest the influence of some local and distant industrial facilities, including some outside the Ells drainage, such as the Muskeg River Mine. The data also suggest the effects of natural stressors, such as temperature. Analyzing these

ecological data further suggest further analyses in complex exposure environments like the Athabasca tributaries in the OSR may need to integrate environmental (i.e., water temperature, air temperature and stream discharge) and industrial covariates (i.e. land disturbance, fuels combusted, stream injection rates, etc.) to better account for variability in the data set and to enhance the sensitivity of the analysis to local human activities. Detailed data from this work will be discussed.

2.15.P-Th-210 Engineering an Incubation Box to Monitor Development of Pacific Salmon Embryos

Jeffrey M. Morris¹, Apryle Collison², Heather McKenzie², Jenni Klebesadel², George Schisler³ and Benjamin D Barst², (1)Abt Associates, (2)University of Alaska Fairbanks, (3)Colorado Parks and Wildlife

Salmon are a keystone species and are synonymous with Alaska. They are flourishing in some regions of Alaska and not in others. A major goal of current research is to determine how developing salmon in the freshwater environment are affected by environmental stressors (e.g., elevated water temperatures and contaminants). Pacific salmon embryos develop over many months in gravel nests (redds). This period of development is extremely important, during which embryos and larvae are extremely sensitive to chemicals and other environmental stressors. However, monitoring development at this critical time is challenging. We are developing a field-deployable incubation box for Pacific salmon embryos that is self-contained, having adequate power and storage capacity to take photos and measurements, as well as storing photos/data until the salmon fry emergence. We recently deployed one of our systems in a sockeye spawning area located in a tributary to Iliamna Lake, Alaska, which is one of the largest sockeye salmon nurseries in the world. We will present our system design, modifications, and preliminary data to date.

2.15.P-Th-211 Environmental Organic Contaminant Body Burdens and GC-MS Based Untargeted Metabolomics in Mediterranean Mussels from Port Phillip Bay, Australia

Hao Shen¹, Damien Nzabanita², Chantel Foord², Stephen Grist² and Dayanthi Nugegoda², (1)Hong Kong Baptist University, (2)RMIT University

Mussels were collected from four coastal sites around Port Phillip Bay, Australia in March and April 2021. Body burdens of Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs) were measured, and the possible sources of these toxicants discussed. In addition, a gas chromatography-mass spectrometry (GC-MS) based untargeted metabolomics analysis was performed using the mantle tissues of mussels. Correlations between the results of contaminant body burdens and metabolic variations were investigated. The results demonstrated that high accumulations of low-molecular-weight PAHs were found in mussels. High body burdens of PCBs and OCPs were only found at mussels from the site close to the river mouth. Some of the metabolic pathways were correlated with the accumulation of PAHs. No correlations were found between PCB and OCP accumulations and metabolic abundances. According to the food and environmental standards of the European Union (EU), the PAH, PCB, and OCP accumulation in mussels in this study are a serious food safety concern.

2.15.P-Th-212 Comparison of Statistical Endpoint Results Using Three Environmental Protection Agency Recommended Statistical Approaches to Evaluate Short-term Chronic Sublethal *Ceriodaphnia dubia* Toxicity Test Data

Laura J Phillips, U.S. Environmental Protection Agency

Aquatic toxicity testing is used by the Environmental Protection Agency (EPA) and other authorized National Pollutant Discharge Elimination System (NPDES) permitting authorities to assess possible impacts to aquatic life protection water quality standards. This presentation provides a comparison of three statistical approaches used to analyze valid data from 310 short-term chronic *Ceriodaphnia dubia* (freshwater water flea) toxicity tests. The short-term chronic test for *Ceriodaphnia dubia* measures reproduction rates. The comparison included determining the concordance, or rate of agreement, between the results from three recommended statistical approaches. For each set of test data, the concentration-response relationship was reviewed to

determine whether such a quality assurance check influences the test results and thereby affects the concordance of the toxicity test statistical endpoints. The three EPA recommended statistical approaches compared included: point estimates (specifically, the effluent concentration that results in a 25% decrease in *C. dubia* reproduction compared to the control or 25% inhibition concentration [IC₂₅]), hypothesis testing (specifically, the highest effluent concentration determined to not be significantly different with respect to *C. dubia* reproduction when compared to the control or the no observed effect concentration [NOEC]), and hypothesis testing using the Test of Significant Toxicity (TST) (specifically, comparing the biological response at the in-stream waste concentration and the control to determine if there is a significant difference). This evaluation indicated that there is a very high rate of concordance amongst these three EPA recommended statistical approaches compared based on the valid short-term chronic *C. dubia* reproduction data analyzed. EPA conducted an additional evaluation of the non-concordant tests, and these results will be presented in the poster. *The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or policies of the Agency.*

2.15.P-Th-213 Evaluation of Nanobubble Ozone Technology for Cyanobacterial Harmful Algal Bloom Control

Heather Alane Raymond, *Ohio State University*

Effective Cyanobacterial Harmful Algal Bloom (CHAB) control strategies are needed to address recreational and drinking water impacts. Nanobubble ozone technology (NBOT) is an emerging treatment, but CHAB efficacy studies are limited. Lab and mesocosm NBOT experiments and full-scale field NBOT treatment trials were conducted June 2021 through October 2023. Lab studies evaluated nanobubble size distribution, density, lifetime, aqueous ozone and hydroxyl radical production, and impact of organic matter on dose response. Lab studies confirmed production of high densities of nanobubbles and the total hydroxyl radical production from nanobubble collapse alone (no ozone) was insufficient for the oxidation demand for most treatments. These results indicate that nanobubble technology without ozone gas, claiming to work through the action of hydroxyl radical production, is not a suitable treatment. Mesocosm studies compared efficacy of NBOT to traditional algaecide cyanobacteria treatments and evaluated impacts to non-target organisms. Algaecides had severe detrimental effects on the zooplankton community while the lower dose NBOT treatment had a positive impact on zooplankton community (increased abundance) and the higher dose NBOT treatment only had minor negative impacts. NBOT treatment reduced CHABs at all doses. Multi-month NBOT trials were conducted at Lake Sylvan and Grand Lake Saint Marys West Beach. At Sylvan Lake, following increased NBOT dose, cyanobacteria concentrations decreased, the community shifted, and recreational advisories were avoided. Cyanobacteria and toxin concentrations were higher during the Grand Lake Saint Marys beach NBOT trial and treatment effects were localized. Recreational advisories at the treatment beach were reduced compared to neighboring beaches.

2.15.P-Th-214 PFAS in the Chesapeake Bay and Maryland Coastal Bays

Michella Paige Salvitti¹, **Camden G. Camacho**², **John A Bowden**², **Joseph Pitula**¹ and **Eguono Wayne Omagamre**¹, (1)*University of Maryland, Eastern Shore*, (2)*University of Florida*

The presence of Per- and Polyfluoroalkyl substances, PFAS, are a group of synthetic chemicals that are prevalent in everyday consumer use. However, their widespread use has led to PFAS becoming an emerging environmental contaminant. Known mostly for its widespread use in firefighting foams, pesticides, and not stick cookware; PFAS are present in our drinking water, seafood, and our environment. PFAS presence is not well understood in the Chesapeake Bay and Maryland Coastal Bays. The objective of this study was to test surface water for PFAS at nine sites throughout the Chesapeake Bay and six sites throughout the Maryland Coastal Bays. All 15 sites were strategically selected based on their proximity to known PFAS sources and consecutively sampled once a month from March to August. Opportunistic sampling was employed at all sites for the biological samples at the Chesapeake Bay sites. The Atlantic Blue Crab, *Callinectes sapidus*, was

sampled once a month at each site in the Maryland Coastal Bays. All of the Atlantic Blue Crabs had claw and hepatopancreas tissues tested for PFAS. The presence of PFAS in both the Chesapeake Bay and Maryland Coastal Bays will be presented.

2.15.P-Th-215 Assessing Whitefish Tissue Composition via Community Based Monitoring in the Peace-Athabasca Delta

Erin Ussery¹, Mark Ervin McMaster¹, Keegan Hicks², Oana Birceanu³, Caroline Bampfylde⁴, Kelly R. Munkittrick⁵ and Bruce Maclean⁴, (1)Environment and Climate Change Canada, (2)Alberta Environment and Parks, (3)University of Western Ontario, (4)Maclean Consulting, (5)University of Calgary

The Peace-Athabasca Delta (PAD), located in northeastern Alberta, is the largest freshwater delta in North America. The delta encompasses the area where the Peace and Athabasca rivers converge on the Slave River and Lake Athabasca. The PAD is partially located within Wood Buffalo National Park where its marshes, lakes and mud flats are inhabited by over 200 species of birds, 40 species of mammals, and 20 fish species during some part of their lifecycle. Additionally, the PAD is home to Dene, Cree and Métis Nations who have occupied the delta for generations, using its resources for sustenance. Traditional use of the delta by these communities continues today with hunting, trapping, fishing, berry-picking, and spiritual, medicinal and cultural uses of the land. Elders and residents of the communities are concerned with the ecological changes they currently see in the PAD and worry that if changes persist, the ecological health of the delta will deteriorate further, threatening their traditional way of life. In 2007 the Mikisew Cree First Nation (MCFN) began a Community Based Monitoring (CBM) program, created with the objective of tracking changes in water quality and fish health in the traditional areas of the MCFN. The Athabasca Chipewyan First Nation (ACFN) subsequently began CBM work in 2011, adding water quantity, and winter ice condition monitoring; and Fort Chipewyan Métis joined whitefish sampling in 2020. To achieve their objectives, the programs rely on combining Western Science and Indigenous Knowledge monitoring methods to allow both scientists and community members to better understand the environmental changes they see at both local and regional scales. Elders have found that an increasing percentage of tissues harvested from Whitefish had an unusual texture, looked and smelled different than past years. To investigate this change, scientists and community members from MCFN and ACFN collected Whitefish muscle tissue from traditionally fished areas of the PAD in fall of 2019-2022. The body composition of most fish species is predominantly composed of water, lipid and protein, all measurable endpoints that would suggest the health of Whitefish in the PAD. Thus, the body composition of harvested Whitefish was assessed by investigating the percentage of the muscle tissue is composed of water, lipid and protein. This poster will summarize results found from the 2019-2022 studies of Whitefish muscle tissue composition.

2.15.P-Th-216 Lessons from the Implementation of OECD Aquatic Toxicology Guidelines When Working with Difficult to Test Substances

Amber M White and Patricia Tcaciuc, 3M Company

Standardized ecotoxicity tests outlined by OECD guidelines are regularly relied on to characterize aquatic organisms. However, substances that have low solubility, high volatility, or are unstable in water can be challenging to work with under conditions described by standard guidelines, including the modifications provided in OECD “Difficult to Test Substances” guideline. Additional method development, validation, and procedural modifications are required to ensure consistent and reliable concentrations throughout these exposures. We evaluated six “difficult to test” compounds in five different aquatic ecotoxicity tests (OECD 201, 202, 203, 211, 218) using modified OECD protocols for their effectiveness in generating consistent concentrations, valid test results, and generating toxicity endpoints. The OECD 218 test (Sediment Toxicity-Spiked Sediments) required the use of a solvent-sand mixture for dosing of poorly soluble and fluorinated compounds 1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulfonamide (FBSA), 1,1,2,2,3,3,4,4,4-Nonafluoro-N-methylbutane-1-sulfonamide (MeFBSA), 1,1,2,2,3,3,4,4,4-Nonafluoro-N-(2-hydroxyethyl)-1-

butanesulfonamide (FBSE), and 1,1,2,2,3,3,4,4,4-Nonafluoro-N,N-bis(2-hydroxyethyl)butane-1-sulfonamide (FBSEE diol) while the phosphonium ions benzyltriphenylphosphonium chloride (TPBP) and tributyl(2-methoxypropyl) phosphonium chloride (TBBP) were successfully directly added to sediment. The mass balance for all compounds in sediment, porewater, and overlying water in the OECD 218 test had a 74-129% recovery on day 0. However, only TPBP, TBBP, and FBSA had total recoveries greater than 70% by day 28, likely due to significant binding of TPBP and TBBP to the solid phase (71-99% of total compound applied) and preferential stability of FBSA in the overlying water (67-90% of total compound applied). MeFBSA, FBSE, and FBSEE diol had recoveries below 50% of total applied dose, suggesting loss of the compound over 28 days. Despite the low recovery observed in the OECD 218 test, the concentration of MeFBSA was maintained within $\pm 20\%$ of initial concentration over 21 days in the OECD 211 (*Daphnia magna* Reproduction Test) by implementing solution renewal every 24 hours. The remaining five compounds retained consistent concentrations with 48–72-hour renewals. The results reported here demonstrate how test design modifications can be used to improve dosing of “difficult to test” substances and generate more reliable data in ecotoxicology testing.

2.15.P-Th-217 The Impact of Various PFAS-free Aqueous Film-Forming Foams on Growth in Hard Clams, *Mercenaria mercenaria*

*Jonathan Stewart*¹, *Katy W. Chung*², *Ed Wirth*², *Peter B. Key*² and *Marie E. DeLorenzo*², (1)Consolidated Safety Services (CSS), (2)National Oceanic and Atmospheric Administration

Aqueous film-forming foams (AFFFs) are fire suppression products used in multiple industries and have been identified as a major source of environmental per- and polyfluoroalkyl substances (PFAS). PFAS are a large class of compounds that have been reported in nearly every environmental ecosystem globally. In addition to the wide spread distribution of PFAS, reports are continuing to identify chronic and sub-lethal effects on multiple biological systems at very low concentrations. Given this, there has been a mandate within the US Department of Defense to phase out the use of PFAS and to better understand how replacement compounds will act in the environment. The objective of this study is to understand the toxicological effects of six novel PFAS-free AFFFs relative to a PFAS-containing AFFF using bivalve growth as an endpoint. The impacts will be evaluated on juvenile clam (*Mercenaria mercenaria*) growth over a 2-3 week exposure window. Previously generated data using shell growth in the Eastern oyster (*Crassostrea virginica*) suggests that growth will be acutely affected by exposure to these products and that PFAS-free AFFFs will affect this endpoint more than the reference PFAS-containing AFFF. These juvenile hard clams are benthic organisms thus have a different ecological niche versus the Eastern oyster (epibenthic). Thus, responses to exposure may differ between these two bivalve species. The additional data generated here will serve to better illuminate any potential exposure related impacts these PFAS-free AFFFs may have on commercial bivalve species.

2.15.P-Th-219 Quantifying Microplastic Dispersion Due to Density Effects

Ben Stride, *Jonathan Pearson*, *Soroush Abolfathi* and *Gary Bending*, *University of Warwick*

One of the most important physical properties that determine microplastic retention within fluvial systems is particle density, yet a significant knowledge gap remains on how polymer density affects the transport and fate of microplastics in aquatic flows. The movement of spherical polymers with densities ranging from 0.9 g/cm³ to 1.4 g/cm³ within the 20 – 200 μm size class was quantified in a laboratory flume scaled to simulate open-channel flows in fluvial systems. Polypropylene, polyethylene, polymethyl methacrylate, polyetheretherketone, and polyvinyl chloride were chemically stained and tested in transitional and turbulent flows representing velocities present within rivers in the natural environment (0.016 – 0.361 m/s). Fluorometric techniques and solute transport models based off the advection-dispersion equation were implemented to quantify both solute and microplastic mass recovery and dispersion. In most conditions tested, microplastics exhibited similar transport characteristics to solutes regardless of density and established solute transport models were successfully implemented to predict their transport and fate. For all conditions, polyethylene at 0.94 g/cm³

followed the same transport pathways as solutes but polypropylene at 0.9 g/cm³ ascended to the water surface indicating a significant density threshold occurs between 0.9 – 0.94 g/cm³. Microplastic deposition and resuspension was strongly associated with polymer density below a velocity threshold ≤ 0.101 m/s. The velocity boundary for polymers with densities 1.18 g/cm³ ≤ 1.32 g/cm³ was ≤ 0.031 m/s (≤ 7254) and for polymers with a density nearer to 1.4 g/cm³ was ≤ 0.101 m/s (≤ 24018) demonstrating a density threshold occurs between 1.32 – 1.4 g/cm³ where faster velocities are required for microplastic suspension. When density becomes the dominant force at these slower velocity thresholds, concentrations of denser than water microplastics will be momentarily or permanently deposited in sediment beds. Therefore, microplastic exposure potential may increase for benthic organisms during low flows and pelagic organisms during high flows in fluvial systems.

2.15.P-Th-220 Lead Exposure Associated with Limited Physiological Effects in Urban Lizards

Annelise Blanchette and Alex Gunderson, Tulane University

Lead contamination can have serious negative health implications for humans and wildlife. That said, there is a gap in our understanding on how natural chronic lead exposure affects wildlife beyond key taxa like birds of prey. We tested tissue lead levels and physiological performance across an urban lead contamination gradient in the brown anole lizard (*Anolis sagrei*) in New Orleans, LA. We predicted tissue lead would correlate with environmental lead, and that tissue lead would negatively affect physiological performance, measured as sprint speed, balance, and endurance. Anoles from the high lead neighborhoods had on average 8.5x higher bone lead and 25.5x higher blood lead levels than those from the low lead neighborhoods. Despite the extreme tissue lead levels, and contrary to our predictions, we found little association between lead level and performance. On average, males but not females from high lead neighborhoods had worse balance. However, neighborhood lead level was not associated with sprint speed or endurance. Furthermore, individual lead level was not associated with individual performance for any performance metric. Lead exposure had a limited effect on the physiological performance of the anoles in the current study. Lizards are underrepresented in studies on the effects of heavy metal pollutants, but the brown anole is a model system in urban ecology and physiological ecology, amenable to field and experimental approaches that will continue to elucidate their physiological tolerance of lead exposure.

2.15.V Late Breaking Science: Aquatic Toxicology, Ecology and Stress Response

2.15.V-028 The Role of Natural Biofilms in Metals Retention of Constructed Wetlands

Raven Bier^{1,2}, Destiny Willard^{1,2} and McKenzie Shea Cromer^{1,2}, (1)Savannah River Ecology Laboratory, (2)University of Georgia

Constructed wetlands are frequently used to reduce macronutrient concentrations to prevent eutrophication, but in some cases they are designed to reduce metal contamination of a water source by creating metal sulfides. Biofilms that occur in constructed wetlands and in waterbodies at the inflow and outflow are created by microbial organic polymers and may adsorb metals and keep them bioavailable. Here we sought to determine how natural biofilms influence metal sequestration over time as they occur upflow, within, and downflow from a constructed wetland. We deployed a common substrate within an upflow retention basin, at the outflow of a constructed wetland, and at three locations downstream to grow naturally occurring biofilms. Over ten sampling events, we collected commonly grown biofilms and biofilm from stones and wood in the stream. We evaluate over time, how biofilms from different habitats retain metals (copper and zinc). We anticipate that biofilms retain metals as a function of biofilm mass and that the mass is controlled by changes in water velocity, temperature, macronutrients, and time. By further understanding the controls for metals retention in naturally occurring biofilms around constructed wetlands designed to reduce metal contamination, we can expand our view to include the effects of these broadly occurring biosorbents beyond the constructed wetland boundaries.

Track 3: Wildlife Toxicology, Ecology and Stress Response

3.01.P-We A Frog, a Snake, and an Ecotoxicologist Walk into a Pond (or Advances in Ecotoxicology and Risk Assessment of Amphibians and Reptiles)

3.01.P-We-051 Assessment of Risk to Tiger Salamander Populations on Mines with Elevated Selenium

Carolyn Meyer, Timothy Alan Walker, Bonner Anthony and Holly McChesney, ARCADIS

We used an advanced tool for risk assessment, metapopulation modeling, to assess risk of selenium exposure on two mine sites in southeast Idaho to tiger salamanders, the main amphibian species on the sites. Tiger salamanders breed in a variety of seasonal and permanent ponds on the sites, with the metamorphosed juveniles and adults migrating between ponds every year. A metapopulation model was created in R to integrate effects of selenium on all life stages of this species, including neotenic forms. To quantify selenium impacts, we first developed literature-based dose-response curves on larval and adult survival that account for amphibian (1) deformities during the hatchling stage from maternal transfer and (2) mortality from dietary exposure. Exposure was estimated using mean pond and soil invertebrate selenium concentrations and amphibian bioaccumulation models. The metapopulation model was stochastic and incorporated literature-based dispersal rates varying with distance between ponds. We calibrated the model life history parameters so the baseline output matched real population dynamics of unexposed tiger salamander populations in the United States. We ran the model for 50 years 10,000 times on scenarios at baseline and with observed selenium concentrations to evaluate the effect on population size and probability of adults being reduced below a quasi-extinction threshold (< 1,000). Density-dependent (competition) and density-independent (e.g., early pond drying) factors were incorporated into the model. On one mine, selenium exposure reduced the metapopulation size by 3% on average and reduced 25 local pond populations by 0 to 10% with the exception of one small, intermittently dry pond that was reduced by 26%. The other mine's metapopulation size reduction was similarly small, reduced by 6% (0 to 11% on 16 local ponds). Probability of quasi-extinction was unaffected. We also conducted a sensitivity analysis to evaluate the effect of uncertainty in some model parameters. Survival of adults, dose-response curves, and age at first reproduction were the most sensitive parameters. Adjustments in the expected uncertainty range of those parameters did not change conclusions of small and acceptable risk to the metapopulation from selenium.

3.01.P-We-052 ZnO Nanoparticles Affect Growth, Development, and Thyroid Histopathology in African Clawed Frog Tadpoles

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The production of nanomaterials is on the rise, and metal oxides nanomaterials especially the being employed for a wide range of applications. However, nanomaterials have the potential to negatively impact the environment, because they may affect the health of aquatic organisms. For example, previous research has shown that exposure to high concentrations of ZnO particles can lead to retardation of growth and metamorphosis in the African clawed frog, *Xenopus laevis*. It is well-known that thyroid hormones are critical to amphibian metamorphosis. Therefore, the main objective of this study is to determine how the exposure to ZnO nanoparticles affect growth, development, and thyroid histopathology of *X. laevis*. Tadpoles were exposed starting in ovo to 0.125, 0.25, 0.5, 1.0 and 2mg/L (nominal concentration) ZnO nanoparticles for up to 35 days. Survival, growth (final body mass), and developmental (Neukoop-Faber [NF] stage) were recorded. In addition, histopathological analysis was carried out to determine effects on the thyroid gland; endpoints included thyroid follicle wall thickness, hyperplasia score, and total follicle area. After 35 days of exposure, survival, growth and NF stage were all reduced compared to control at concentrations of 0.5 mg/L and above. Thyroid follicular hyperplasia was increased at all concentrations, indicating disruption of the hypothalamic/pituitary/thyroid signaling axis. In addition, follicle wall thickness and total follicle area were

reduced at concentrations of 0.5 mg/L and above, indicating retardation of thyroid development. These findings are significant because there are relatively few reports of the effects of nanoparticles on thyroid hormone disruption.

3.01.P-We-054 Monitoring Accumulation and Potential Effects of Per- and Polyfluoroalkyl Substances (PFAS) in Diamondback Terrapins (*Malaclemys terrapin*) in the Chesapeake Bay

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Per- and polyfluoroalkyl substances (PFAS) are a widely used group of chemicals that are of global concern due to their persistence and accumulation in humans and wildlife. Internationally, there is a paucity of data related to PFAS accumulation, distribution, and potential impacts in reptiles. Reptiles are important components of natural ecosystems and can serve as effective bioindicators of local contamination. Similar to other aquatic turtles, diamondback terrapins (*Malaclemys terrapin*) have been shown to be useful for monitoring various pollutants in estuarine habitats. Terrapins exhibit many characteristics that make them ideal for monitoring PFAS contamination in their surrounding environment, including a long-life span, moderate home ranges, and a relatively high trophic position. Because terrapins generally consume crustaceans and mollusks, and dietary preferences may vary by region, non-lethal blood sampling could provide an effective matrix for identifying potential exposure risk and negative health outcomes in this vulnerable species. For this presentation, we will report preliminary results of PFAS concentrations in diamondback terrapins sampled throughout portions of the Chesapeake Bay. All sites are located in Maryland and include Naval Air Station Patuxent River (NAS PAX), Poplar Island, and Frenchtown-Rumbly. Our project represents an opportunity to provide critical PFAS bioaccumulation data for a vulnerable reptile species, as well as a foundation for our future studies focused on the potential effects of PFAS and other contaminants in the diamondback terrapin.

3.01.P-We-055 Response of Multiple-Biomarkers in Amphibians from an Environmental Emergency Region of Mexico

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Recently, the Mexican government, through the Science and Technology Council, declared zones or regions as regions of environmental emergency. In this type of region, one or several industrial, mining, urban and agricultural activities converge. The region of the lower basin of the Coatzacoalcos River is distinguished because three of these activities converge, but above all, the industrial-petrochemical activity. This is one of the most contaminated sites in Mexico. This work aims to show the first results of a monitoring carried out with amphibians (giant toads) and biomarkers that reflect the damage and defense against exposure to pollutants in the region. Monitoring was carried out in two seasons of the year, with the determination of biomarkers of defense and damage in blood and tissue of amphibians. The results allowed us to show that the toads that lived in places closer to petrochemical or industrial complexes had a greater alteration of biochemical enzymes related to tissue damage. The giant toads served as perfect bioindicators of human exposure, since they were found to be closely related to human settlements.

3.01.P-We-056 Spatial Variation of Mercury Exposure in Painted Turtles (*Chrysemys picta*) and Common Snapping Turtles (*Chelydra serpentina*) from Onondaga County, New York: Pre-Remediation in Onondaga Lake

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Onondaga Lake in Syracuse, New York has been one of the most polluted lakes in the United States with

mercury a primary contaminant of concern. Mercury concentrations in the lake's fish have improved following extensive remediation efforts, including sediment dredging and capping, and continual hypolimnetic nitrate additions, but mercury exposures in the lake's high trophic, semiaquatic predators are not well studied. Turtles, which are long-lived omnivores that feed on a diversity of prey items including plants, crustaceans, and fish, can function as a bioindicator of high trophic level predators due to the propensity of the organic form of mercury to bioaccumulate in them. The goal of this study is to examine spatial and temporal differences in mercury exposures in two species of native turtles – common snapping turtle (*Chelydra serpentina*) and the painted turtle (*Chrysemys picta*) – by 1) comparing spatial variation in mercury concentrations in turtle toenails among different sites throughout the region 15 years ago and 2) comparing temporal change in mercury concentrations in turtle toenails from pre-remediation efforts (dredging and capping) and post-remediation efforts in Onondaga Lake and the Seneca River as well as one control site. Here we present mercury concentrations in turtle toenails that were collected between 2009 and 2010 from painted turtles (*Chrysemys picta*; n = 183) and common snapping turtles (*Chelydra serpentina*; n = 262) from various sites in Onondaga County NY, including Onondaga Lake and the Seneca River Outlet and outline plans for future sampling to evaluate the effects of Onondaga Lake remediation on mercury concentrations in these important native species.

3.01.P-We-057 Dietary Exposure and Toxicity of Per- and Polyfluoroalkyl Substances (PFAS) Using Representative Invertebrate and Reptilian Models

Taylor S Anderson and Christopher J. Salice, Towson University

Per- and polyfluoroalkyl substances (PFAS) are a large class of manufactured, persistent chemicals that have been used in commercial and industrial applications due to their useful properties such as water, oil, and fire resistance. A key occurrence are ingredients in Aqueous Film-Forming Foams (AFFFs), which have been used at military installations for fire suppression and emergency response. AFFFs have been linked to soil, groundwater and surface water contamination for a variety of pertinent PFASs, including PFOS and PFHxS. While a large number of studies have reported PFAS in abiotic and biotic samples, there are still critical uncertainties regarding PFAS mixtures, PFAS toxicity to terrestrial invertebrates and reptiles, as well as realistic exposure routes. Thus, it is imperative to characterize PFAS mixtures and toxicity in ecologically realistic exposure scenarios. The goal of this study aims to develop “ecologically realistic” experimental designs that incorporate a systems-based ecotoxicology approach, including relevant exposure conditions representative of invertebrate and reptilian species in the actual environments. Two, 60-day studies utilizing the Falcon Dress method assessed the toxicity of PFOS and a PFOS + PFHxS mixture to male and female brown anoles (*Anolis sagrei*) via live PFAS-exposed house crickets (*Acheta domesticus*) as prey. Preliminary results suggest that liver masses were significantly different between treatment groups in the PFOS only study. Liver masses were not significantly different between treatments, but were significantly different between males and females in the PFOS + PFHxS study. Utilizing a systems based approach in ecotoxicology studies will lead to more accurate environmental data which can help us to better understand the vast variety of PFAS chemicals and how they interact in our ecological environment.

3.01.P-We-058 Ecotoxicity of Fluorine-Free Foams to Brown Anoles (*Anolis sagrei*)

Liam Odean, Christopher J. Salice and Taylor S Anderson, Towson University

Aqueous film forming foams (AFFF) are fire extinguishing products that have traditionally included per- and polyfluoroalkyl substances (PFAS) as key ingredients. Research has indicated that PFAS can adversely impact human and ecological health. The carbon-fluorine bond characteristic of PFAS resists degradation which has led to its ubiquitous occurrence and long-term persistence throughout the environment. Fluorine-free AFFFs potentially offer effective alternatives to the PFAS-based fire suppressants but, potentially, without the negative ecological and human health impacts and environmental persistence. The aim of this research was to assess the toxicity of six fluorine-free AFFFs, namely Buckeye, FOMETEC, BIOEX, Nation Foam Avio Green, NRL 502W and SOLBERG, on brown anoles (*Anolis sagrei*). A 60-day chronic study was performed for each of

these fluorine-free AFFFs. During each study, brown anoles were split into five separate dosing groups with the following exposure solution concentrations: control, 15mg/L, 45mg/L, 150mg/L, and 450mg/L. Because the foam ingredients are mixtures and proprietary, exposure solutions were all nominal but equal on a mass per volume basis and represented very high potential environmental exposures to anoles. Each anole was dosed Monday, Wednesday, and Friday using the common pseudo-gavage method. Mass and snout-to-vent length data were collected on a weekly basis, bite force and evaporative water loss data were collected at the 30-day and 60-day points, and organ masses were collected at necropsy. Statistical analyses are still ongoing but preliminary analyses have not suggested any significant impacts of the fluorine-free foams on any endpoints. In particular, there were no apparent impacts on lizard mass which has, historically, been a sensitive indicator of PFAS toxicity. Results will further the understanding of the toxicity of these substances which will be important in determining if any can replace the PFAS-based AFFFs.

3.01.P-We-059 Working Toward a Mercury Dietary NOAEL and LOAEL for Frogs

Paige A. Leitman, Carla Beals, Katrina B. Leigh, Phyllis C. Fuchsman and Mary Sorensen, Ramboll

The presence of environmental mercury in ecosystems is of increasing environmental concern due to its global distribution, its ability to methylate, and its proclivity to bioaccumulate and, subsequently, biomagnify in food chains. Amphibians can be sensitive to environmental conditions and chemical pollutants, such as mercury, can potentially impact their growth, development, reproduction, and populations. Despite this sensitivity, ecological risk to amphibians via diet is often not included in ecological risk assessments due to a paucity of toxicity information. Therefore, risk to amphibians is either qualitatively assessed or assumed to be comparable to another site-specific aquatic or terrestrial wildlife receptor with a more robust toxicity data set. In this study, a focused literature review on the effects of mercury toxicity in amphibian diets was conducted with a focus on those studies which provide sufficient information to calculate dietary No Observed Adverse Effect Levels (NOAELs) and Lowest Observed Adverse Effect Levels (LOAELs) in milligrams per kilogram food per day. The calculation of NOAELs and LOAELs is of particular importance because it allows frogs to be represented in food web models to determine their potential ecological risk due to the dietary pathway. While frogs are also exposed to environmental mercury through water consumption, incidental sediment ingestion, and dermal uptake, the dietary pathway can be a particular concern as mercury biomagnifies through the food web. The mathematical comparison between studies was used to normalize test concentrations to calculate NOAELs and LOAELs from several studies and then these values are combined into a consensus NOAEL and LOAEL value for dietary intake of mercury. The literature review also includes a critical review of study methods and results, as well as a review of the toxicological information. Creation of mercury dietary toxicity values to frogs will enable ecological risk assessors to start looking at risks to frogs via the food web in addition to uptake from abiotic media.

3.01.P-We-060 Evaluation of Anticoagulant Rodenticides (ARs) Effects in Sea Turtles by Metabolome and Lipidome Analysis

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Although anticoagulant rodenticides (ARs) are effectively used for the control of invasive rodents, nontarget species are also frequently exposed to ARs and secondary poisonings occur widely. However, little data is available on the effects of ARs, especially on marine organisms. To evaluate the effects of ARs on marine wildlife, we chose green sea turtles (*Chelonia mydas*), which are one of the most common marine organisms around the Ogasawara islands, as our primary study species. The sensitivity of these turtles to ARs was assessed using both in vivo and in vitro approaches. We administered 4 mg/kg of warfarin sodium either orally or intravenously to juvenile green sea turtles. We also administered 4 mg/kg of diphacinone intravenously. We then conducted an in vitro investigation using the liver microsomes from green sea turtles, and two other turtle species (softshell turtle and red-eared slider) and rats to compare metabolic capacity of diphacinone. In addition,

we performed metabolome and lipidome analysis using the sea turtle plasma. The turtles exhibited slow pharmacokinetics, and prolongation of prothrombin time (PT) was observed only with intravenous warfarin administration. Concentration of diphacinone in green turtle plasma did not decrease and about 50% of the C_{max} value was still observed after 216h of administration of the drug. About two-fold prolongation of PT was observed 9 days after dosing compared to before dosing, suggesting that diphacinone exposure causes hematologic toxicity in green turtles. The cytochrome P450 metabolic activity in the liver of green sea turtles was lower than in rats. Additionally, vitamin K epoxide reductase (VKOR), which is the target enzyme of ARs, was inhibited by warfarin in the turtles at lower concentration levels than in rats. Metabolome and lipidome analysis suggested that five substances were identified by metabolome analysis and four substances by lipidome analysis as being associated with the blood diphacinone levels. The effects of fluctuations in these substances are currently being carried out on the basis of metabolic pathway analysis and other statistical analyses. Our data indicated that turtles may be more sensitive to ARs than rats. We expect that these findings will be helpful for sea turtle conservation following accidental AR-broadcast incidents.

3.01.P-We-061 Using a Noninvasive Sampling Technique for Profiling the Biomarker Response of Marbled Salamanders (*Ambystoma opacum*) Across a Rural to Urban Land Use Gradient

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Obligate vernal pool breeding amphibians provide essential ecosystem services in wetlands and forests. Habitat alteration and pollution near these ecosystems may place these species at risk. Biomarkers of amphibian health are well-established, but sampling is typically lethal and difficult to justify for small or declining populations. To evaluate the use of dermal swabbing as a nonlethal technique for biomarker analysis in amphibians, marbled salamanders (*Ambystoma opacum*) from eight wetland ecosystems in Maryland's Coastal Plain were sampled during fall breeding migrations in 2021 and 2022. Wetlands occurred across a landscape gradient that included urban/suburban development, agriculture and forest to determine if the land use in a 1000m buffer zone impacted biomarkers of interest. Sediment samples from each wetland were analyzed for common-use pesticides, metals and organic carbon content. To obtain dermal mucus samples, cotton swabs were gently rubbed ~50 times over the dorsal and ventral body surface. Salamanders were also sexed, categorized as juvenile or adult, and photographed in the field for morphometric (snout vent length (cm) and total surface area (cm²)) analysis using ImageJ then subsequently released at the site of capture. Dermal swabs were analyzed for disease (*Batrachochytrium dendrobatidis* (*Bd*), *Batrachochytrium salamandrivorans* (*Bsal*), ranavirus), biochemical profiling using GC/MS-based metabolomics, and glutathione stress response. Distinct biomarker profiles were observed across wetlands and differed with land use types within the 1000m suggested buffer zone. Given the unique dermal properties of amphibians and their susceptibility to environmental stressors, obtaining mucus samples may be an invaluable tool for conservation practitioners. Insights provided by amphibian dermal swabs regarding bioindicators of population health may be helpful in understanding land use impact and aid in shaping subsequent *in situ* conservation practices.

3.01.T A Frog, a Snake, and an Ecotoxicologist Walk into a Pond (or Advances in Ecotoxicology and Risk Assessment of Amphibians and Reptiles)

3.01.T-01 Impact on Anuran Metamorphosis of Neonicotinoid and Anthranilic Diamide Insecticides

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In the agricultural region of the Argentine Pampa, the neonicotinoid (NEO) and anthranilic diamides (AD) families of insecticides are widely used on numerous crops, including soybean and corn. Both families of

insecticides are persistent in the environment and can reach the aquatic environment by lixiviation and runoff. The objective of the present study was to analyze the impacts on amphibian species native to the Pampa region of the commercial formulations Actara and Confidor containing the NEOs, thiamethoxam (THIA) and imidacloprid (IMI), and the commercial formulations Coragen and Fortenza containing the ADs, chlorantraniliprole (CHLO) and cyantraniliprole (CYAN). Impacts on development were examined in tadpoles of the toads *Rhinella arenarum* at a range of concentrations. Overall, the effects occurred mainly between stages 39 and 42. In general, NEO and AD acted differently at environmentally-relevant concentrations. In the case of NEO, exposure to THIA caused a delay in the time to metamorphosis of *R. arenarum* starting from the lowest concentration tested (5 µg/L), while the percentage of animals completing metamorphosis was not affected. For its part, exposure to IMI had no major effects on the development at the concentrations tested. Exposure to AD, CHLO and CYAN, altered the time to metamorphosis in a non-monotonic manner (increased or decreased depending on the concentration) and significantly reduced the percentage of animals completing metamorphosis but, in this case, the effect exhibited a linear dose-response relationship. Because most effects were triggered after the tadpoles had reached Stage 39, when metamorphosis is strictly dependent on thyroid hormones, an effect of both families of insecticides on the hypothalamic-pituitary-thyroid axis is suggested. Overall, both anthranilic diamide and neonicotinoid insecticides may impair the metamorphosis success of wild amphibians as effects were observed at low, environmentally relevant concentrations.

3.01.T-02 Biochemical and Behavioral Effects in Amphibians Exposed to Herbicides and Microplastics

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In this work, we exposed leopard frog (*Lithobates pipiens*) larvae to nominal and ambient concentrations of three herbicides (glyphosate, atrazine, msma) used in agricultural fields in Mexico. Additionally, we exposed the larvae to ambient concentrations of microplastics, and to herbicity mixtures with microplastics. We measure biochemical and defense biomarkers: antioxidants, oxidative stress; and damage: tissue damage, DNA damage and neurotoxicity. Likewise, the behavior (average speed, average distance) after exposure in amphibian larvae was evaluated. The results obtained showed an alteration in biochemical biomarkers, especially tissue damage by atrazine. While microplastics turned out to have a greater effect on the behavior of amphibians. Mixtures of herbicides with microplastics were shown to be more harmful to tadpoles in their behavior. This study contributes to the knowledge of the toxicological effects that these contaminants can have on amphibians.

3.01.T-04 From the Laboratory to the Field: Evaluating Amphibian Immunity in a Multi-Stressor Environment

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Infectious diseases are a major driver of the global amphibian decline. In addition, many factors, from genetics and stress to pollution and climate change, can influence the response to pathogens. Therefore, it is important to be able to evaluate amphibian immunity in both the laboratory and in the field. The phytohemagglutinin (PHA) assay is an inexpensive and relatively non-invasive tool that has been used extensively to assess immunocompetence, especially in birds, and more recently in amphibians. However, there is substantial variation in experimental methodology among amphibian PHA studies in terms of species and life stages, PHA doses and injection sites, and use of experimental controls. Here, we compile and compare all known PHA studies in amphibians to identify knowledge gaps and develop best practices for future work. We found that research has only been conducted on a limited number of species, which may not reflect the diversity of amphibians as a whole. There is also a lack of validation studies in most species, so that doses and timing of PHA injection and subsequent swelling measurements may not effectively evaluate immunocompetence. Further, there is limited information on how the assay performs under field conditions which is essential to

adequately assess the potential exposures to and effects of mixtures of contaminants, disease, climate, etc. Based on these and other findings, we put forward a set of recommendations to make future PHA studies more consistent and improve the ability to utilize this assay in wild populations, where immune surveillance is greatly needed.

3.01.T-05 Assessment of Heavy Metal Contamination in Amphibians from Otofure Dumpsite, Edo State, Nigeria

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Dumpsites have been implicated in the storage of heavy metals. Amphibians are typically drawn to the dumpsite due to the abundance of permanent food/prey items and the presence of appropriate moisture, particularly during the wet season. The vulnerability of amphibians to heavy metal pollutants in open dumpsites is worrisome. As a result, an atomic absorption spectrophotometer (AAS) was used to examine heavy metals contamination in amphibian tissues and soil from the Otofure dumpsite in Edo State, Nigeria. Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Lead (Pb), and Nickel (Ni) are among the metals evaluated. Two species of amphibians (*Ptychadena pumilio* and *Sclerophrys maculata*) were handpicked within the dumpsite and at ≥ 100 m away towards human habitation in the community. Except for Cd, which was exclusively detected in the kidney and liver, all heavy metals investigated were found in amphibian organs and tissues. The kidney and liver tissues exhibited the highest Cu concentrations. Copper and Pb predominated in all amphibians from both the dumpsite and the community, while Cu and Cr predominated in the soil. Cadmium was not detected in the thigh muscles of amphibians from either location, nor in the liver or kidney of *P. pumilio* from the community. Copper and Pb concentrations in amphibians from the dumpsite varied from 1.53 ± 0.16 to 3.40 ± 0.16 mg/L and 0.95 ± 0.57 to 5.10 ± 1.67 mg/L, respectively. All six heavy metals identified in soil were above the WHO acceptable limits in plants and soil, with Cu concentrations ranging from 103.95 ± 0.05 to 123.15 ± 0.83 mg/kg. The measured heavy metal concentration trend was Cu>Pb>Cr>Ni>Co>Cd in amphibians and Cu>Cr>Ni>Co>Pb>Cd in soil. The findings of this study show heavy metal pollution in both the dumpsite and the town of Otofure, which is most likely due to waste placed in the dumpsite. This has implications for plants and animals, as well as humans living in the neighborhood, particularly those whose residents live near the dumpsite and scavengers who earn a living off the dumpsite. As a result, the usage of open dumpsites should be discouraged, and the dumpsite should be relocated far from human habitation.

3.01.T-06 US Endangered Species Risk Assessment- What Do We Do About Amphibians and Reptiles?

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In January 2022, the United States Environmental Protection (US EPA), published a new policy to provide a framework to meet obligations specified in the US Endangered Species Act (ESA) where all new pesticide active ingredients would complete an Effects Determination for endangered and threatened (i.e., listed) species as a part of the registration, and now by extension the reregistration process. As with FIFRA when there are no hazard tests available for amphibians or reptiles, taxa-level risk assessments use fish as surrogates for aquatic phase amphibians, whereas birds are used as surrogates for terrestrial phase amphibians and reptiles. There are over 80 listed amphibians and reptiles in the US, each of which may require a species-specific risk assessment depending on its proximity to a potential pesticide use area. For the dietary route of exposure, these assessments typically employ allometric equations to determine daily caloric requirements, which are then coupled with estimated residues on potential food items. If the estimated body concentrations of a given chemical exceed the toxicity value, an adverse effect is potentially indicated, which can lead to preliminary predictions of jeopardy/adverse modification to the species or its critical habitat under ESA. These assessments use worst-case, conservative assumptions about body mass, potential residues on food items, and dietary composition. For example, using allometric equations for iguanid lizards to cover all amphibians and reptiles and comparing estimated body burdens to avian endpoints can lead to overestimates of potential risk and unnecessary label

restrictions. However, within the structured endangered species risk assessment process, there are opportunities for additional refinements that illustrate more representative exposure scenarios, thereby facilitating more accurate risk assessments to listed amphibian and reptiles. This presentation provides an overview of the current risk assessment process, as well as identifies improvements that more accurately assess potential risk to listed amphibians and reptiles in a transparent and scientific manner.

3.01.V A Frog, a Snake, and an Ecotoxicologist Walk into a Pond (or Advances in Ecotoxicology and Risk Assessment of Amphibians and Reptiles)

3.01.V-005 Effects and Bioconcentration of Bisphenol A and S on Chronically Exposed Tadpoles

Stacey A Robinson¹, Jade Pon^{1,2}, David J Carpenter¹, Sarah Young¹, Mark R Forbes² and Eve Gilroy¹, (1)Environment and Climate Change Canada, (2)Carleton University

Bisphenol A (BPA) is a chemical used in a variety of plastic products. Toxicity concerns led to its removal from some products; however, the replacement chemicals have similar structure and function to BPA and suspected similar toxicity. Very little information is available to determine the relative toxicity of the replacement products compared to BPA. Wildlife are exposed to BPA (and related replacement chemicals) through leaching from products and discharge in waste-water effluents. Hence, there is a concern for wildlife, particularly aquatic species sensitive to endocrine-disruption, such as amphibians that rely on properly functioning hormonal communication for larval growth and development. To assess the potential effects of BPA and a current BPA replacement chemical, bisphenol S (BPS), we conducted chronic aquatic exposures using the northern leopard frog (*Lithobates pipiens*). Specifically, Gosner stage 25 to 42 tadpoles were exposed to either BPA or BPS at concentrations ranging from 0.8 to 500 µg/L. These encompass measured concentrations in the environment up to a concentration where sub-lethal effects are expected based on effect concentrations found for other amphibians. Tadpole survival, Gosner stage of development, body mass, hepatosomatic index (HSI) and sex ratio were assessed after up to 45 days in a static-renewal laboratory exposure. As expected, there was minimal mortality in all test concentrations, ranging from 0-10%. Among treatments, tadpole body mass ranged from 2.4 ± 0.7 g to 2.9 ± 1.1 g (mean ±SD), with tadpoles exposed to BPS at 4, 20 and 500 µg /L being significantly heavier than the controls (GLMM, p < 0.05). Tadpole development ranged from Gosner stage 37 ± 3 to 39 ± 3, with 2 to 6 tadpoles/treatment reaching Gosner stage 42 or higher by the end of the experiment. HSI ranged from 3.1 ± 1.0 to 3.6 ± 1.4 and the sex ratio ranged from 58 ± 20% to 81 ± 16% females. However, there were no statistical differences when compared to controls for Gosner stage, HSI or sex ratio (p-values > 0.05). Tadpoles bioconcentrated BPA (BPS not assessed) in their tissues with a bioconcentration factor of 2.5 L/kg wet weight. In conclusion, we found tadpoles were 4% to 21% heavier with exposure to BPS, suggesting possible effects on lipid accumulation or metabolism. Further work on the ~30 other currently used or proposed replacement chemicals for BPA is still required with specific consideration for amphibians.

3.01.V-006 Effects of Elevated Temperatures and Exposure to Atrazine on Amphibian Health and Immune Systems

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Amphibians are declining on a global scale, some of which are due to climate change, pesticides and emerging diseases. Importantly, these stressors may combine to have exacerbated effects on amphibian health. Amphibians commonly breed in surface waters, resulting in the exposure of vulnerable life stages to contaminants such as pesticides from agricultural runoff. Pesticides can be lethal to amphibians but can also have sub-lethal effects such as reduced immunity, which can increase susceptibility to emerging diseases. Furthermore, as ectotherms, amphibian physiology, including immunity, is highly influenced by changes in temperature. As such, changes in temperature due to climate change are also expected to increase susceptibility of amphibians to parasites and pathogens. Surprisingly, little work has been done to examine the effects of

elevated temperatures on amphibian immune systems, either independently, or in combination with exposure to pesticides. Our project aims to address this gap by conducting a 2 x 2 factorial mesocosm experiment, looking at the effects of experimentally elevated temperatures and exposure to a pesticide, both independently and in combination on amphibian health. We expose northern leopard frog (*Lithobates pipiens*) tadpoles to environmentally relevant concentrations of atrazine (2µg/L) or dechlorinated water, until they have completed metamorphosis. Greenhouses are used to elevate mesocosm temperatures, with an anticipated temperature differential of 3°C between ambient and warmed mesocosms. The frogs will be assessed for life-history characteristics (survival, days to metamorphosis, size, weight, hepatosomatic index), and an indicator of stress (blood glucose levels). Furthermore, immunological endpoints such as blood cell profiles and bacterial killing ability of blood plasma will be assessed. Finally, locomotor performance of the juvenile frogs will be measured. The findings of this study will help advance our knowledge of the projected effects of climate change on amphibian health, contributing to the conservation of biodiversity. This study may further contribute to the re-evaluation of atrazine for use in Canada. Finally, results of this study will help to determine whether current methods for amphibian toxicity testing guidelines, which are often held at a single temperature, may be underestimating the toxic effects of contaminants on non-target organisms.

3.02.P-Th Exposure and Effects of Recognized and Emerging Contaminants to Wildlife

3.02.P-Th-036 Assessing the Differences Between Adult and Nymph *Amblyomma americanum* (Lone Star) Ticks as Viable Sentinels for the Detection of Per – and Polyfluoroalkyl Substance Contamination

Timothy Walsh, GHD

Per- and polyfluoroalkyl substances (PFAS) are a group of emerging contaminants that have received increased attention in recent years due to their widespread use, particularly in water repellent materials and non-stick cookware, limited breakdown in the environment, potential for bioaccumulation, and health impacts. A recent study demonstrated that ticks, including the Lone Star Tick, were viable sentinels for the detection of background PFAS contamination in the environment. Comparisons between collection sites also provided evidence for how certain remediation strategies are successful in responding to historical PFAS releases and anthropogenic contamination. Sentinel species have long been used in risk assessments to monitor contamination and provide early warnings of unsafe levels for certain chemicals. Ticks present a sufficient system for the detection of PFAS due to their widespread habitat range, reliance on blood meals, lack of preferential hosts, and increasing duration of questing seasons. While past research provided a proof of concept that various tick species could be viable sentinels for the detection of PFAS in the environment, there was no direct comparison between species or between different life stages within a single species. Both nymph and adult ticks require blood meals. Based on differences in vector competency between these life stages due to host seeking behavior and evasion of host detection, there is merit in determining if variations in PFAS detection between nymphs and adults exist. Future research should focus on a single tick species, such as AMA, and compare PFAS concentration between collected nymphs and adults. Based on differences in host seeking behavior and ability to evade host detection, there would be an expected differences in PFAS detection viability and efficiency between the nymphs and adults within a given species of ticks. A greater percentage of collected nymph samples could be reasonable anticipated to contain at least one PFAS compound compared to adult samples for example. These results would further parse out the specifics of ticks as a useful sentinel species for the detection of environmental PFAS contamination beyond proof of concept offering a more robust understanding of this overall relationship. Additional direct comparisons between the life stages of a single species and between different species should be carried out to determine the most efficient sentinels.

3.02.P-Th-037 Long-Term Monitoring and Assessment of Population, Reproductive, and Immune Effects in Colonial Waterbirds Breeding at Contaminated Great Lakes Sites in Michigan

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Fish-eating birds are excellent sentinel species for assessing and monitoring effects of persistent, bio-accumulative toxic chemicals (PBTs), including reproductive problems, deformities, and immunotoxicity. The Great Lakes Water Quality Agreement (GLWQA) recognizes “contaminants of mutual concern,” including both legacy (i.e., recognized) pollutants (e.g., polychlorinated biphenyls [PCBs]) and contaminants of emerging concern (CECs; e.g., per/polyfluoroalkyl substances [PFAS compounds]). Two wildlife-related Beneficial Use Impairments at Areas of Concern (AOCs) and across lakes are addressed by the GLWQA: 1) bird or animal deformities or reproductive problems and 2) degraded fish and wildlife populations. Current questions under the Great Lakes Restoration Initiative (GLRI) include whether these impairments continue and their potential associations with legacy pollutants and CECs. This monitoring program assessed immune function, reproduction, and populations of fish-eating birds in the Saginaw Bay and River Raisin AOCs and Grand Traverse Bay in 2010-22. 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 (both infertility and mortality) in gull embryos were 2-3 fold higher than reference in both AOCs and Grand Traverse Bay. Deformities associated with PCBs and dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) were observed in embryos and chicks only at contaminated sites. Productivity of 4-week-old tern chicks in Saginaw Bay was 34% lower than reference. In the River Raisin AOC, productivity of 4-week gull chicks was poor in 8 of 12 years. Breeding herring gulls decreased significantly in the River Raisin AOC, and breeding Caspian terns, a state-threatened species, declined in the Saginaw River and Bay AOC. The mean phytohemagglutinin skin response (T cell-mediated immunity) was suppressed 50-57% in gull chicks in both AOCs and Grand Traverse Bay, and 50% in terns and 39% in herons in Saginaw Bay. Antibody responses in gull chicks in the River Raisin AOC and Grand Traverse Bay were 1.5-1.9 fold lower than reference. Biological effects did not improve in the most recent years of study (2021-22). Tissue analysis 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.

3.02.P-Th-038 Occurrence and Species-Specific Variations of Per- and Polyfluoroalkyl Substances (PFAS) in Sharks from the Southeastern Coastline of the United States

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Per- and polyfluoroalkyl substances (PFAS) have gained widespread commercial usage across the globe in various industrial and consumer products, such as textiles, firefighting foams, and surface coating materials. Studies have shown that PFAS exhibit a strong tendency to accumulate within aquatic food webs, primarily due to their higher bioaccumulation potential and resistance to degradation. Despite such concerns, the impact of PFAS accumulation in apex predators, especially marine species, such as sharks, remains limited. Therefore, this study aimed to investigate the presence of PFAS in five shark species inhabiting the southeast coastline of the United States by analyzing their plasma samples (n=407). Bonnethead sharks (*Sphyrna tiburo*) exhibited the highest concentrations of \sum PFAS ($30.9 \pm 18.2 \text{ ng g}^{-1}$ plasma), followed by Atlantic Sharpnose (*Rhizoprionodon terraenovae*, $24.6 \pm 9.75 \text{ ng g}^{-1}$), Sandbar (*Carcharhinus plumbeus*, $20.1 \pm 7.49 \text{ ng g}^{-1}$), Blacknose (*Carcharhinus acronotus*, $17.1 \pm 6.55 \text{ ng g}^{-1}$ and Finetooth (*Carcharhinus isodon*, $13.8 \pm 8.78 \text{ ng g}^{-1}$). No significant differences in PFAS concentrations were determined by sex. Additionally, there was no significant variance in individual PFAS concentrations in relation to shark length; however, a minor decrease of \sum PFAS was observed as length increased, which implies that longer (more mature) sharks may experience a higher degree of elimination. Despite declines in the manufacturing of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the long-chain (C8 – C13) perfluoroalkyl acids (PFAAs) were highly detectible

with PFOS retaining its status as the dominant compound. The results of our study underscore the urgency for more extensive biomonitoring of PFAS in aquatic environments to obtain a comprehensive understanding of the impact of these emerging pollutants on marine fauna.

3.02.P-Th-039 Bioaccumulation Patterns of Individual Per- and Poly-Fluoroalkyl Substances and Binary Mixtures in the Brain of Northern Bobwhite Quail

Kenneth Kikanme, Adcharee Karnjanapiboonwong, Rameshkumar Angappan and Todd A Anderson, Texas Tech University

Per- and poly-fluoroalkyl substances (PFAS) are highly persistent chemicals commonly found in surface protectants and fire-fighting foams. Research has revealed that exposure to environmentally relevant concentrations of PFAS can reduce reproductive success and have immunological effects in laboratory animals, including birds. Furthermore, these chemicals can be passed down from parents to offspring, posing a threat to sensitive life stages. At the same time, studies have shown that different animal classes can exhibit diverse degrees of vulnerability to PFAS; avian species may be at particular risk due to their foraging habits. PFAS can enter the brain by disrupting tight junctions or binding to transporters, producing cognitive disorders and alteration of neurotransmitters, but our overall understanding of the interactions and accumulation of individual PFAS and simple mixtures in the avian brain is limited due to the lack of data. Our study investigated the presence and interaction of selected PFAS (PFOS, PFHxS, PFHxA, PFBS, PFHpA, PFOS:PFHxS, and PFOS:PFHxA) in adult quail brain to determine possible relationships. We had access to male:female pairs from chronic toxicity studies where adults were exposed to individual PFAS or binary mixtures through drinking water for at least 60 days. Whole brains were removed from the skull, extracted using QuEChERS salts, and then analyzed by LC-MS. PFAS were detected in the brains of exposed adult quail, while brains from control birds had only occasional detections of PFAS (mostly PFOS), likely due to their presence in feed. Despite similar average daily intakes for both males and females, we observed higher concentrations of most PFAS in the brains of males. For example, a significant effect of sex ($p = 0.016$) on brain concentrations of PFHxS following adult exposure (Males = 132 ± 22 ng PFHxS/g brain, Females = 43 ± 5 ng PFHxS/g brain). Sex had no effect ($p = 0.493$) on brain concentrations of PFHpA following adult exposure. For the binary mixture exposures, the presence of PFOS appeared to reduce brain concentrations of PFHxS in males and females to levels that could not be explained by ADI differences alone. Next, we will examine PFAS uptake and distribution in chick brains hatched from eggs laid by these same adults. Our goal is to understand better how factors such as PFAS exposure time, dosage, functional groups, and chain length influence the accumulation of PFAS in avian brain tissue.

3.02.P-Th-040 Chronic Reproductive Toxicity of Five Fluorine-Free Firefighting Foams and a Short Chain Fluorinated Foam to Northern Bobwhite Quail (*Colinus virginianus*)

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The development of novel, PFAS-free, firefighting foams with the potential to replace PFAS-containing firefighting foams is a subject of continuing research. These fluorine-free firefighting foams need to undergo toxicology studies, to assess their ecotoxicity and avoid unfavorable outcomes. In this study, we used Northern Bobwhite quail (*Colinus virginianus*) to assess oral chronic toxicity of five fluorine-free foams (National Foam Avio Green KHC, National Foam NFD 20-391, Solberg Re-Healing Foam, BioEx ECOPOL A, and NRL 502W) and a short chain PFAS-containing foam (Buckeye Platinum Plus C6). Exposure concentrations were 0.01%, 0.1% and 0.25% individual foam in drinking water, which was used to determine average daily intake (ADI) based on water consumption. We made observations on adult survival, growth, and reproduction, as well as hatching success, survival, and growth of chicks following parental exposure to the test foams. Adult survival following oral exposure was 100% for National Foam NFD 20-391, Solberg Re-Healing Foam, BioEx ECOPOL A, and Buckeye Platinum Plus C6, and 94% for National Foam Avio Green KHC and NRL 502W.

There were no statistically significant treatment effects observed on adult body weight change for these six foams. Further, no significant treatment effects were observed for eggs laid by adults, hatching success, hatching weight, and juvenile survival rate among treatment groups in comparison to controls. A statistically significant increase in the number of cracked eggs was observed from the 0.25% Buckeye-exposed adult birds ($p < 0.001$). Adult exposure to Buckeye, Avio, and NRL 502W caused embryos in unhatched eggs to be arrested earlier in development compared to control eggs ($F_{3,114} = 62$, $p < 0.01$; $F_{3,88} = 21$, $p < 0.01$ and $F_{3,72} = 17$, $p < 0.01$ for Buckeye, Avio, and NRL 502W, respectively). This study provides avian ecotoxicity data for risk assessment and further investigation of the potential environmental implications of fluorine-free firefighting foam alternatives.

3.02.P-Th-041 Mercury Concentrations in the Eggs of the Common Loon (*Gavia immer*) in the NYS Adirondack Park

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The Common Loon (*Gavia immer*) is an indicator of mercury (Hg) pollution in the waterbodies of the New York State Adirondack Park, as loons are long-lived, top trophic-level predators in their environment. Female loons bioaccumulate Hg and depurate a portion in their eggs, making eggs a useful tool to predict Hg burdens in the mother. Several factors may change Hg exposures to loons in a changing climate. Lakes with smaller areas tend to heat up at a more rapid rate than larger lakes, which may be a factor in changing loon Hg concentrations in the face of climate change. Higher water temperatures could lead to faster metabolic rates and more prey consumption, leading to increased Hg bioaccumulation from the fish they consume. It was hypothesized that smaller lakes would correlate to higher Hg concentrations in loon eggs. Past studies also show that lakes that are affected by human disturbance (e.g., reservoirs or dammed lakes) contain higher Hg concentrations, leading to the prediction that man-made structures such as dams change the hydrology of a lake and affect loon Hg exposure. Lake area was found to predict loon egg membrane Hg concentrations, confirming previous studies showing the importance of watershed and lake size in predicting loon Hg exposure. Lakes affected by human disturbance (e.g., dams) were found to result in higher exposure of Hg to loons. Ongoing work is assessing per- and poly-fluoroalkyl substances (PFAS) in the eggs of the Common Loon to evaluate multiple contaminant stressors on this charismatic and important top predator for the Adirondack Mountain lakes.

3.02.P-Th-042 Evaluation of Options for an Avian Reproduction Study Protocol to Meet Global Data Needs

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Avian reproduction study protocols typically harmonize the requirements of OECD 206 and OCSPP 850.2300 guidelines to meet global data needs. The EFSA's recent update to their guidance document for bird and mammal risk assessment has created a situation in which different endpoints for risk assessment will be used in Europe and North America. EFSA now requests that the benchmark dose (BMD) for a 10% effect be used while other regulatory authorities, including US EPA and PMRA will still use the NOEL (No-observed effect level). Only when it is not possible to obtain a reliable BMD would EFSA recommend a NOEL and even then, it would be calculated using a different (recently updated) statistical decision tree. Avian reproduction study designs place emphasis on power to detect a NOEL (i.e., comparisons against the control) and typically include high replication with few groups (e.g., 18 pairs/group and only 3 treatment groups plus a control). Indeed, a 2015 EFSA opinion rated the avian reproduction study as having serious limitations using a regression-based approach due to the low number of groups. Thus, if registrants are to provide EFSA with statistically robust BMD10s, then avian reproduction study designs will need to increase the number of treatment groups from the

current standard of three groups. However, there are five factors that must be balanced when making such adjustments: 1) Updates to regulatory guidelines typically take years; therefore, any changes to protocols should work within the constraints of the current guidelines (i.e., minimum 16 pairs/group for OCSSP 850.2300 and minimum 12 pairs/group for OECD 206). 2) Additional vertebrate usage should be minimized. 3) Only 80 duck cages can fit in one study room at the laboratory conducting almost 100% of the testing and splitting the study across multiple rooms is undesirable. 4) The modified design must retain power to detect a NOEL. 5) Any adjustments must not impact the ability of the study to meet existing guideline validity criteria (e.g., at least 29 eggs/hen in control). This presentation will review the results of computer simulations to compare potential model parameters and evaluate factors such as statistical power and variability under the alternative protocol designs (e.g., fewer pairs, reduced egg laying period) that satisfy the five considerations. Based on the outcome of the simulation study, a proposed path forward will be recommended for future avian reproduction study protocols.

3.02.P-Th-043 Development and Application of a Liver Perfusion System to Evaluate the Biotransformation Capability of Juvenile American Alligators

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The American alligator (*Alligator mississippiensis*) is an important sentinel species that can serve as an indicator of environmental health. Previous studies report alligators' susceptibility to environmental pollutants, and we therefore initially determined levels of various anthropogenic compounds in the blood plasma of wild juvenile alligators. Results showed the presence of commonly used human pharmaceuticals in the blood plasma of alligators, however, little is known about their biotransformation capability to metabolize pollutants or pharmaceuticals. Whole organ perfusion systems have been frequently used to study tissue/organ functions under stressed conditions in various vertebrates. The organ perfusion system is a favorable method in terms of preserving the integrity and physiological functions of the complex organ structure without interference from nervous and humoral systems. In this presentation, we will discuss the development and optimization of a novel liver perfusion system using Juvenile American alligators. The goal is to assess its usefulness in evaluating the ability of the liver to metabolize anthropogenic compounds in these ancient reptiles. To determine the perfusion system's robustness a comparison was made with S9 assays. Overall, the findings of this research will aid in our understanding of the hepatic physiology of the American alligator and their susceptibility of exposure to environmental pollutants.

3.02.P-Th-045 Validation of a Method for Determining Polycyclic Aromatic Compounds in Seabird Feathers

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Because of their ease to collect and transport from the field and that they accumulate pollutants, bird feathers are increasingly being used as a non-invasive biomonitoring tool for environmental monitoring programs. Polycyclic aromatic compounds (PACs) are a diverse class of environmental pollutants and because of their deleterious impacts on biological species monitoring these compounds in wildlife is of high importance. Current approaches to measuring PACs in bird feathers involves a time-consuming acid treatment step with a concomitant solvent extraction step. Here we present a validated method for the measuring a suite of PACs in bird feathers using accelerated solvent extraction and identification and quantitation by gas chromatography-tandem mass spectrometry. Chicken feathers were purposely fortified with a suite of 30 PACs separately at three dosing levels and placed inside an ASE extraction cell containing silica gel/ deactivated alumina to clean up the sample matrix. The accuracy of the method ranged from 70-120% and limits of detection and quantitation ranged from 0.4-5 and 2-16 pg/uL, respectively. The method exhibited good

repeatability with both inter- and intra-day repeatability <30%. The developed method represents an effective and efficient approach to extraction of PACs from bird feathers.

3.02.P-Th-046 Characterizing Fluoride Exposure in *Zalophus californianus* on the North Central California Coast

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For years, researchers have been concerned about fluoride (F) accumulating in marine environments as a result of anthropogenic activities (i.e. fluoridated drinking water, industrial waste, and agricultural run-off) and natural sources such as local geology. While often fatal in animals, elevated F concentrations in tissues of aquatic species could increase exposure to humans and risk of disease development. Recently, a stranded subadult male California sea lion (*Zalophus californianus*) was found to have periosteal new bone growth on several long bones. Bone F concentrations were 3-6 fold higher than F concentrations in controls. Bone lesions and F concentration elevations were consistent with a diagnosis of fluorosis. Subsequently, two other stranded *Z. californianus* with similar but less severe bone lesions, and lower F concentrations than the first animal, but 2-3 fold higher than control concentrations, were identified. All affected animals stranded in the same northern California county. Identification of elevated bone F concentrations consistent with excessive F exposure is challenging because expected ranges for bone fluoride concentrations have not been established in *Z. californianus*. Assessment is further complicated by variable deposition of F in different bones, differences in F accumulation reported with age and gender, incomplete skeleton recovery due to predation or weathering, and the lack of bone samples available for toxicology in existing tissue archives. Recognition of excessive F exposure, whether localized or wide spread, cannot be determined without establishing background bone F concentrations. To establish reference ranges, we chose five bones readily recoverable from beached carcasses and during postmortem examinations from 100 male and female *Z. californianus* across NOAA age-group classes. A standardized method using a fluoride-selective electrode was utilized to measure the bone fluoride concentrations and establish gender and age-group class specific background F concentrations. Future research to begin to unravel the source and route of the excessive F exposure will explore diet and geographical trends to facilitate containment of this outbreak.

3.02.P-Th-047 National Assessment of PFAS in Livers of White-Tailed Deer

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Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals that are used in countless industrial and consumer applications due to their properties including stain, oil, water, and fire resistance. Wildlife exposure to these chemicals is mainly via water and food that may have become contaminated from a variety of direct and indirect sources including atmospheric deposition, application of contaminated biosolids, contaminated water, or contaminated lands from aqueous fire-fighting foams (AFFF). For instance, 'Do Not Eat' advisories have been issued in several areas of the United States (U.S.) populated by white-tailed deer (*Odocoileus virginianus*) because of high levels of PFAS measured in their tissues. The goal of this study was to assess PFAS exposures in white-tailed deer across the U.S. We partnered with permitted hunters to obtain select deer components (i.e., liver, spleen, and scat) following legal harvest of white-tailed deer from a range of land use and potential chemical exposures. Between September 2022 and February 2023, biological samples from 55 white-tailed deer from 24 U.S. states were submitted for analysis. This study is part of a larger effort to characterize the prevalence of anthropogenic contaminants (i.e., PFAS, pesticides, microplastics, and antibiotic resistant genes) in white-tailed deer across the U.S. Herein, we present the results for 40 targeted PFAS compounds measured in liver tissues using ultra-performance liquid chromatography tandem mass spectrometry. Concentrations and compositional profiles are compared to evaluate PFAS spatial trends. Information from this study will help elucidate the need for further research to better understand contaminant uptake in white-tailed deer and potential exposure and effects to humans.

3.02.P-Th-048 Adapting a Bioenergetics-based Dosimetry Model to Predict Bioaccumulation and Biomagnification of Per- and Polyfluoroalkyl Substances in an Insectivorous Bird Species

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Bioaccumulation and biomagnification of per- and polyfluoroalkyl substances (PFAS) are known to occur in avian food webs, but the fate and transport dynamics of PFAS at different trophic and taxonomic levels is poorly understood. Insectivorous birds are reported to have high PFAS exposure because of their diverse feeding guild originating from contaminated sediment, surface water, and soils. While other research efforts are underway to understand the mechanisms by which PFAS exposure leads to adverse apical outcomes, predictive tools to estimate PFAS dosimetry and uptake from the food web are lacking. The objective of this research effort was to develop an *in silico* tool to estimate the dosimetry of select PFAS chemicals from the food web in an insectivorous bird species as a means to ultimately predict downstream effects based on an internal dose. To this end, an existing bioenergetics-based dosimetry model was adapted to compare whole nestling PFAS residues of Tree Swallows (*Tachycineta bicolor*) to model predictions based on the consumption rates of contaminated invertebrate prey. The original model simulates the accumulation of polychlorinated biphenyls (PCBs) by nestling Tree Swallows and served as the starting point from which chemical partitioning behavior was re-parameterized given the unique behavior of PFAS partitioning (i.e., by accounting for various lipid fractions and protein-binding affinity). As a means to establish and/or verify PFAS partitioning behavior, an ongoing field study was leveraged that consisted of nine Tree Swallow nesting sites with various levels of PFAS exposure in Duluth, MN. Data generated from the field study to parameterize and calibrate the model included nestling growth and respiration rates, as well as nestling and dietary invertebrate PFAS concentrations, and caloric densities of nestlings and prey. The adaptation of an existing bioenergetic-based dosimetry model has broadened the applicability of the model to evaluate unique aspects of PFAS partitioning behavior. As a result, we can compare existing food web bioaccumulation models, support downstream predictions of *in vivo* effects, and provide PFAS-relevant predictive tools for contaminated site 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.*

3.02.P-Th-049 Effects of In Ovo and Ex Ovo Exposure to Two “Alternative” Flame Retardants on Chick Embryonic Development

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Although the use of alternative flame retardants (FRs) in homes and workplaces has increased substantially in the last two decades, the potential for adverse effects of these chemicals on embryonic cardiovascular development has not been thoroughly investigated. Several studies have detected the presence of alternative FRs in placental fluid and breastmilk of human mothers, raising the concern that *in utero* exposure to alternative FRs may interfere with critical developmental windows. We used the chicken (*Gallus gallus*) embryo model to investigate whether *in ovo* and *ex ovo* exposure to triphenyl phosphate (TPP) and tris(2-chloroethyl) phosphate (TCEP) affected vasculogenesis and embryonic development. To measure extraembryonic branching *ex ovo*, chicken eggs were preincubated for 2 days. On embryonic day (ED) 3 embryos and egg contents were transferred to a shell-less incubation vessel and TPP and TCEP were injected into the albumin. On ED 6, we measured extraembryonic vascular branching. To measure embryonic heart and metabolic rates *in ovo*, we exposed chick embryos to TPP and TCEP *via* egg-injection on ED 3, then measured embryonic heart and metabolic rates on ED 18. Preliminary results suggest exposure to TPP and TCEP reduces extraembryonic branching, heart rate, and metabolic rate.

3.02.P-Th-050 Changes in Mercury, Stable Isotopes, and Polyunsaturated Fatty Acid Values Due to Aquatic Insect Metamorphosis and Insect-Mediated Contaminant Flux

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Aquatic emergent insects serve as vectors of bioaccumulative contaminants that can be transported from aquatic to terrestrial habitats due to their biphasic life cycles. In their aquatic larval stage, they accumulate contaminants in their aquatic habitat, then undergo metamorphosis, and in their adult stages they are a part of a terrestrial food web, making retained contaminants available to predators, such as riparian tetragnathid spiders, birds and bats. The type of emergent insects available to predators throughout a year as well as the process of metamorphosis may impact the amount of energy and potential contaminants being transferred to terrestrial habitat. In this study, we collected samples of benthic and emergent insect populations and compared the values of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes, polyunsaturated fatty acids, and mercury (total and methyl) in an emergent mayfly species (*Heptageniidae*) (larvae and adult) and a riparian spider (*Tetragnathidae*). Samples were taken from June to August 2022 within a 100m reach of the East Fork Stones River in Cannon Co., TN. Benthic and emergent insect populations varied in composition over the sampling period. It was found that $\delta^{15}\text{N}$, % carbon, and % nitrogen differed significantly between mayfly life stages, while $\delta^{13}\text{C}$ did not. It also was found that mayfly $\delta^{15}\text{N}$ varied over time and was reflected in tetragnathid spider values. $\delta^{13}\text{C}$ was found to decrease in tetragnathid spiders, but not vary significantly over time in mayflies. Mercury and polyunsaturated fatty acid values were found to vary over time in mayflies and spiders, as well as vary with mayfly life stage. This work further investigates the food web dynamics occurring during aquatic to terrestrial subsidy via emergent aquatic insects and illustrates the effects of population composition over time and metamorphosis on values of carbon and nitrogen stable isotopes, polyunsaturated fatty acids, and mercury in mayflies and a riparian spider predator.

3.02.P-Th-052 Characterizing Invertebrate Prey Diets of Songbirds Using DNA Metabarcoding: A Non-invasive Approach to Understanding Avian Diets and Potential Exposure to Persistent Organic Pollutants

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Previous studies indicate a wide variety of avian species are exposed to per- and polyfluoroalkyl substances (PFAS), a group of persistent organic chemicals that are known to cause reproductive toxicity in both human and animal models. Some PFAS are known to bioaccumulate and bio-magnify, but the distribution of PFAS in avian dietary items is unknown. Traditionally, determining diets of insectivorous birds is invasive and challenging, relying on the use of ligatures on nestlings, obtaining stomach contents, or intercepting provisioning adults en route to feeding their young. Using advances in DNA sequence analyses, we have developed a high resolution, non-invasive method to identify and characterize prey diets of insectivorous birds using DNA metabarcoding of dietary DNA (dDNA). Discarded fecal sacs were collected from nests at 6 sites with variable amounts of PFAS contamination, including a subset dosed with PFOS under semi-controlled conditions. Multiple loci were evaluated for utility in characterizing nestling tree swallows (*Tachycineta bicolor*) and house wrens (*Troglodytes aedon*) dDNA. Two of these loci, representing a total of 382 bp of the “barcode of life” cytochrome c oxidase 1 mitochondrial gene, have been optimized to successfully amplify songbird dDNA. Metabarcoding of fecal sac dDNA generated of arthropod OTUs representing of insect species at 100% identity when referenced to NCBI DNA databases. In summary, at least 23 Orders, 106 families, and 264 genera were identified using our approach. This research will advance our understanding of food web dynamics and routes of exposure to PFAS in songbirds and ultimately inform food web exposure-models.

3.02.P-Th-053 Exposure to the Flame Retardant Isopropylated Triarylphosphate Esters (ITP) Alters Microbiota Diversity, Metabolome and Immune Transcriptomic Responses of American kestrel (*Falco sparverius*) Nestlings

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Isopropylated triarylphosphate esters (ITP) are a type of organophosphate flame retardant (OPFR) that has been increasingly integrated in consumer products since the phase-out of polybrominated diphenyl ethers over the past decade. OPFRs may elicit detrimental effects on numerous non-avian species, resulting in behavioral, developmental, neurological and cardiac abnormalities. However, research on avian species, and specifically how elevated ITP exposure can influence various metrics of host fitness like microbiota composition, metabolomic profiles and transcriptomic responses have not been investigated. To further understand the potential toxicological effect of environmentally-relevant concentrations of ITP, we exposed American kestrel (*Falco sparverius*) nestlings to ITP and challenged them with polyinosinic:polycytidylic acid (Poly I:C), to simulate viral infection. Shotgun metagenomic sequencing on genomic DNA isolated from cecal small intestine rinsate were used to quantify microbiota composition. A section of the small intestine and plasma samples were submitted for global metabolomic analysis using a non-targeted UPLC-MS/MS approach. Preserved spleen tissue was used for transcriptomic analysis. Our data show that while microbiota richness did not vary among treatments, nestlings exposed to ITP had significantly lower Shannon diversity index and a higher prevalence of avian-associated viruses. Observed biochemical changes indicated that several biochemical pathways were altered in Poly I:C challenged nestlings and Poly I:C nestlings treated with ITP, including Vitamin A and Ascorbate metabolism, Purine Metabolism and Fatty Acid Degradation. We observed a higher number of differentially expressed genes (DEGs) in Poly I:C challenged individuals. However, Poly I:C challenged nestlings exposed to ITP had the highest number of downregulated DEGs that were associated with immune function. Moreover, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses indicated that the DEGs were enriched in biological processes related to the regulation of innate immune response, the regulation of response to biotic stimulus, toll-like receptor signaling pathways and glutathione metabolism. Taken altogether, these results demonstrate that exposure to environmentally relevant levels of ITP can alter metagenomic, transcriptomic and metabolomic profiles in birds, and may have much broader-reaching implications on host health than previously reported.

3.02.P-Th-054 Contaminant Burdens in Common Loon (*Gavia immer*) Eggs Associated with Reduced Eggshell Thickness and Egg Size in New Hampshire, USA

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As a long-lived, high-trophic level species, Common Loons (*Gavia immer*) are important indicators of contaminants in aquatic ecosystems. We quantified per- and polyfluoroalkyl substances (PFASs), brominated diphenyl ethers (BDEs), polychlorinated biphenyls (PCBs), dioxins/furans, organochlorine pesticides, and trace elements in 92 Common Loon eggs collected between 1993-2021 from 28 New Hampshire lakes. Additionally, we measured productivity and egg morphology for all nests and eggs. Whole egg burdens of Σ_9 PFAS ranged from 58.4-1587.4 ng/g ww, Σ_6 BDEs from 9.3-336.5 ng/g ww, Σ_{209} PCBs from 398-12,849 ng/g ww, Σ dioxins/furans from 5.4-55.5 pg/g ww toxic equivalency factor, Σ DDT from 132.8-974.0 ng/g ww, and Σ chlordanes from 10.4-131.1 ng/g ww. Seventy-two percent of eggs contained contaminant levels for at least one contaminant class that exceeded the lowest observed effects levels seen in other bird species. While we did not detect associations between egg contaminant burdens and loon breeding success, we found eggshell thickness and egg size were negatively correlated with concentrations of BDEs, organochlorine pesticides, and dioxins/furans. All contaminant classes were detected in all eggs, however, there was substantial variation

within and among lakes and sampling years. Further monitoring of contaminants in loon eggs in New Hampshire would further understanding of spatial and temporal variation in exposure risk and how co-exposure to multiple contaminant classes could influence the health of loon populations.

3.02.T Exposure and Effects of Recognized and Emerging Contaminants to Wildlife

3.02.T-02 Relationships between Spatial Behaviour, Habitat Use and Contaminant Exposure in Urban-Adapted Ring-Billed Gulls

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The movement of animals through space and their habitat use strategy can have a significant impact on their exposure to environmental contaminants, which could potentially affect their fitness. A few studies have determined that differences in habitat use are predictive of certain contaminant concentrations, however, it is not well understood whether differences in fine-scale animal movement (i.e., differences in home-range or number of foraging locations) can lead to differences in contaminant exposure. Additionally, it is not clear whether the reversal of this relationship is of significance, that is, could higher levels of contaminants lead to potential effects on spatial movements including decreased home-ranges or number of foraging locations? We studied fine-scale spatial movement and brain concentrations of 45 halogenated flame retardants (HFRs) and 16 trace elements in 65 ring-billed gulls (*Larus delawarensis*) breeding in a highly urbanized environment close to Montreal, Canada. We quantified several measures of their spatial behaviour, including home-range area, number of foraging locations, track repeatability, and maximum distance from the breeding colony. We found a large inter-individual variations for all those variables as well as in brain concentrations of several HFRs and trace elements. We also found that females have a higher number of foraging trips and foraging sites than males, likely to replenish resources that were depleted by egg laying. Preliminary findings showed an increase in brain concentrations of the brominated diphenyl ether (BDE)-99 with increasing number of foraging locations, while other measures of spatial behaviour were not related to brain contaminant concentrations. This study is important in highlighting how animal behaviour, specifically movement, could affect contaminant exposure, ultimately allowing for more targeted conservation efforts to protect vulnerable populations.

3.02.T-03 Assessment of Per- and Poly- Fluoroalkyl Substances from Multiple Tissues of North Pacific Killer Whales

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Killer whales (*Orcinus orca*) are apex predators that are exposed to high levels of contaminants. Per- and poly-fluoroalkyl substances (PFAS) are a group of chemical contaminants widely used in industrial and commercial products. They are persistent, are toxic to people and animals, and are mobile in aquatic ecosystems. In the North Pacific, there are three genetically different ecotypes of killer whales, namely, Offshore, Transient, and Resident, each with distinct feeding ecology and social dynamics. Among these, the Southern Resident Killer Whale (SRKW) is an endangered population under the Endangered Species Act. We measured 30 PFAS compounds from multiple tissues collected from stranded individuals, including a Transient killer whale (liver, kidney, skeletal muscle, intestine, blubber), a suspected Northern Resident killer whale (liver, kidney), and two SRKW (liver, blubber). Perfluorooctanoic acid (PFOS) was the most dominant PFAS detected across all tissues in all but one individual, an SRKW. In this individual, perfluorooctanesulfonamide (FOSA) predominated, contributing to 67% of the total PFAS in the blubber, and 59% of the total PFAS in the liver. Between tissues, liver had the highest concentration of total PFAS on a wet weight basis, followed by kidney, intestine, skeletal muscle, and blubber. This study highlights the exposure to PFAS faced by the endangered SRKW and discusses PFAS partitioning between various tissues.

3.02.T-04 Dietary Per- and Polyfluoroalkyl Substances (PFAS) Mixture Uptake and Elimination in the American Toad (*Anaxyrus americanus*)

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Quantifying receptor exposure to mixtures of PFAS remains a nebulous challenge in ecological risk assessment. Current methods largely use PFAS-specific generalizations to avoid the conflation of variability with uncertainty (i.e., PFOS data to speak for sum PFAS). Fortunately, some exposure scenarios are relatively well-characterized and can be captured in controlled studies. Aqueous film-forming foam impacted Department of Defense soil PFAS mixtures were used to create a model contaminated soil. Earthworms were raised in this soil plus a control soil for 28 days, collected, rinsed, and homogenized. Earthworm homogenate was then fed to American toads (*Anaxyrus americanus*) in a standardized volume on a time-weighted average schedule. PFAS-contaminated worms were fed for 28 days and then control worms were fed for 28 days. Toads were sampled prior to exposure, weekly during 28 days of uptake, and weekly during 28 days of elimination. Liver, whole body, and blood were collected from each individual toad. Liver and whole body PFAS concentrations were used to fit uptake and elimination kinetic models to relate PFAS-specific static and kinetic transfer coefficients tissue-to-tissue and PFAS-to-PFAS. Models fit were non-compartmented (fit via nonlinear least squares regression) and a two-compartment differential system (whole body and liver compartments) fit via Levenberg-Marquardt algorithm and a residual based model cost function. Starting parameter estimates in each model type were based on linear regression techniques in OECD protocol 305 (diet only portion). Notably, some PFAS show slow kinetics (PFOS) and some show very fast kinetics (6:2 FTS). Others do not appear to accumulate in toads (PFOA). PFOS appears to be the only PFAS to not reach steady state in 28 days of exposure, but liver concentrations did continue to rise during elimination phase—indicating kinetics into the liver may be slow. Relating these observations to other mixture studies to infer about competitive binding/excretion processes is underway. This study appears to be the first kinetics study of a herptile exposed to a PFAS mixture relevant to AFFF-impacted sites.

3.02.T-05 Imidacloprid Exposure is Detectable in Over One Third of Wild Bird Samples from Diverse Texas Ecoregions

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Avian decline is occurring globally with neonicotinoid insecticides posed as a potentially contributing factor. Birds can be exposed to neonicotinoids through coated seeds, soil, water, and insects, and experimentally exposed birds show varied adverse effects including mortality and disruption of immune, reproductive, and migration physiology. However, few studies have characterized exposure in wild bird communities over time. We hypothesized that neonicotinoid exposure would vary temporally and based on avian ecological traits. Birds were banded and blood sampled at eight non-agricultural sites across four Texas counties. Plasma from 55 species across 17 avian families was analyzed for the presence of 7 neonicotinoids using high performance liquid chromatography-tandem mass spectrometry. Imidacloprid was detected in 36 % of samples ($n = 294$); this included quantifiable concentrations (12 %; 10.8–36,131 pg/mL) and concentrations that were below the limit of quantification (25 %). Additionally, two birds were exposed to imidacloprid, acetamiprid (18,971.3 and 6844 pg/mL) and thiacloprid (7022.2 and 17,367 pg/mL), whereas no bird tested positive for clothianidin, dinotefuran, nitenpyram, or thiamethoxam, likely reflecting higher limits of detection for all compounds compared to imidacloprid. Birds sampled in spring and fall had higher incidences of exposure than those sampled in summer or winter. Subadult birds had higher incidences of exposure than adult birds. Among the species for which we tested more than five samples, American robin (*Turdus migratorius*) and red-winged blackbird (*Agelaius phoeniceus*) had significantly higher incidences of exposure. We found no relationships between exposure and foraging guild or avian family, suggesting birds with diverse life histories and

taxonomies are at risk. Of seven birds resampled over time, six showed neonicotinoid exposure at least once with three showing exposures at multiple time points, indicating continued exposure. This study provides exposure data to inform ecological risk assessment of neonicotinoids and avian conservation efforts.

3.02.T-06 Polyhalogenated Carbazoles in the Food Web of the St. Lawrence Estuary Beluga Population

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Polyhalogenated Carbazoles (PHCZs) are contaminants of emerging concern. These contaminants may be impurities in halogenated indigo dye formulations or carbazole-containing polymers used in electronic devices. Also, fungi are possible natural sources of PHCZs in the aquatic environment. PHCZs are structurally similar to halogenated dibenzofurans, raising concerns about their potential dioxin-like toxicity and their ability to bioaccumulate and biomagnify in the food web. Some PHCZs have previously been reported in sediment and fish of the Great Lakes of North America, but there is no information about PHCZs in the St Lawrence Estuary (SLE) (Canada), which is downstream of the Great Lakes and critical habitat for the endangered SLE beluga. To fill this knowledge gap, tissues were collected from 17 known and potential prey (n=183 including 15 fish species and 2 invertebrates) of SLE beluga in 2019 and 2020 to investigate the occurrence and trophodynamics of eleven PHCZs in the SLE beluga's food web. Preliminary results of 40 biota samples indicate the presence of all target PHCZs except for BCZ-1368. The concentrations of total PHCZs in samples were in the range of 9.8±1.6-49.1±19.8 ng/g (mean±SE) (lw), which were lower than previously reported concentrations in the fish from Great Lakes (66.1±20.3-181±141 ng/g (lw)). The trophodynamics of the target contaminants with 100% detection were evaluated. CCZ-3, CCZ-36 and total PHCZs were positively correlated to $d^{15}N$, indicating biomagnification of these contaminants in the SLE food web. The biomagnification of PHCZs may be attributed to the weak biotransformation of these contaminants in fish. To test hypotheses, PHCZs were incubated with liver S9 fractions of lake trout, Atlantic salmon and rats for 1 hour. The results showed that rats could biotransform PHCZs with one chlorine or bromine substitute, whereas fish could not biotransform any PHCZs. The estimated toxic equivalent (TEQ) concentration of total PHCZs in SLE prey species (mean range: 0.77-11.3 pg/g lw) was lower than fish from Great Lakes (8.7-25.7 pg/g lw) and San Francisco Bay (2.5-34.8 pg/g lw) as well as harbor seals from San Francisco Bay (7.6-174 pg/g lw). This study will provide a baseline for monitoring PHCZs in the SLE food web and contribute to a better understanding of the mechanisms underlying the biomagnification of PHCZs in the aquatic food web.

3.03.P-We Linking Molecular, Cellular, Tissue and Organ Level Effects to Apical Endpoints in Wildlife Toxicology

3.03.P-We-064 Risk Assessment and Biomonitoring for Exposure and Effects of Legacy Persistent Organic Pollutants and Contaminants of Emerging Concern in Colonial Waterbirds of the Great Lakes

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This long-term risk assessment and biomonitoring program (2010-2019) found ongoing developmental, physiological, and population effects consistent with exposure to polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins, and dibenzofurans (PCDD/Fs) in fish-eating birds in the Saginaw River and Bay and River Raisin Areas of Concern (AOCs) and Grand Traverse Bay. Reference sites were in the lower St. Marys River (herring gulls and Caspian terns), eastern Lake Superior (terns), and eastern Lake Huron (black-crowned night herons). Relative risk ratios for embryonic nonviability (both infertility and mortality) in herring gull embryos were 2-3 fold higher than reference in both AOCs, (contaminated primarily by PCBs) and Grand Traverse Bay (primarily PCDD/Fs). Deformities associated with PCBs and PCDD/Fs were observed in embryos and chicks only at

contaminated sites. The phytohemagglutinin skin response (T cell-mediated immunity) was suppressed 50-56% in gull chicks in both AOCs and Grand Traverse Bay, and 49% in terns and 39% in herons in Saginaw Bay. Antibody responses in gull chicks in the River Raisin AOC and Grand Traverse Bay were 1.6-2 fold lower than reference. Tern chick productivity in Saginaw Bay was 35% lower than reference, and gull chick productivity in the River Raisin AOC was poor in 7 of 10 years. These two populations with low productivity also experienced significant decreases breeding adults: 90% decline for River Raisin herring gulls ($r=-0.96$) and 49% decline for Saginaw Bay Caspian terns, a state-threatened species ($r=-0.64$). In River Raisin gulls, none of the impaired endpoints (total embryonic nonviability, infertility, embryonic death, chick productivity, T cell response, and antibody titers) changed over time despite remedial dredging of contaminated sediments in 2014 and 2016. In Saginaw Bay gulls, no impaired endpoints (total embryonic nonviability, infertility, embryonic death, and T cell response) improved with time on either island. Embryonic death increased on the CDF. In Saginaw Bay terns, T cell responses and chick productivity did not improve over time. In Grand Traverse Bay gulls, most impaired endpoints did not improve (infertility, embryonic death, T cell response, and antibody titers). Only total embryonic nonviability improved marginally. Analysis of legacy pollutants in eggs and plasma demonstrated ongoing significant exposure to PCBs, with fluorinated and brominated contaminants also elevated in tissues at most sites.

3.03.P-We-065 The Use of Japanese Quail EcoToxChips to Improve the Understanding of the Mechanism(s) of Action of Ethinyl Estradiol (EE2) in Early-Life Stage Embryos

Emily Boulanger¹, Nil Basu¹, Doug Crump² and Jessica Head¹, (1)McGill University, (2)Environment and Climate Change Canada

Ethinyl estradiol (EE2) is a synthetic estrogen, which is widely used in birth control pills. While monitoring studies have documented that various avian species are exposed to this chemical, much less is known about the molecular mechanisms by which EE2 (and more generally, estrogenic chemicals) affect birds, particularly during early-life stages. The objective of this study was to increase understanding of EE2's mechanism(s) of action in an early-life stage avian model using the EcoToxChip Test System. Briefly, fertilized unincubated Japanese quail (JQ) eggs were injected on embryonic day 0 with DMSO (solvent control), 6 or 54 ug/g EE2 into the air cell (35 eggs per dose group). Eggs were artificially incubated at 37.5 ± 0.5 °C and $60 \pm 5\%$ humidity. At embryonic day 9 (mid-incubation for JQ), liver samples from 5 viable embryos were harvested per dose group. Hepatic gene expression was assessed using the 384-gene Japanese Quail EcoToxChip (www.ecotoxchip.ca). The qPCR data generated from the JQ EcoToxChips were analysed with EcoToxXplorer (www.ecotoxxplorer.ca) and all quality control criteria were met when 2/5 housekeeping genes were excluded from the analysis. There was no mortality observed in any of the exposure groups. The number of differentially expressed genes (DEGs) was 39 and 41 in the 6 and 54 ug/g EE2 groups, respectively. Several genes expected to be perturbed by EE2 exposure were dysregulated (e.g. VTG2, CYP2H1), and some of these genes were found in Adverse Outcome Pathways (AOPs) associated with CAR (AOP #107) and ER Antagonism (AOP #30). A pathway-level investigation of the gene expression data suggests that the main biological pathways affected by this exposure were related to metabolism. In general, these findings increase understanding of how avian models may be affected by early-life exposure to EE2. The molecular responses are expected based on an analysis of literature from other vertebrate species exposed to EE2. These results also provide increased confidence that the EcoToxChip Test System using early-life stage Japanese quail may serve as an effective New Approach Method.

3.03.P-We-066 The Use of Japanese Quail EcoToxChips to Improve the Understanding of the Mechanism(s) of Action of Hexabromocyclododecane (HBCD) in Early-Life Stage Embryos

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Hexabromocyclododecane (HBCD) is a brominated flame retardant listed under the Stockholm Convention.

Owing to its environmental persistence and continued use under certain exemptions (e.g., polystyrene), ecological concerns remain. While monitoring studies have documented that various avian species are exposed to this chemical (particularly through the analysis of egg content), much less is known about the molecular mechanisms by which HBCD may affect birds particularly during early-life stages. The objective of this study was to increase understanding of HBCD's mechanisms of action in an early-life stage avian model using the EcoToxChip Test System. Briefly, fertilized unincubated Japanese quail (JQ) eggs were injected on embryonic day 0 with DMSO (solvent control), 0.7 or 11 ug/g HBCD into the air cell (35 eggs per dose group). Eggs were artificially incubated at 37.5 ± 0.5 °C and $60 \pm 5\%$ humidity. At embryonic day 9 (mid-incubation for JQ), liver samples from 5 viable embryos were harvested per dose group. Hepatic gene expression was assessed using the 384-gene Japanese Quail EcoToxChip (www.ecotoxchip.ca). The qPCR data generated from the JQ EcoToxChips were analysed with EcoToxXplorer (www.ecotoxxplorer.ca) and all quality control criteria were met. There was no mortality observed in any of the exposure groups. The number of differentially expressed genes (DEGs) was 81 and 49 in the 0.7 and 11 ug/g HBCD groups, respectively. Some of these genes were found in Adverse Outcome Pathways (AOPs) associated with PPAR (AOP #6) and ER Antagonism (AOP #30). A pathway-level investigation of the gene expression data suggests that the main biological pathways affected by this exposure were those related to metabolism. In general, these findings increase understanding of how avian models may be affected by early-life exposure to HBCD. The molecular responses are ones that would be expected based on an analysis of literature from other vertebrate species exposed to HBCD. These results also provide increased confidence that the EcoToxChip Test System using early-life stage Japanese quail may serve as an affective New Approach Method.

3.03.P-We-067 Effect of UV Stabilizers and Filters in Early-Life Stage Japanese Quail and Double Crested Cormorant

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UV stabilizers and filters are emerging contaminants of concern that are ubiquitous in the environment. They biomagnify and have been detected in seabirds and their eggs. Their effects have yet to be understood in avian species; studies in mice, crustaceans, fish and cell cultures demonstrate UV stabilizers and filters have immune and endocrine disrupting properties and are potential aryl hydrocarbon receptor ligands. Here, we use avian embryos as a model to assess the effects of three UV stabilizers and one UV filter in birds. Japanese quail (JQ; *Coturnix japonica*) eggs were obtained from a commercial farm and double-crested cormorant (DCCO; *Phalacrocoax auritus*) eggs were collected from an island near Cornwall, Ontario. We injected test chemicals into the air cell (n=20/dose group for both species) on embryonic day 0 (ED0). We tested the chemicals at their upper solubility limit in safflower oil (5.25 ug/g to 12.59 ug/g), and at a lower dose of 1 ug/g. We artificially incubated the eggs and dissected a sub-set at mid-incubation (n= 5, day 9 for JQ and 14 for DCCO) and the remainder just prior to hatching (n= 10-15). We collected liver tissue at both timepoints and measured morphological changes including embryo, liver, and gallbladder mass and tarsus and head plus bill length at the latter time point. We analyzed hepatic gene expression in mid-incubation livers using EcoToxChips, a species-specific 384-well qPCR array comprising a curated set of genes that are relevant to ecotoxicology. Genes involved in endocrine function, xenobiotic metabolism, immune function, and cell regulation were differentially regulated in Japanese quail. Cormorant hepatic gene expression has not yet been analyzed. Japanese quail embryos treated with the high dose of UV9 had a significant number of enlarged gallbladders. There were no significant changes in other apical endpoints. The dysregulated genes and morphological changes observed in Japanese quail embryos may indicate biliary duct malformation in the liver. This study provides novel toxicological data for two avian species regarding chemicals of emerging concern.

3.03.P-We-068 Association of Hepatic Gene Expression Changes with Tissue Residue Concentrations in Wild-Collected Double-Crested Cormorant Embryos Using an EcoToxChip Gene Array

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Laboratory ecotoxicity studies commonly use embryos from avian models such as chicken, pheasant, quail, or mallard. However, avian field studies and contaminant monitoring programs that use eggs typically employ very different wild species. Furthermore, whether chemical residues measured in eggs elicit adverse effects is seldom certain and assessment methods are limited. To address some of those limitations, we evaluated spatial and correlational relationships between whole embryo contaminant concentrations and mRNA expression in embryonic hepatic tissue from a common diving waterbird with a wide breeding range in North America, the double-crested cormorant (*Nannopterum auritum*). We collected viable eggs from three sites with distinct proximal human land use and known contamination history (one site in the Laurentian Great Lakes and two sites in Pacific Canada; n = 6/site). Eggs were artificially incubated in the lab and at pipping, hepatic tissue was sampled and RNA was extracted for cDNA synthesis and qPCR array analysis using an EcoToxChip containing 354 unique gene assays. The remaining embryo carcass and yolk sac were analyzed for total mercury and organic contaminants: 20 organochlorine pesticides, 17 perfluoroalkyl substances, 35 polychlorinated biphenyls, and 22 halogenated flame retardants. Contaminant concentration data and qPCR normalized gene expression data ($2^{-\Delta Cq}$) are being analysed with a combination of linear models and multivariate approaches. Preliminary data suggest gene-contaminant trends across individuals and among sites, indicating that targeted, high-throughput screening methods designed for a native North American avian species can support wildlife risk assessment and contaminant monitoring. Furthermore, paired gene expression and chemical residue observations provide data for one of the lines of evidence necessary to elucidate adverse outcome pathways (AOPs) and indicate where existing AOPs described for model organisms have relevance to wild species.

3.03.T Linking Molecular, Cellular, Tissue and Organ Level Effects to Apical Endpoints in Wildlife Toxicology

3.03.T-01 Predicting Avian Toxicity of Pesticides- Where Are We At and Where Should We Go?

Audrey Bone, Bayer AG - Crop Science Division

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. However, these study designs collectively require the use of thousands of birds and are not amenable to use for screening of large numbers of substances, for example on the scale needed for screening novel candidate pesticides (thousands-hundreds of thousands). New approach methods (NAMs) offer alternatives to traditional *in vivo* ecotoxicity testing. For example, the rainbow trout gill cell line assay (OECD TG 249) is now an OECD validated test guideline that is fit-for-purpose for predicting acute toxicity of substances to fish in many use cases. However, research on avian NAMs, either for the purpose of high-throughput screening or for regulatory use, has lagged behind research in the human and aquatic NAMs arenas. In addition, while method development and proof-of-concept experiments are a key piece of NAM development, the *in vivo* data that is intended to be predicted should be well-characterized and understood to guide NAM development and implementation. This presentation will cover the current state of avian NAM research, discuss challenges in this research area, and present preliminary results of *in vivo* avian toxicity data analysis including comparisons of sensitivity between the mallard duck and bobwhite quail and comparisons of acute and chronic endpoints by chemical class and mode-of-action.

3.03.T-02 Reproducibility of Transcriptomics Experiments; Performance of Japanese quail EcoToxChips in a Multi-lab Ring Test

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The field of chemical risk assessment is increasingly moving towards New Approach Methods (NAMs) that reduce animal use and incorporate 'omics endpoints. These approaches hold promise, but uptake of NAMs by the regulatory community will require demonstration of their reproducibility. Here, we use a multi-lab ring test to evaluate the reproducibility of transcriptomic responses in early-life stage Japanese quail (*Coturnix japonica*). Three independent laboratories (McGill University, Environment and Climate Change Canada (ECCC) and U.S. Geological Survey Eastern Ecological Science Center (USGS)) participated in the ring test. At each lab, fertilized, unincubated quail eggs were exposed via air cell injection to DMSO (solvent control) or 7 concentrations (0.04–40 µg/g egg nominal) of chlorpyrifos (CPF) on embryonic day 0 (ED0) (10 eggs per dose group). Eggs were incubated at 37.5 ± 0.5 °C and $60 \pm 5\%$ humidity until mid-incubation (i.e. ED9). Hepatic gene expression in liver samples from 5 embryos per dose group was assessed using the 384-gene Japanese quail EcoToxChip. Standard operating procedures, reagents, dosing solutions, and methods for transcriptomic dose-response analysis were common between the three laboratories, but the strain of Japanese quail used by the USGS lab was different. The number of differentially expressed genes (DEGs) increased across dose groups in samples from all three labs, with a higher number of DEGs at the maximum dose for McGill (69 DEGs) and ECCC (80 DEGs) compared to USGS (27 DEGs). Despite these differences, the transcriptomic points of departure (tPOD) calculated by each lab were similar (17.5, 16.5, and 16.1 µg/g for McGill, ECCC, and USGS respectively), and corresponded to the tPOD previously calculated from RNASeq data (21.6 µg/g). All tPODs were below our previously published organismal lowest observable effect level of 41.1 µg/g for CPF in Japanese quail embryos. Overall, our results suggest that transcriptomic dose-response analysis holds promise as a NAM that is reproducible and of potential interest to the regulatory community.

3.03.T-03 Toxicity of Acetaminophen in Bird Species: Induction and Activity of Phenol-type UDP-glucuronosyltransferases

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The commonly used anti-inflammatory drug acetaminophen was found to be extremely toxic to snake species and is therefore used to manage invasive snakes like the brown treesnake (*Boiga irregularis*) on the island of Guam. Recent studies have revealed that toxicity in snakes is the result of the lack of phenol-type UDP glucuronosyltransferase (UGT), which effectively breaks down the drug in most mammalian species. Because bird species may become exposed to acetaminophen in areas where treated bait is distributed for snake control, studies were needed to assess its toxicity in bird species. A dosing experiment was performed with chicken as a model for other bird species. Chickens were exposed to increasing acetaminophen doses, up to 237 mg/kg/day. No acute mortality related to the exposure was encountered, however, blood plasma analysis showed that in the higher dosage groups some biomarkers for liver damage and oxidative stress were significantly different from the control group. Analysis of induction of phenol-type UGT isoforms demonstrated that there were no significant differences in enzyme activities between the dosage groups. Analysis of the expression of all known UGT isoforms in chicken, using qPCR, showed a very moderate upregulation (up to 1.9x) for four of those genes in the dosed chickens. In addition, liver samples from 16 different bird species were analyzed for phenol-type UGT activity. This revealed that the collected bird species can be divided into two groups: a group with high UGT activity (chicken, rail, ring-billed gull, barred owl, screech owl and American crow) and a group with very low or no significant activity (pigeon, mourning dove, brown pelican, blue heron, snowy egret, turkey vulture, red-tailed hawk and red-shouldered hawk). This indicates that there is not a clear relationship between diet and phenol-type UGT, nor is there a taxonomic relationship with enzyme activity. Further investigations are performed to identify which specific UGT isoform in birds is responsible for the metabolism of

acetaminophen, and which bird species express phenol-type UGT genes. Based on these results, it appears that chickens are not highly susceptible to acetaminophen toxicity, but the results of enzyme activity measurements in wild bird species suggest that there is significant variability between species, which warrants caution for application of acetaminophen to control snake species.

3.03.T-04 Associations Between Persistent Organic Pollutants, Altered Immune Function, and Apical Endpoints in Colonial Waterbirds of the Great Lakes

Keith Grasman, Calvin University

Developmental and immunotoxic effects of polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins, and dibenzofurans (PCDD/Fs) have 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 information on immunotoxic effects of PCDD/DFs and PCBs at the genomic, cellular, and physiological levels continues to emerge after 5 decades of research. Developmental and reproductive effects PCBs and PCDDs/Fs include chick edema disease in chickens and Great Lakes Embryo Mortality, Edema, and Deformities Syndrome (GLEMEDs) in wild waterbirds. Many of the immune and developmental effects of PCBs and PCDD/Fs are mediated by the aryl hydrocarbon receptor (AhR), a regulator of gene expression and cell cycling. This talk will focus on immunological, reproductive, and developmental endpoints in two populations of waterbirds whose breeding numbers have declined significantly in areas with high PCBs: 90% decline in herring gulls in the River Raisin Area of Concern (AOC) ($r=-0.96$) and 49% decline in Caspian terns, a state-threatened species, in the Saginaw Bay and River AOC ($r=-0.64$). Population level effects at the end of adverse outcome pathways (AOPs) build on cellular and physiological mechanisms and individual-level apical endpoints such as development and survival. These two populations frequently show poor chick productivity, an apical measure of egg and chick survival through 4 weeks after hatch. Caspian tern chick productivity in Saginaw Bay was 34% lower than reference, and herring gull chick productivity in the River Raisin AOC was poor in 8 of 12 years. Relative risk ratios for embryonic nonviability (both infertility and mortality) in gull embryos were 2.5-3X higher than reference in River Raisin gulls. Deformities associated with PCBs and PCDD/Fs were observed in embryos and chicks only at contaminated sites, including these two populations. The mean phytohemagglutinin skin response (T cell-mediated immunity) was suppressed 50% in River Raisin gull chicks and Saginaw Bay tern chicks. 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 the population declines observed in this study, other studies in avian immunoeology have shown that birds with lower immune responses using these types of assays have lower survival rates.

3.03.T-05 Considerations for Selecting Assays Relevant to Wildlife for Ecological Risk Assessments: The Role of Toxicology, Ecology, and Assumptions

Mark S. Johnson, U.S. Army Public Health Center

Regulatory policies based on expert opinion focus ecological risk assessments at the population biological level of organization. Ecological risk assessments for wildlife redefine population to be relevant to the assessment population which can be considered equivalent to the metapopulation in contemporary ecology. Often these risk assessments for wildlife assume that toxicity benchmarks based on mortality, growth, and reproduction directly affect population density. Although these parameters are useful in simple population models, movements of wildlife between metapopulations (i.e., immigration and emigration) and community dynamics (e.g., predator composition, availability of nesting sites) can also significantly affect metapopulation densities. Conceptually, alternative toxicity endpoints (e.g., neurological effects) can result in behavioral changes that can increase mortality events and decrease reproductive success. New tools for investigating toxicity in vertebrate models provide the opportunity to elucidate biological mechanisms that can assist in interspecies extrapolation and provide new insights on adverse effects that may be relevant to metapopulation densities. This presentation will

provide conceptualization for understanding the influence of these tools and alternative toxicological endpoints that may improve the estimation of risk for wildlife species to enable more accurate decision making.

3.03.T-06 Critique of Lower-Level Toxicological Response Measurements With Linkage to Apical Effects for Wildlife Ecological Risk Assessments

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Ecological risk assessments (ERAs) involving wildlife have historically focused on apical endpoints at the level of the individual organism, including survival, growth, and reproduction. However, since the 1960s, adverse effect measurements at levels of biological organization below the individual (e.g., molecular, cellular, tissue, organ) have been studied and developed for use in wildlife (e.g., alterations in DNA and RNA, inhibition and induction of enzymes, changes in protein synthesis, presence of metabolic products, impaired immune function, histopathological lesions, and physiologic and nonspecific response biomarkers). While these lower-level toxicological effect endpoints have been extensively used in retrospective ERAs for evaluation of chemical spills and contaminant site assessments, they have rarely been applied in prospective ERAs involving approval of pesticides, industrial chemicals, and pharmaceuticals focused on wildlife. We will summarize and critique lower-level measurements used in wildlife toxicology studies and ERAs with discussion of the rapidly evolving new approach methodologies (NAMs), adverse outcome pathways (AOPs), gene-protein reaction and ecological effect models that have direct application to terrestrial wildlife. If and when such biomarker responses, NAMs, AOPs, and models are clearly linked to ecologically relevant key events and relationships, then lower-level effect endpoints could be incorporated when identifying sources of chemical risk to wildlife populations. A high degree of accuracy is required to build confidence in the use of such predictive tools, not only to ensure that wildlife populations are protected, but to avoid overly conservative or erroneous risk decisions that could stifle innovation. *This abstract does not necessarily reflect the views or policy of the US EPA.*

3.03.V Linking Molecular, Cellular, Tissue and Organ Level Effects to Apical Endpoints in Wildlife Toxicology

3.03.V-026 Development of New Approach Methodologies (NAMs) for Avian Risk Refinement

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As part of regulatory requirements for pesticide risk assessment, both acute and chronic avian toxicology tests are conducted. These in vivo studies using zebra finch, northern bobwhite, and mallard, provide relevant endpoints for screening level risk assessments. However, risk refinements are often limited due to the specific design of these tests. Additional in vivo tests are not recommended due to animal welfare issues. In order to better understand realistic risks and provide refinement options, the U.S. Environmental Protection Agency (U.S.EPA) and European Food Safety Authority (EFSA) support the development of new approach methodologies (NAMs) as a promising way forward for vertebrate wildlife risk assessments. The limited work available in this area highlight the need for developing case studies to illustrate the use of NAMs in improving mechanistic understanding of the internal kinetics, enabling cross-species extrapolation, and risk refinement for avian species based on in vitro, in silico, and historical in vivo avian residue data. In this study, we developed in vitro to in vivo extrapolation (IVIVE) and physiologically based kinetic (PBK) models for three avian species exposed to thiamethoxam. In vitro kinetic measurements of hen, mallard and bobwhite, including metabolic stability and plasma protein binding, were collected for the target compound. Those in vitro kinetic measurements were used to parameterize IVIVE-PBK models. An in vivo residue study using laying hens was used to validate the laying hen model. Mallard and bobwhite simulations were conducted with the same avian PBK model structure but considered differences in species physiology. The time-course of internal concentration predictions by the PBK model will serve as input into toxicodynamic (TD) and physiological

models, such as those based on the Dynamic Energy Budget (DEB) theory, to predict the effects on survival, growth or reproduction from exposure under realistic conditions. The integrated DEB-PBK-TD approach can greatly increase the utility of in vivo studies conducted for avian risk assessment and allow for a more realistic assessment of potential risks to individuals and populations in the field.

3.04.P-Th Toxic Effects of Per- And Poly-Fluorinated Compounds: From the Molecular to Ecosystem Levels

3.04.P-Th-055 Exposure to Per- and Polyfluoroalkyl Substances (PFAS) and Cardiovascular Disease in the Central Savannah River Area

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Recent environmental investigations have raised concerns regarding the high per- and polyfluoroalkyl substances (PFAS) accumulation in the underrepresented Central Savannah River Area (CSRA). CSRA is situated in the “Black Belt” of the Southeastern states of Georgia and South Carolina, in the top 10 overweight and obese areas in the United States where cardiovascular disease (CVD) is the leading cause of death. This pilot study measured PFAS concentrations in archived blood samples collected from CSRA adolescents (i.e., a well-established and well-published Lifestyle, Adiposity, and Cardiovascular Health in Youth (LACHY) cohort) and explored the health impacts of PFAS on obesity and cardiometabolic risk using measured cardiometabolic risk factors. The results show that PFAS are bioaccumulated in the blood of people from the CSRA region, with several compounds present at significantly higher levels than in the general US population. Twenty-six targeted PFAS compounds were detected in all participants. Different PFAS compounds presented different accumulation levels. PFHxS (0.79–12.3 µg/L), PFNA (0.19–1.79 µg/L), and PFOA (1.62–32.1 µg/L) demonstrated high accumulation levels as all concentrations are statistically significantly higher than the general US population (PFHxS: 1.18 µg/L; PFNA: 0.58 µg/L; PFOA: 1.56 µg/L), especially PFOA and PFHxS that are about three times higher than the general US population. Notably, sex differences in PFAS bioaccumulation were also observed as males had significantly higher PFOA and PFHxS concentrations than females, suggesting that sex is a biological variable in PFAS exposure in the CSRA, which is consistent with prior studies. Given its small sample size (n=20), the pilot study also found a promising positive correlation between PFAS exposure (i.e., PFOA and PFPeA) and measures of obesity (i.e., Body mass index, BMI) and CVD (i.e., supine systolic blood pressure, SBP), which may contribute to the CSRA region’s status as one of the top 10 areas for overweight and obesity with high CVD risks. These findings highlight the need to identify PFAS exposure and its health impacts on people in the CSRA, as well as to understand the toxicological mechanisms of PFAS. Our preliminary study provides the initial data on PFAS bioaccumulation and health impacts for the CSRA population, underscoring the critical need for ongoing research in this area.

3.04.P-Th-056 Comparative Toxicity of Legacy and Short-chain Replacement PFAS on Early Life Stage Estuarine Fishes

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Estuaries in close proximity to industrial and military facilities and urban centres 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 – including nearly all tested humans and wildlife. One of the most studied legacy PFAS, perfluorooctanoic acid (PFOA), is a known endocrine disruptor that leads to developmental and reproductive toxicity in fishes. Effort has been made to transition to short-chain PFAS (i.e., GenX compounds), intended to be less toxic due to their lower bioaccumulative potential. However, data on the presence and behaviour of GenX compounds (e.g., hexafluoropropylene oxide dimer acid [HFPO-DA]) in estuarine environments and biota are lacking. Here, we

investigated the toxicity of both PFOA and HFPO-DA on early life stage (ELS) red drum – an important estuarine-dependent fish found in habitats receiving industrial and commercial runoff. Fish were exposed to PFOA or HFPO-DA for the first 72h of development. Environmentally relevant concentrations of both compounds led to morphological impacts associated with reduced fitness, including smaller hatch size. These impacts may result in changes to population structure, as size-selection is the primary driver of mortality in ELS fish.

3.04.P-Th-057 Hazard Metrics, Including Transcriptomic-Based Points of Departure, for Fathead Minnow (*Pimephales promelas*) Exposed to 22 PFAS

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The presence of per- and polyfluoroalkyl substances (PFAS) in aquatic environments has led to concern for their potential impacts to these ecosystems. Aquatic hazard data for PFAS are sparse with few exceptions (ex., PFOS and PFOA). This study evaluated the effects of 22 different PFAS with varying chain lengths and functional head groups on larval fathead minnow (*Pimephales promelas*) utilizing a 24-hour high throughput microplate assay. Fish were exposed to eight concentrations per chemical, at ½ log spacing, in 1 mL 96-well microplates. Survival data were collected post-exposure to calculate traditional hazard metrics (i.e., LOEC and EC50). In addition, whole larvae were homogenized, and pooled homogenates were analyzed with targeted transcriptomics using Biospyder's TempO-Seq platform. The resulting transcript abundance data were analyzed using BMDEExpress3 and a custom analysis pipeline in R statistical software to obtain transcriptomic points of departure (tPODs) and a list of differentially expressed genes (DEGs). Of the 22 chemicals tested, five (PFHxS, FHxSA, N-EtFOSA-M, FC10diol, and 6:1FTOH) significantly reduced survival and 17 of the 22 had tPODs within the range of test concentrations. The tPODs were more sensitive (i.e., protective) than apical endpoints. The most potent chemical in this study was 6:1FTOH, a fluorotelomer alcohol, that had a survival EC50 of 250 ug/L and a tPOD of 148 ug/L. These data will be compared to similarly derived results for PFAS tested in other species (*Daphnia magna*, *Chironomus dilutus*, and *Raphidocelis subcapitata*) to investigate possible impacts that PFAS structural features have on toxicity as well as evaluate potential suggested mode(s) of action from the lists of derived DEGs. The present study exemplifies the possible use of high-throughput assays for transcriptomics in fathead minnow to aid in effectively screening environmental contaminants with limited data in a timely manner. *The contents of this abstract neither constitute, nor necessarily reflect, official US EPA policy.*

3.04.P-Th-058 Uptake and Elimination for a Suite of Per- and Poly-fluoro Alkyl Substances (PFAS) in a Soil-Plant-Mammal Model

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Per/polyfluoroalkyl substances (PFASs) have been linked to accumulation in soil, terrestrial plants, and mammals in previous studies with the potential for biomagnification in terrestrial food webs. We investigated the uptake and of a suite of PFAS compounds in Timothy grass (*Phleum pratense*) and kale (*Brassica oleracea*) from soil to estimate the biomagnification potential in a soil-plant-rabbit food web. Plants were grown in a modified Organisation for Economic Co-operation and Development (OECD) constructed soil amended with PFAS compounds to generate bioconcentration factors (BCF). The shoot BCFs increased as the C chain length decreased except for Perfluorobutanoic acid (PFBA) and were greater for kale than for Timothy grass. PFBA had the greatest BCF in plant shoots. Root BCFs generally increased with C chain length except for PFBA. PFAS compounds with shoot BCF values ≥ 1 were selected for the soil-plant-rabbit biomagnification study. For the biomagnification study, kale plants were grown in an environmentally controlled greenhouse in a modified OECD constructed soil amended with 0.01 mg/kg each of 11 PFAS compounds for 56 days. Kale leaves were

harvested from mature plants. Certified contaminant-screened Timothy hay was purchased and amended via atomizer with PFAS compounds at concentrations achieved in the BCF studies. Plant material was fed to Dutch-belted rabbits weekly for a 28-day uptake phase and 28-day elimination phase. The experiment was repeated two additional times to include three groups of rabbits. Accumulation of the 11 compounds in soil, plants, and rabbits in both the uptake and elimination phases of all three groups were analytically determined. The final results and statistical analyses will be reported. Ecologically relevant data developed in this research will fill several knowledge gaps regarding biomagnification potential for PFAS compounds released into the terrestrial environment.

3.04.P-Th-059 Molecular Profiling of Tree Swallow (*Tachycineta bicolor*) Nestlings Exposed to Environmental Per- and Polyfluoroalkyl Substances in Support of Adverse Outcome Pathway Development

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Lab and field studies indicate that per- and polyfluoroalkyl substances (PFAS), a group of highly persistent chemicals, impair avian reproduction. However, the molecular mechanisms and modes of action through which PFAS cause adverse apical outcomes in avian species have not been elucidated. Existing knowledge from the ECOTOX Knowledgebase and Adverse Outcome Pathway (AOP) Wiki suggest that vertebrate species exposed to various PFAS experience changes to the thyroid system, pathways controlled by estrogen, retinoid X, and peroxisome proliferator-activated receptors (PPARs), and measures of oxidative stress. Avian studies on PFAS exposure and effects are limited and are generally restricted to only 8 model species that are not representative of the diversity of birds. The objective of this research is to determine potential PFAS mechanisms and modes of action by leveraging an existing avian, field-based food-web study on the reproductive success of Tree Swallows at sites with varying levels of environmental PFAS contamination. Tree Swallow nest boxes in Duluth, Minnesota were monitored bi-weekly during the breeding season and nestlings were collected 12 days post-hatch to determine PFAS whole body residues and associated changes in specific plasma and tissue molecular profiles. Plasma thyroid hormone (TH) levels were measured using high performance liquid chromatography–tandem mass spectrometry to determine whether higher PFAS body burden correlates with aberrant circulating TH levels. Global transcriptomic profiles in liver tissue were measured using non-targeted RNA-sequencing to determine whether changes in relative transcript abundance varied with levels of PFAS body residue. Subsequent analyses evaluated the concordance between PFAS-responsive genes and pathways known to be perturbed by PFAS based on literature reviews. Results from this study will provide support for delineation of putative AOPs, molecular biomarker identification, and may provide insight into new PFAS modes of action in avian species. Understanding the linkages between PFAS exposure and the mechanisms leading to apical effects is essential for developing predictive frameworks to support risk assessment at contaminated sites with the potential for extrapolation to other vertebrate species susceptible to PFAS exposure. *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.*

3.04.P-Th-060 Effects Observed in Algae (*Raphidocelis subcapitata*) after 24-hours of Exposure to 22 Per- and -Polyfluoroalkyl Substances in a High Throughput Assay

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Per- and polyfluoroalkyl substances (PFAS) have been detected in numerous aquatic ecosystems, resulting in ongoing concern for their potential toxicity. However, except for a few well studied PFAS, hazard data required to evaluate risks that various PFAS pose 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 a

green algal species, *Raphidocelis subcapitata*. The tested PFAS included carboxylic acids, diols, a fluorotelomer alcohol, fluorotelomer sulfonates, sulfonamides, and sulfonates. Algae in log-phase of growth were exposed in a 96-well microplate format to 8 concentrations (nominally, 100 to 0.03 μM) of each PFAS, in a $\frac{1}{2}$ log dilution series. After 24 hours of exposure, several endpoints were assessed including cell density/biomass/growth rate, viability, concentration of photopigments, and photosynthetic efficiency using microplate reader-based methods. The concentrations of each PFAS were quantified at the beginning and end of the exposure. Twelve of the 22 PFAS significantly affected growth rate and/or viability of the algae after 24 hours of exposure. Benchmark effect concentrations ranged from 4 $\mu\text{g/L}$ to greater than 55 mg/L . The most potent PFAS tested was N-ethyl perfluorooctane sulfonamide (N-EtFOSA; CAS 4151-50-2), which lowered the growth rate after exposure to 4 $\mu\text{g/L}$ (0.008 μM). After 24 hours exposure on a 96-well microplate, free concentration of PFAS in the media ranged as high as 90%, for several chemicals, to as low as 1% or less with perfluorotridecanoic acid (PFTrDA; CAS 72629-94-8). In addition to the apical and biochemical measurements described above, total RNA was extracted and is being sequenced to support ongoing efforts to quantify the transcriptomic point of departure in algae after PFAS exposure. Algal toxicity data will be compared to hazard metrics for the same list of PFAS collected in similar assays with other aquatic species (a fish, a crustacean, and an insect) to provide insights into structural features that are potentially related to toxicity in aquatic species across various trophic levels. *The contents of this abstract neither constitute, nor necessarily reflect, official US EPA policy.*

3.04.P-Th-061 Exposure to Short-Chain Perfluoroalkyl Carboxylic Acids (PFCAs) Increases Northern Leopard Frog (*Rana pipiens*) Tadpole Growth and Body Condition

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Short-chain perfluoroalkyl carboxylic acids (PFCAs) are prevalent replacements for toxic long-chain PFCAs such as perfluorooctanoic acid (PFOA). As such, these compounds have been increasingly detected in the environment, which increases the risk of wildlife exposure to PFCAs. However, the effects of short-chain PFCA exposure on wildlife are relatively unknown. Therefore, our objective was to investigate the chronic toxicity and bioconcentration of two short-chain PFCAs, perfluorobutanoic acid (PFBA) and perfluorohexanoic acid (PFHxA) using northern leopard frog (*Rana pipiens*) tadpoles. We measured tadpole growth and development metrics and PFCA concentrations in tissues of tadpoles after 43-46 days of exposure to PFBA and PFHxA (as individual chemicals) at nominal concentrations of 0.1, 1, 10, 100, and 1000 $\mu\text{g/L}$. We used general linear mixed models (GLMMs) to assess treatment effects on tadpole growth metrics. Tadpoles exposed to 0.1, 1, 10, and 100 $\mu\text{g/L}$ of PFBA had significantly increased mass and body condition relative to control tadpoles. PFHxA-exposed tadpoles had significantly increased snout-to-vent length, mass, and body condition at low (0.1 $\mu\text{g/L}$) to mid (10 $\mu\text{g/L}$) exposure concentrations relative to control tadpoles. Multiquantal Jonckheere-Terpstra (MQJT) tests indicated no effect of PFCA exposure on tadpole Gosner stage of development. We detected PFBA and PFHxA in tadpole whole-body and liver tissues. Concentrations of PFCAs in liver were approximately 3X higher than in whole-body samples. Preferential accumulation of PFCAs in liver may provide rationale for reported hepatotoxicity of PFAS in amphibians. However, the measured bioconcentration factors (BCFs) for both compounds were <10 L/kg, wet weight, suggesting low bioconcentration potential for PFBA and PFHxA in tadpoles. Our results suggest that short-chain PFCAs may influence lipid metabolism and/or endocrine processes linked to amphibian development at low exposure concentrations. Overall, exposure to replacement short-chain PFCAs at ecologically relevant concentrations may influence amphibian growth and development, and further investigation is required before these compounds can be deemed safe alternatives to their long-chain counterparts.

3.04.P-Th-062 A Critical Review Amphibian PFAS Ecotoxicity Research Studies: Identification of Screening Levels in Water and Other Useful Resources for Site-specific Ecological Risk Assessments
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With the goals of aiding risk assessors conducting site-specific risk assessments at PFAS-contaminated sites, this critical review synthesizes information on the ecotoxicity of PFAS to amphibians in 10 amphibian species, and 16 peer-reviewed publications. The studies in this review consisted of spiked-PFAS chronic toxicity experiments with PFOS, PFOA, PFHxS, and 6:2 FTS that evaluated apical endpoints typical of ecological risk-based decision making (survival, growth, and development). Body mass was the most sensitive endpoint, showing clear and biologically meaningful population level adverse effect sizes ($\geq 20\%$ adverse effects). From these results, we recommended chronic no observed effect concentration (NOEC) screening levels of 620 $\mu\text{g/L}$ for PFOS and 130 $\mu\text{g/L}$ for PFOA. At or above recommended chronic LOEC screening levels of 1100 $\mu\text{g/L}$ PFOS and 1400 $\mu\text{g/L}$ PFOA, there is an increased chance of adverse biologically relevant chronic effects. Biologically relevant adverse effects were not observed for PFHxS and 6:2 FTS, so unbounded NOECs of 1300 $\mu\text{g/L}$ PFHxS and 1800 $\mu\text{g/L}$ 6:2 FTS are recommended. Screening levels for are also provided for concentration of PFAS in amphibian diet, amphibian tissue, and moss substrate. Additionally, bioconcentration factors that can be useful to predict concentrations of PFAS in amphibians using concentrations in water are recommended; these values are useful for food web modeling to understand risks to vertebrate wildlife that prey on amphibians. Overall, this study provides a guide to the wealth of ecotoxicological research on PFAS conducted by this research group and highlights the need for additional work that that would improve the understanding of chemical risks to amphibians.

3.04.P-Th-063 Measuring the Metabolic Effects of Environmental Per- and Polyfluoroalkyl Substance Exposure on Tree Swallows (*Tachycineta bicolor*) In Ovo Using Novel Field Respirometry Methods
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Most avian toxicity studies of per- and polyfluoroalkyl substances (PFAS) have investigated apical endpoints within limited species of non-wild birds in laboratories. Significantly fewer studies have investigated the effects of PFAS exposure in wild birds in natural settings. The lack of avian diversity and real-world systems in toxicity studies make accurate extrapolation of the effects of environmental PFAS exposure in wild birds especially difficult. The difficulty is compounded by confounding effects from other non-target stressors and contaminants that exist in natural environments. There is substantial evidence that several metabolic pathways are disrupted by PFAS exposure. Metabolic effects may be difficult to detect with traditional apical endpoints (e.g., LD50) but can affect the fitness of an organism, nonetheless. In this study, portable field respirometers were utilized as a novel technique to estimate the metabolic rates of wild tree swallow eggs at regular intervals throughout their incubation. Four sites with varying degrees of environmental PFAS contamination were set up with 20-50 nest boxes per site, as well as one site with high levels of PFAS and PCB contamination, offering the potential for exploration of confounding effects. We hypothesize that the metabolic rates of eggs with higher PFAS will be significantly lower than the metabolic rates of eggs with lower burdens of PFAS during the incubation period. Allometric equations and curves of metabolic rates will be constructed for each egg over the incubation period. Egg contents will be analyzed for PFAS concentrations, and metabolic allometry will be compared across all samples to determine if significant differences exist between eggs bearing varied PFAS concentrations. Any divergence point(s) of metabolic rates during the incubation period could potentially help pinpoint adverse outcome pathways in the development of eggs. The approach used in this study has the potential to be utilized for future field-based toxicity studies in areas of environmental contamination. 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.

3.04.P-Th-064 Trophic Transport Pathways for Per- and Poly-Fluoroalkyl Substances in a Terrestrial System With Historical Aqueous Film-Forming Foam Contamination

Morgan Eldridge, Adcharee Karnjanapiboonwong, William Jackson and Todd A Anderson, Texas Tech University

Among different applications, per- and poly-fluoroalkyl substances (PFAS) were used as the key ingredient in aqueous film-forming foam (AFFF) formulations for hydrocarbon fire suppression. As a result, AFFF were a source of PFAS to environments where they were applied for emergency use or fire and rescue training purposes. The objectives of this study were to determine the concentrations and the distribution of PFAS in environmental samples and attempt to understand the potential trophic transport pathways for PFAS from soil to higher organisms at sites within Joint Base Cape Cod (JBCC), MA. Surface soils, vegetation, arthropods, earthworms, and small mammals were collected at four locations. Depending on location, the sum of PFAS concentrations (Σ PFAS) were 1.6-130 ng/g in soils, ND to 739 ng/g in plant stems and leaves, ND-2,742 ng/g in plant roots, ND-1,609 ng/g in arthropods, *Zapus hudsonius* and *Peromyscus leucopus* captured at one site contained almost exclusively PFOS, suggesting that food items (phloem-fed plant tissues, earthworms) and direct soil ingestion are the most likely trophic transport pathways from soil to higher organisms for that time of year. *P. leucopus* captured at a second site contained almost exclusively PFOS in their liver; they also accumulated some PFHxS. The dominant presence of those two PFAS and the general absence of other PFAS in *P. leucopus* from this location is supportive of the idea of exposure through ingestion of soils during burrowing, nest building, and grooming.

3.04.P-Th-065 Relationships Among Structural Characteristics and Aquatic Toxicity for Per- and Poly-fluoroalkyl Substances, Part 1: Patterns in Sublethal Effects of Single Chemicals and Mixtures

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A significant challenge for ecological risk assessment of per- and polyfluoroalkyl substances (PFASs) is the existence of numerous, structurally diverse compounds, many of which have limited or no toxicological data. Understanding how PFAS toxicity varies across chemical structures and species may facilitate estimation and prioritization for data-poor chemicals and sensitive taxa. To this end, our team has conducted 7-d sublethal toxicity tests with *Ceriodaphnia dubia*, *Chironomus dilutus*, and *Hyalella azteca* for 17 PFASs of high ecological priority, including perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs), perfluoroalkyl sulfonamides (FASAs), and fluorotelomer sulfonic acids (FTSAs). Potency of the studied chemicals varied widely, with median effect concentrations (EC50s; based on reproduction or growth) ranging over five orders of magnitude, though only a few EC50s were below 0.1 mg/L (PFOS and PFNS [*C. dilutus* only], FOSA [*C. dilutus* and *H. azteca*], and FHxSA [*H. azteca* only]). Evaluating structure-toxicity patterns across species, chemical classes, and chain lengths, we propose three groups that may represent different mechanisms of toxicity: 1) that associated with the broadly similar toxicity of PFSAs to *C. dubia* and *H. azteca*, and PFCAs and FTSAs in all tested species, 2) PFSAs in *C. dilutus*, and 3) FASAs across all tested species. Our ongoing work is focused on advancing understanding of organisms and PFASs that are likely to result in the highest sensitivity. We have expanded our testing program with binary mixture studies, new species (i.e. fathead minnow), and additional PFASs with diverse structural characteristics (e.g. diols, betaines, ether linkages, cyclic compounds). A separate presentation from our team evaluates these patterns through the lens of bioaccumulation factors and resulting estimates of critical body burden. *This abstract neither constitutes nor necessarily reflects US EPA policy.*

3.04.P-Th-066 Effects of Perfluoroalkyl Substances on Amphibian Body & Hepatic Condition: Is Dysregulation of Lipid Metabolism a Driver?

Anna Grace Bushong, Tyler D. Hoskins, Abigail Corrine Valachovic, Elizabeth Allmon and Marisol S. Sepulveda, Purdue University

Per- and polyfluoroalkyl substances (PFAS) are a class of persistent environmental contaminants that have become ubiquitous in the environment, resulting in widespread exposure among humans and wildlife. Amphibians are regularly exposed in the field, making them susceptible to sublethal effects of PFAS exposure. In amphibians exposed to PFAS, body condition, measured using the scaled mass index (SMI), and hepatic condition have been negatively affected. *In vitro* and rodent studies have demonstrated that PFAS may dysregulate lipid metabolism through altering signaling cascades regulated by peroxisome proliferator activated receptors (PPARs). However, whether PPAR-mediated changes in fat stores can explain previously observed PFAS-mediated changes in amphibian SMI and hepatic condition remains underexplored. Since lipids provide a valuable fuel source for anuran metamorphosis and reproduction, understanding whether altered lipid metabolism can explain effects on SMI and hepatic condition is critical. The central objectives of this work were to (a) determine if PFAS alter lipid homeostasis and whether this correlates with changes in SMI or hepatic condition for the amphibian model, *Xenopus laevis*, (b) investigate relative expression for downstream genes regulated by PPARs that are hypothesized as mediators of PFAS-induced dysregulation of lipid homeostasis, and simultaneously (c) utilize MRM-profiling to assess the hepatic lipidome for PFAS-mediated changes in class abundance. We chronically exposed *X. laevis* to environmentally relevant concentrations of perfluorooctanesulfonic acid (PFOS, 0.5 ppb), perfluorohexanesulfonic acid (PFHxS, 0.5 ppb), perfluorooctanoic acid (PFOA, 0.5 ppb), perfluorohexanoic acid (PFHxA, 0.5 ppb), a binary mixture of PFOS and PFHxS (0.5 ppb of each), or a negative control, beginning at Nieuwkoop Faber (NF) stage 52. We sampled animals at NF 58, 62, and 66. Data collected included morphometric data (length, mass, SMI, hepatic condition), time-to-stage, genetic sex, and individual *X. laevis* livers bisected for molecular and lipidomic work. Our data will provide understanding whether environmentally relevant PFAS exposures influence development, body condition, or hepatic lipid profiles in *X. laevis*. Importantly, we will link any observed changes in lipid content or phenotypes to PPAR expression and signaling cascades, which may be an important mechanism of PFAS toxicity broadly in vertebrates.

3.04.P-Th-067 Investigating the Effects of Chronic 6:2 Fluorotelomer Sulfonic Acid Exposure on *Xenopus laevis* through Metamorphosis

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Per- and polyfluoroalkyl substances (PFAS) are emerging contaminants that are distributed across the globe and are widely used for their grease, oil, and water-resistant properties. Legacy PFAS, such as perfluorooctanoic acid (PFOA), have received much attention in recent literature. However, less attention has been given to effects of PFAS replacements for legacy compounds, such as 6:2 fluorotelomer sulfonic acid (6:2 FTS), and ecological risk remains associated across both terrestrial and aquatic taxa, especially as they degrade to more toxic terminal products. Amphibians are vulnerable to PFAS exposure, including fluorotelomers, and have high likelihood of contact in natural environments via proximity to various anthropogenic sources. In ranids and ambystomatids exposed to various PFAS, body condition, measured using the scaled mass index (SMI), was shown to be reduced. Information for effects of 6:2 FTS on amphibians is lacking, but deleterious effects have been observed in other aquatic species, ranging from dysregulated immune function and thyroid hormone transport. Using an established amphibian model, *Xenopus laevis*, the central objective of our study was to determine if and how 6:2 FTS exposure affects responses of survival, morphometric measurements (body length, body mass), developmental stage, and time-to-metamorphosis. We chronically exposed animals to environmentally relevant concentrations for impacted surface water of 6:2 fluorotelomer sulfonic acid (6:2 FTS; 1.2 ppb, 12 ppb, 120 ppb, and 1200 ppb) or a no PFAS control, beginning at Nieuwkoop Faber (NF) stage 51

until 10 days after all control animals reached NF 65. Upon reaching NF 62, morphometric data was collected (mass, SVL) and time-to-stage recorded, and then transferred to internal enclosures until lethally sampled at NF 65, recording morphometric data and time-to-stage. A subset of animals from each tank were randomly assigned for body burden/6:2 FTS metabolite analysis. Additionally, one hindlimb was taken from each animal for genetic sex. Data will allow for the development of toxicity reference values for growth and development and screening for evidence of thyroid disruption caused by aquatic exposure to a broad range of 6:2 FTS concentrations. Furthermore, our data will test whether sex plays a role in growth with any observed changes linked to potential differences in 6:2 FTS toxicity in amphibians.

3.04.P-Th-068 PFAS Mixture and Full Life-Cycle Exposures To Fathead Minnows

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Per- and polyfluoroalkyl substances (PFAS) have been introduced to the environment via industrial practices, products and application of aqueous film forming foams (AFFF). PFAS have been detected in all abiotic media and biota, becoming an emerging environmental concern. Perfluorooctanesulfonate (PFOS) and perfluorohexanesulfonic acid (PFHxS) are PFAS most frequently detected together at sites that have been impacted by AFFF release. The focus of these studies is to determine the combined effects of PFOS and PFHxS to fathead minnows *Pimephales promelas*, over critical life stages of growth and reproduction and development. Two separate experiments were carried out, the first is a reproduction-developmental study that started with sexually mature fish and the second is a full life-cycle study starting with less than 24-hour old fish, exposing them to a mixture of PFOS and PFHxS. In the repro-developmental exposure, five-month-old sexually mature fish were exposed to PFOS, PFHxS and a mixture for 42 days, eggs were collected through the study and allowed to hatch and grow for 21 days. Larval (F1) fish biomass was measured, adult fish (F0) were analyzed for brain and ovary PFAS concentrations. Larval fish experienced a greater decrease in biomass in mixtures than PFOS and PFHxS alone. Adult fish had a had measurable (and comparable) PFOS concentrations in brain and ovary tissue. To study the chronic effects given PFAS are not subjected to degradation, fish less than 24 hours were exposed for 300 days to PFOS and a low and high mixture of PFOS and PFHxS. When whole body tissues were analyzed, we found an increase in PFOS and PFHxS in mixture than PFOS alone, independent of mixture concentration. These studies will increase our knowledge of how PFAS mixtures interact and can affect organisms differently in comparison to single chemical exposures.

3.04.T Toxic Effects of Per- And Poly-Fluorinated Compounds: From the Molecular to Ecosystem Levels

3.04.T-01 PFAS Bioaccumulation and Trophic Transfer in Linked Stream and Riparian Food Webs

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Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic substances that have warranted concern due to their environmental persistence, ability to bioaccumulate, and potential toxicity. PFAS are commonly detected in aquatic ecosystems where aquatic emergent insects can serve as vectors of waterborne contaminants, such as PFAS, to adjacent riparian consumers. Despite the recent and high level of scientific focus on PFAS research, few studies have investigated PFAS trophodynamics in linked stream and riparian food webs. Here, we report PFAS concentrations, compositions, and metamorphic and trophic transfer factors for food webs representing five stream sites in the Farmington River watershed, Connecticut, USA from 2022 and 2023. Surface water, seston, detritus, biofilm, sediment, aquatic larval and emergent insects, and riparian spiders were collected and analyzed for 28 PFAS (8 short-chain and 20 long-chain). Stable isotope analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) results are forthcoming and will be used to identify diet and trophic level position. Results from the first project year show that water samples had the highest number of compounds detected and reflected potential nearby PFAS sources, such as a firefighter training site. PFAS concentrations in

sediment and aquatic insect food sources (i.e., seston, detritus, and biofilm) were highly variable and differed between sites, however, PFAS composition still reflected potential source contributions. Aquatic emergent insects had the highest mean Σ PFAS concentrations and bioconcentration factors suggested uptake of several PFAS, including PFOS and PFNA, during larval life stages. Moreover, metamorphic transfer factors indicated retention of PFOS, PFNA, and PFUnA in several insect orders. These same compounds were also detected in riparian spiders within 1 m of the stream, suggesting a risk to higher order organisms that consume aquatic emergent insects. Overall, our results suggest that PFAS are transferred from the aquatic ecosystem to the riparian food web via aquatic prey subsidies, however stable isotope analysis and data collected in 2023 will help further elucidate these results.

3.04.T-02 Relationships Among Structural Characteristics and Aquatic Toxicity for Per- and Polyfluoroalkyl Substances, Part 2: Evidence from Uptake, Bioconcentration, and Critical Body Burdens

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The large number of structurally diverse per- and polyfluoroalkyl substances (PFASs) poses a substantial challenge for ecological risk assessment. Understanding patterns of toxicity across chemical structure and species is an obvious need to allow toxicological estimation and hazard prioritization for numerous data-poor PFASs. In a separate talk, our group presents water-column sublethal effect concentrations for single PFASs and PFAS mixtures on aquatic organisms and proposes three distinct groups of chemical-species pairs that we hypothesize share common mechanisms of toxicity. In this presentation, we discuss results of whole-body tissue measurements and explore how bioconcentration and internal dose at the effect concentration (critical body burden; CBB), can provide additional insights into these groupings. Bioconcentration factors (BCFs) in *Hyalella azteca* and *Chironomus dilutus* were determined for 12 PFASs from the homologous series of perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAAs), perfluoroalkyl sulfonamides (FASAs), and fluorotelomer sulfonic acids (FTSAs). BCF increased linearly with chain length across each homologous series for both species, although times to steady state were radically different for *H. azteca* (>32 days) and *C. dilutus* (generally 1-2 days). BCF values were then used to estimate CBBs (water column effect concentration times BCF), which tended to cluster into three groups associated with: 1) the high body burden and low water-column toxicity of PFCAs, PFSAAs, and FTSAs in *H. azteca* and of PFCAs and FTSAs in *C. dilutus*; 2) the much lower body burdens and higher water-column toxicity of three PFSAAs (PFHpS, PFOS, and PFNS) in *C. dilutus*; 3) the moderate body burden and high water-column toxicity of FASAs in both species. These patterns reinforce our proposed groupings and demonstrate that the high sensitivity of *C. dilutus* to certain PFSAAs is not the result of higher accumulation compared to other PFASs but instead reflects a greater internal potency of these compounds. Analysis of chemical potency on an internal dose basis thus reveals unique features of PFAS toxicity and aids in efforts to group chemicals by common modes of action. *This abstract neither constitutes nor necessarily reflects US EPA policy.*

3.04.T-03 Characterization of the Replacement PFAS, Perfluoroethylcyclohexane Sulphonate (PFECHS) and Perfluorobutane Sulphamide (FBSA) in vitro Individually and in Mixture with Perfluorooctane sulphonate (PFOS)

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The widespread application of poly- and per-fluoroalkyl substances (PFAS) has resulted in some substances being ubiquitous in environmental matrices with associated toxic effects. While certain substances of concern have been phased-out or banned, new substances continue to be produced. Two such substances are perfluoroethylcyclohexane sulphonate (PFECHS) and perfluorobutane sulphamide (FBSA), both of which are developed analogues and replacements of perfluorooctanesulphonic acid (PFOS) which have recently been

detected in multiple environmental media around the globe. However, there is little information on the toxic potency of PFECHS and FBSA relative to legacy substances especially, but also other known PFAS replacements. Initially, this research aimed to characterize PFECHS and elucidate its effects in the aquatic environment using *in vitro* techniques. The fish liver and gill cell lines RTL-W1 and RTgill-W1 were used to predict the exposure response of PFECHS in rainbow trout, and further analyses focused on membrane effects were completed with rainbow trout leukocytes. PFECHS exposure did not result in apical adverse effects at environmentally relevant concentrations apart from decreasing plasma membrane polarity. However, molecular alterations were observed at exposure concentrations nearing those of environmental relevance, regardless of the chemical analyses that supported PFECHS is not as acutely potent, nor as potentially bioaccumulative as legacy congeners. These results raised concerns about other replacement PFAS, and FSBA was further studied in response. While FSBA was determined to have less toxic potency than PFOS and PFECHS *in vitro* using the fish gill cell line RTgill-W1, concerns surrounded how they may interact in environmental mixtures containing multiple replacements and legacy substances. Therefore, the cytotoxic and phospholipidosis effect of PFECHS and FSBA was determined individually *in vitro* in relation to PFOS before their effect in binary and tertiary mixtures was assessed *in vitro* using the equipotency framework. These experiments were also conducted alongside sub-lethal *in vivo* exposures to potentially identify the molecular basis of PFAS toxicity, and how mixtures can potentially impact the toxic pathways. Determining the toxic potency of emerging replacement PFAS is an important step that will help better inform the viability of replacements as a strategy for PFAS management in the future.

3.04.T-04 Do Perfluoroalkyl Acids Influence Reproductive Success of Tree Swallows? Going beyond PFOS and PFOA

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Bioaccumulative perfluoroalkyl acids (PFAAs) and precursors are found ubiquitously in the environment and are a large sub-group of synthetic compounds collectively known as per-/polyfluoroalkyl substances (PFAS). PFAAs have been detected in several wild bird species, however, to date, there has been limited study of the potential toxic effects of PFAAs on avian reproductive success. As we previously showed, Tree swallow (*Tachycineta bicolor*) nestlings raised near a wastewater treatment plant outfall in southwestern Ontario, Canada, had elevated concentrations of most measured PFAA compounds relative to nestlings at a nearby reference site. The objectives of this study were to 1) compare reproductive measures of tree swallows between the same WWTP and reference sites previously studied, and 2) assess potential associations between concentrations of individual PFAAs and reproductive measures, including initial lay date, clutch size, hatching success, fledging success, and nestling mass. In 2019-2022, reproductive data and tissues of tree swallow eggs (2019, 2020) and nestlings (liver: 2019, 2021, 2022; carcass: 2019, 2022) were collected, and tissues were analyzed for 18 major PFAAs, i.e., 13 perfluorocarboxylic acids (PFCAs) and 5 perfluorosulfonic acids (PFSAs). There were significant reproductive differences between sites, with swallows near the wastewater treatment plant outfall requiring more time to lay their complete clutches and experiencing longer incubation periods, than reference birds. Overall, although perfluorooctanoic acid (PFOS) was dominant in eggs and nestlings similar to the previous study, the egg concentrations of the long-chain replacement PFCAs, perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFUdA), were positively correlated with both clutch size and incubation period; as egg concentrations of PFDA and PFUdA increased, there were associated increases in the number of eggs laid per pair (clutch size) and longer incubation periods from clutch completion until hatching. In addition, PFDA concentrations in nestling livers or carcasses were significantly and negatively associated with body mass: chicks that accumulated higher PFDA levels appeared to be of lower mass. These preliminary results will be presented as well as results from additional modelling to further investigate potential influences of individual PFCAs and PFSAs on avian reproduction.

3.04.T-05 Accumulation Of Per- and Polyfluoroalkyl Substances and their Association with Immune Parameters in Juvenile Osprey (*Pandion haliaetus*) from Chesapeake and Delaware Bays

David Lee Haskins, Natalie K. Karouna-Renier, Sandra Schultz and Barnett A. Rattner, U.S. Geological Survey

Per- and polyfluoroalkyl substances (PFAS) are a class of ubiquitous, environmentally persistent compounds that pose a threat to human and wildlife health. Despite efforts to reduce the use of long-chain PFAS in industrial practices, the persistence and solubility of PFAS have led to the detection of these compounds in aquatic wildlife around the world. Osprey (*Pandion haliaetus*) are a model avian species with a documented history of serving as effective bioindicators of contamination. Here we report the first large-scale evaluation of PFAS and potential health impacts in osprey from the Chesapeake and Delaware Bays. In 2011 and 2015, we collected plasma samples from osprey nestlings throughout the Chesapeake (n = 23) and Delaware Bay (n = 28) watersheds. We quantified 40 PFAS congeners in osprey plasma. PFOS was the most commonly detected PFAS, followed by PFUnA and PFDA. In birds from the Chesapeake Bay, PFOS tended to be a higher contributor to PFAS profiles relative to nestlings from the Delaware Bay. In contrast, long-chain PFCAs such as PFUnA and PFDA comprised larger portions of total PFAS in osprey from Delaware Bay relative to birds from the Chesapeake Bay. In addition to PFAS concentrations, we will also report measures of osprey nestling immune status (haptoglobin and immunoglobulin Y) and how these responses may be associated with PFAS exposure. Overall, our findings aim to provide a more thorough understanding of PFAS presence in the Chesapeake and Delaware Bays, as well as how PFAS exposure may be associated with health impacts in raptors. *"These data are preliminary or provisional and are subject to revision. They are being provided to meet the need for timely best science. The data have not received final approval by the U.S. Geological Survey (USGS) and are provided on the condition that neither the USGS nor the U.S. Government shall be held liable for any damages resulting from the authorized or unauthorized use of the data."*

3.04.T-06 Tissue Distribution of PFAS in Wildlife Species and their Toxicological Significance: Relevance to Mustelids Evaluations

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. In terrestrial wildlife, toxicological effects can be evaluated in several ways, as an exposure that takes into account ingestion absorption and inhalation of PFAS from their environment, or via the use of tissue-based concentration data that can be related to adverse effects, these are known as Tissue-based Toxicity Values (TRVs). In the TRV approach, toxicity and tissue concentration 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 can, with varying capacities, bind to various proteins as well as to partition into different lipid classes in a species-specific manner. In addition, tissue distribution of PFAS can also vary based on the chemical structure including chain length and type of terminal group as well as with changes in environmental conditions including temperature and the presence of other chemicals. As a result, PFAS tissue distribution and their potential to adverse effects in these tissue can be species-specific. To address this issue, a critical examination of tissue distribution data from mammalian laboratory and field studies was conducted that focused on species/tissue specific differences in metabolic capacity, protein binding characteristics and lipid classes. Normalization approaches that take into about the capacity of tissues to accumulate PFAS were investigated to better understand some of the underlying factors that contribute to species related differences in tissue PFAS distribution and accumulation. Insights into how this information can be used to establish TRVs in mustelids as well as other wildlife will be discussed.

3.05.P-Mo Bridging Ecotoxicology and Risk Assessment for Aquatic and Terrestrial Plants

3.05.P-Mo-081 Comparison of Mercury (Hg) Bioaccumulation with Mono- and Mixed- *Lemna minor* and *Spirodela polyrhiza* Cultures

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Mercury (Hg) is a prevalent and harmful contaminant, persisting in soil, water, and the atmosphere. Phytoremediation can offer an eco-friendly and cost-effective solution to reducing Hg in the ecosystem. For phytoremediation purposes, it is important to discover which plants can bioaccumulate meaningful amounts of Hg while being able to tolerate its toxicity. Species of duckweed have been shown to be able to do both. Increasing biodiversity could create a more resilient and self-sustaining system for Hg remediation. This study explores whether mixed populations of *Lemna minor* and *Spirodela polyrhiza* can better bioaccumulate and tolerate Hg than monocultures of either species. Mono- and mixed cultures of *L. minor* and *S. polyrhiza* were grown in mesocosms of 0.5 ug/L or 100 ug/L Hg (HgCl₂) spiked water for 4 days. Hg concentrations in the duckweed and water were analyzed at the end of the experimental period. Change in weight of the duckweed populations was used to assess the tolerance to Hg. Diffusive gradients in thin films (DGTs) were also used as surrogate monitoring devices for bioavailable levels of Hg. For biomass growth, the only significant difference between duckweed mixes was the mixed culture of the *L. minor* being greater than the monoculture at the high dose. The *L. minor* accumulated more Hg in the mixed culture at the low dose while the *S. polyrhiza* was higher in the mixed dose at the high dose. Overall, the mixed cultures performed as well as or better than the monocultures when tolerating or bioaccumulating Hg. However, practically, the monocultures of *S. polyrhiza* accomplished the same as the mixed cultures while also being a better bioaccumulator than *L. minor*. Hg speciation in water was modeled using Windermere Humic Aqueous Model 7 (WHAM7) to compare the bioavailable species (e.g., dissolved organic matter complexes and inorganic speciation) indicated by the DGTs. Potentially due to the controlled water conditions, the WHAM7 output of bioavailable Hg was almost 1:1 to that estimated by the DGTs. In conclusion, this study showed that differences can be made by mixing species, though the mixed effects for these specific species may not be practically significant. Both DGT and WHAM7 can be applied to study Hg speciation and bioavailability in such designed studies. Increasing the length of time to examine system sustainability or mixing more different species could help further the use of phytoremediation for Hg contamination.

3.05.P-Mo-082 LED vs. Fluorescent Lighting Systems for the Culturing and Testing of *Raphidocelis subcapitata* under OCSPP 850.4500 and OECD 201

Maegan Elizabeth Teets, Smithers

Lighting type and quality is a critical variable in experiments conducted with photosynthetic organisms such as micro-algae. Algal toxicity guidelines (OCSPP 850.4500 and OECD 201) do not currently specify the type of lighting that should be used in test systems under the validity elements (acceptability criteria). The guidelines contain statements that algae should receive “continuous illumination” from “cool-white fluorescent lights” providing photosynthetically active radiation. The scope of this project is to determine the viability of using an LED lighting system to culture algae under these current guidelines. Since the finalization of the guidelines, OCSPP in 2012 and OECD in 2006, lighting technology has improved to where LED bulbs to culture algae has become a suitable option. LED bulbs offer a longer lifespan, minimizing the number of times they need to be replaced in a year and are more energy efficient than their fluorescent counterpart. Due to the wording of the guidelines, fluorescent bulbs are the light source used in most algal toxicity tests conducted at Smithers. Other studies have been published observing the growth of algae under LED systems demonstrating that LED lighting is sufficient to support growth, however these studies were not conducted under regulatory guideline test methods. It is important to ensure that changing an input that is critical for algal growth will not negatively impact future testing. The goal of this project is to verify that algal growth under LED lights is equivalent to, or an improvement on, growth observed under fluorescent lights and can reliably meet guideline performance

criteria. To evaluate the acceptability of LED systems compared to traditional fluorescent systems, the growth of *Raphidocelis subcapitata*, a species of green alga suggested in both guidelines, is evaluated in 72- and 96-hour test durations. A set of test group vessels were maintained under an LED lighting system and another set of test group vessels were maintained under a fluorescent lighting system. Endpoints evaluated were average specific growth rate, yield, and area under the growth curve. Additionally, the impact on the sensitivity of *R. subcapitata* to a reference toxicant was evaluated under LED and fluorescent systems to determine if the response is comparable. Based on the cell density at termination of these reference tests, an EC50 was determined and compared to in-house historical reference test data.

3.05.P-Mo-083 Higher-Tier Non-Target Plant Bioassays for Compost Risk Assessment

Chloe de Perre, Yunjie Ding, Travis Bui, Dave Barnekow, Brian Beato and John Marton, Corteva Agriscience

Composting is the natural process of recycling organic matter and contributes to soil health when applied in agricultural fields and gardens. Compost feedstock is diverse and may include manure and/or vegetative matter such as grass, hay, or even food scrap. When off-label herbicide use happens, residues may accidentally enter feedstock, resulting in non-target exposure when the contaminated compost is utilized as soil amendments and/or top dressing (mulching) treatments. Therefore, it is important to evaluate the persistence of herbicide throughout the composting process and assess the risk to non-target plants (NTP), should compost containing herbicide residues be applied to areas for growing sensitive plants. Non-target plant bioassays are routinely performed for herbicide product registrations and mainly follow two guidelines to evaluate toxicity of residues to seeds via soil exposure (for pre-emergence herbicide application) and to seedlings via spray application (for post-emergence herbicide application). However, the guideline NTP studies may not directly relate to plant's exposure route through compost use. Composts are almost exclusively used as mulching materials or nutrient supplements through soil incorporations, where both uses are bringing compost-inherited residues directly into soils. Therefore, the NTP study through foliar spray (vegetative vigor study) is not relevant to compost route of exposure. Three non-target plant dose-response studies were developed to generate toxicity endpoints of auxin herbicides to seeds or seedlings exposed to contaminated compost via mulching (on top of the soil) or amended (mixed) into the soil. Nuances and endpoints of these three studies will be compared to the existing guideline NTP studies for auxin herbicide examples. These higher-tier NTP bioassays result in more accurate risk assessment for compost.

3.05.P-Mo-085 Options for Refining Non-Target Terrestrial Plant Testing and Risk Assessment from Pesticide Uses

Clifford Habig, Compliance Services International (CSI)

US EPA uses the TerrPlant model to evaluate potential off-site risks to non-target terrestrial plants (NTPs) from outdoor pesticide uses. This model compares estimated exposures to non-target plants to data from standard seedling emergence and vegetative vigor testing to estimate potential risks to both endangered and non-endangered non-target terrestrial plants; potential risks are calculated as risk quotients (RQs). In these tests, NTP seeds or seedlings are treated with a single application at the maximum labeled application rate plus a series of lower rates with the goal of obtaining both an EC25 and a NOEL; these tests last for 14-21 days. Testing is generally conducted in a greenhouse, although use of small outdoor plots are an option. Endpoints from these tests include seedling emergence, seedling survival, and seedling growth (weight and length) measured at test termination. Results for the most sensitive endpoints from each test are used for NTP risk assessments. Current endpoints do not include any assessment of potential effects on plant reproduction, such as seed production, seed weight, or seed viability. These endpoints can be assessed in extended tests, either under greenhouse conditions or in small outdoor plots; preliminary work has been conducted under both conditions. There are pros and cons, or limitations, for conducting extended tests in both greenhouses and outdoor plots, which will be discussed. These include factors such as space requirements, test duration, seasonality, weather, pollination, and species selection. Currently there are no clear data on the

relative sensitivity of current NTP test endpoints compared to possible plant reproduction endpoints. The TerrPlant model uses default estimates for estimating both potential runoff exposure and spray drift exposure. Refinements for both types of potential exposure are available through refined modeling (PRZM and AgDrift), and also based on label application instructions. Some of these options will also be presented and discussed.

3.05.P-Mo-086 The Use of Remote Sensing in Assessing Risk to the Terrestrial Plant Community at Large Sites

Jennifer Holder, Melissa Sandoz, Sam Furey and Wade Brunham, ERM

Most ecological risk assessments evaluate risk to terrestrial plant communities by comparing soil chemical concentrations to soil screening benchmarks. While this is a common approach, there is significant uncertainty due to the lack of relevance of the plant species in the underlying laboratory toxicity tests to the plant communities in the field, the unknown extent to which conditions in the laboratory toxicity tests represent conditions in the field, particularly bioavailability, and the focus on effects at the organism level rather than the community level. Specifically at sites contaminated by metals, additional uncertainties include the complexities associated with exposure to mixtures of metals and that some metals are essential nutrients for plants. Field studies of contaminated sites can provide critical lines of evidence to balance these uncertainties. However, field studies with sufficient coverage and statistical power are challenging to design and costly to implement, especially at large, remote sites. The increasing availability of remotely sensed, high-resolution satellite imagery allows for new types of data that can be used in ecological risk assessment. This presentation describes an alternative landscape-level approach for evaluating potential risk to terrestrial plant communities at a large metals site in northeastern Washington state that uses remotely sensed vegetative community metrics that measure: 1) chlorophyll content (Enhanced Vegetation Index) as a surrogate for primary productivity and 2) aboveground biomass. The evaluation compared primary productivity and aboveground biomass at the site to five reference areas. Additionally, concentration-response modeling was performed to evaluate whether relationships exist between remotely sensed vegetative community metrics and soil metal concentrations found at the site. Variation across the landscape (such as dominant plant species, logging, agriculture, and wildfire history) was controlled for where remotely sensed data were available. These lines of evidence established by the remote sensing evaluation were used in conjunction with soil chemistry benchmark comparisons to evaluate potential risk to the terrestrial plant community.

3.06.P-Mo-088 Cytotoxicity and Oxidative Stress Induced by the Water-Soluble Fraction of Agricultural Soils: Evidence from South Africa

Rialet Pieters, Ilzé Horak and Suranie Horn, North-West University

In South Africa, agrochemicals are extensively applied to reduce pest-associated crop losses. However, polar pesticides reach non-target environments via spray drift, run-off and/or leaching. When fractions of agrochemical mixtures are bioavailable, they pose a threat to humans and wildlife. The current study evaluated whether the water-soluble fraction of agricultural soils in South Africa induces toxicological effects in human duodenum adenocarcinoma (HuTu-80) and rat hepatoma (H4IIE-*luc*) cell lines. Composite soil samples were collected in two maize-growing regions of South Africa where agrochemicals are extensively applied (i.e., the Mpumalanga province and Vaalharts Valley). Polar compounds were extracted from the soil using deionised water to mimic environmental conditions. The cytotoxic potential of the soils was assessed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] cell viability assay. The following oxidative stress responses were also evaluated *in vitro*: intracellular reactive oxygen species production, superoxide dismutase content, and catalase activity. Following exposure to the soil extracts (28, 83, and 250 mg/mL) for 24 (HuTu-80) and 72 (H4IIE-*luc*) hours, five of the nineteen samples caused moderate to severe cytotoxicity ($p \leq 0.05$). On the other hand, eight samples increased cell viability to above 100% ($p \leq 0.05$) indicating that compounds present in the soil potentially stimulated the metabolic activities of the cells. Overall, the soil extracts induced

oxidative stress at 83 mg/mL. Following exposure, similar oxidative stress responses were observed in the human intestinal and rat liver cells. However, the H4IIE-*luc* cell line was able to detoxify the compounds present in the samples better. This is likely due to the primary role of the liver which is detoxification of xenobiotic compounds. The findings of this study show that the bioavailable fraction of agricultural soils in South Africa potentially poses a threat to non-target organisms. Aquatic organisms are especially at risk if these compounds are transported to waterbodies following rainfall or irrigation.

3.06.P-Mo-089 Environmental DNA Analysis: Evaluating Arsenic Toxicity through *Allonychiurus kimi* (Collembola)

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Environmental DNA (eDNA) refers to the collective term for DNA present in the environment, including soil, water, and other mediums. The quantity of specific DNA present has the potential to represent the presence, and density of specific organisms. As a result, eDNA analysis is currently being extensively researched in various environmental fields. Collembola, which are widely distributed in soil ecosystems and highly sensitive to environmental changes, are commonly used as model organisms for assessing ecological toxicity. However, conventional Collembola reproduction test is often time-consuming and cumbersome. To overcome this limitation, we proposed a more efficient and rapid approach by utilizing the quantity of eDNA as a novel endpoint for analyzing contaminated soil. Using species-specific eDNA primers, we quantified the eDNA of the model organism *Allonychiurus kimi* (Collembola) in soil through real-time PCR. By determining the EC50 value based on quantity of species-specific eDNA, we were able to conduct toxicity test using less soil and a shorter time frame compared to conventional methods. The results obtained by new method were compared to those of conventional Collembola reproduction test. Our research represents the first case of applying eDNA as an endpoint in toxicity assessments using Collembola, demonstrating the strength of eDNA analysis and indicating the potential for further advancements in toxicity tests.

3.06.P-Mo-090 Refined Parameters for Bumblebee Microcolony Dietary Exposure Tests

Michael-Thomas Ramsey, Emma Danby and Kim Taylor, Labcorp Early Development Laboratories

Honeybees have long been a surrogate for other non-*Apis* bees in laboratory testing owing to the vast understanding of their biology and their compliance with a laboratory setting. Recognising the importance of bumblebees to managed and natural landscapes, there is a pronounced need for bumble bee-specific methodologies to develop a better understanding of their biology and how these bees respond to various stressors. Current testing formats reduce experimental complexity, as they limit investigations to acute exposures in adult bees, and ignore important effects related to colony health and production of new progeny. As a result, microcolony studies have grown in popularity. In this preliminary study, we explore the necessary procedures and parameters required to optimise a GLP laboratory microcolony feeding test to determine whether sugar syrup and pollen contaminated with doses of a test item could affect the food consumption, worker survival and the size of the brood, as well as cause any delay of adult emergence in the bumblebee *Bombus terrestris*. In this work we showcase the results of the 5 individual trials that led us to the optimised parameters and processes for the final study design. These trials consisted of an 85-day study concept trial, a 35-day feeding syrup trial, a 42-day toxic reference trial, a 21-day revised enclosure feasibility trial and a 42-day dietary improvement trial. Over these individual trials, conducted with the aim of minimising untreated control mortality, we successfully established the following: (1) the number of mother colonies required to produce the required amount of queenless micro-colonies, (2) the most appropriate dietary sugar solution composition, (3) the frequency at which the micro-colonies require feeding, (4) the average time duration for worker-dominance and drone laying, (5) the natural mortality of individuals kept in this setting, (6) the most effective method for diet administration, (7) The optimal nest box design and the level of intervention required to maintain hygiene

levels, (8) the sample size required at each rate to accurately determine the lethal concentration for mortality and Effect Concentration for the reproductive output following exposure to a test item in each diet.

3.06.P-Mo-091 The Mealworm Dietary Exposure Lifecycle Test: A Potential Model System in Environmental Risk Assessments

Michael-Thomas Ramsey¹, Emma Danby² and Kim Taylor³, Labcorp Early Development Laboratories

This study was undertaken to explore the necessary procedures required for conducting a higher-tiered Good Laboratory Practice (GLP) Laboratory test to evaluate the effects of a test substance in a diet on the survival and reproduction of the mealworm, *Tenebrio molitor*. *Tenebrio molitor* is a holometabolic insect that lives in flour, bran and similar products. They are being recognised more as a beneficial insect as, economically, they are among the most important species used for the large-scale conversion of plant biomass into protein. They also possess the ability to break down plastic with no ill effects or accumulation of toxic substances. They are a robust species that can thrive under laboratory conditions, which make them an ideal candidate system for EIA testing. In this work, we showcase the four experimental trials from which we successfully determined the final protocol for this test design. Each trial was conducted to determine: (1) the time taken to complete study, (2) the viability of larva in diet (test substrate), (3) the most appropriate assessment techniques, (4) time duration of each life cycle stage, (5) a procedure for egg collection and assessment, (6) the acceptable control performance criteria across each life cycle stage, (7) the rate and reference item to be used for a toxic reference, and (8) the sample size required at each rate to accurately determine the lethal concentration for mortality and Effect Concentration for the reproductive output following exposure to a test item in the substrate. We conclude this work with the results of a full test-run of the final protocol (water control and toxic reference only) that incorporated the optimised parameters determined from the four experimental trials. The exciting results obtained from this final experiment allows us to demonstrate the effectiveness of this test design and the potential of *Tenebrio molitor* as a test system.

3.06.P-Mo-092 Evaluation of Avian Dietary Consumption Rates

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A critical part of assessing risk from contaminants to birds is knowing what dietary consumption rate to use when modelling exposure. Risk assessments frequently use consumption rates that have been reported in the literature and are then applied to a species of interest. Actual measurement of avian dietary consumption in wild birds is less common than using previously reported values. The goal of this research project was to expand the avian dietary consumption database with a focus on small birds where the ingestion rates are reported to be high. The European Food Safety Authority (EFSA), in screening-level risk assessments, use ingestion rates equalling 100% of body weight for some species and food types. These values are based on energetic calculations. The question is whether this assumption is too conservative or not? Dietary consumption data from more than 300 studies of 30 species was reviewed and used to develop relationships between body weight and consumption rate. The information obtained from this review was compared with the literature EFSA Guidance on Risk Assessment for Birds and Mammals. The results of our study support the commonly held premise that smaller birds consume a greater amount of food on a daily basis as a function of their body weight. However, the amount of food consumed per day appears to be less than 50% and most often less than 20% of body weight. This is supported by previously published measurements of wild bird weights in the morning and evening. The data summaries are presented.

3.06.P-Mo-093 *Caenorhabditis elegans* as a Biological Sandbox for Evaluating the Effectiveness of RNA Interference As A Biocide

Cody Robert Lowrie¹ and Adam M. Simpson², (1)Pennsylvania State University, Erie, (2)Pennsylvania State University, The Behrend College

Ecotoxicology examines the risk that contaminants pose to target/non-target organisms and the environment.

These contaminants include insecticides, which are sprayed to combat agricultural pests; however, the effectiveness of these chemicals declines as pests evolve resistance to them. One alternative solution is the use of RNA interference (RNAi) to target and silence gene products that contribute to vital biological processes in a pest species, but it is challenging to find a reliable model upon which to test this technology. *Caenorhabditis elegans* may be an excellent option for testing RNAi due to their convenience as an established model and their conserved physiology. This study aimed to evaluate the logistical constraints of performing RNAi on two strains of *C. elegans* (wildtype and RNAi-sensitive). We quantified the success rate and duration of RNAi-induced suppression of a mobility gene (*unc-22*); RNAi was performed by plating the nematodes with a transformed bacterial lawn. The phenotype of each nematode within the bacterial lawn was scored daily for five days; individuals exhibiting the distinct “twitchin” behavioral phenotype were indicative of successful RNAi. RNAi induction occurred quickly (<48 hours) but the success rate was lower for the wildtype strain (N2; 81.2 ± 0.01%) compared to the sensitive strain (NL2099; 93.0 ± 0.03%). The disparity between strains disappeared after five days, with both strains exhibiting a success rate of approximately 78%. This simple study provides valuable information regarding the reliability of RNAi in *C. elegans* and the practicality of using this species as a model for innovative pest management practices.

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

3.07.P-Th-220 Effects of Multiple PFAS on Early Development of *Xenopus laevis*

Emma Stacy, Sigmund Degitz, Philip DeGoey and Scott Meyers, U.S. Environmental Protection Agency

As part of the US EPA’s PFAS Strategic Roadmap, the agency is committed to increasing our understanding of the potential ecological effects of per- and polyfluoroalkyl substances (PFAS). The objective of this study was to characterize the developmental toxicity of 17 PFAS chemicals in the model amphibian species *Xenopus laevis* throughout the process of organogenesis. Embryos were screened for viability at Nieukoop and Faber (NF) stage 2-4 and were placed in exposure solution starting at NF stage 8 and exposed for 96 hours. Solutions were renewed every 24 hours and chemical concentrations were measured by HPLC-MS/MS before and after renewal. 17 PFAS including multiple homologues of carboxylic and sulfonic acids, sulfonamides, and fluorotelomer sulfonates, among others, were initially screened for effects at single concentration of 5 mg/L. Of these 17 PFAS, 8 compounds showed evidence of impacts on development when compared to controls, as indicated either by increased mortality, delayed development, or malformations. These 8 PFAS were subsequently run in concentration response testing using a 50% dilution series with a high concentration of 5 mg/L. Of these 8 PFAS tested in concentration response, 1H,1H,10H,10H-Perfluorodecane-1,10-diol (FC10diol) showed 100% mortality at the top three test concentrations (5, 2.5, 1.25 mg/L), and perfluorohexanesulfonamine (FHxSA) showed 100% mortality in the top two concentrations (5, 2.5 mg/L), with significant mortality in the third concentration (1.25 mg/L), and delayed development in the fourth concentration (0.625 mg/L). No significant impacts on development were observed for the other PFAS. Overall, these assays demonstrated that while most PFAS studied had little effect on early *Xenopus laevis* development, FC10diol and FHxSA did produce significant effects albeit at concentrations well above what is observed in the environment. *The contents of this abstract neither constitute, nor necessarily reflect, official US EPA policy.*

3.07.P-Th-221 Bioaccessibility to Wildlife of Metals in Plants and Invertebrates at a Former Smelter Site

Kyle Fetters, Phyllis C. Fuchsman, Alison O'Connor and Shuo Yu, Ramboll

Wildlife risk assessments typically assume that the bioavailability of metals in ingested soil, sediment, plant tissue, or prey is the same as in the studies underlying toxicity reference values. A growing body of literature suggests that relative bioavailability can often be much less than 100%, so bioaccessibility analyses are a promising tool to develop more realistic exposure and risk estimates. *In vitro* bioaccessibility (IVBA) methods to estimate the bioaccessible fraction of total metals have been validated through *in vivo* comparisons for certain metals in soil, although comparable validations have not been performed for wildlife food items. Nevertheless,

extending bioaccessibility assessments to wildlife food has been endorsed in an expert workshop on ecological cleanup values for metals, and a variety of researchers have implemented this approach. We present bioaccessibility data for barium, cadmium, lead, and zinc in biota collected from a former zinc smelter site. Tissue types included terrestrial plant components (foliage, seeds, and berries) and aboveground and litter invertebrates. Biota tissues were analyzed using an IVBA method (USEPA Method 9200.1-86). Bioaccessibility was similar across plant tissue types, except that lead was much less bioaccessible in foliage than in seeds and berries. Metal bioaccessibility in plants was generally greater than in invertebrates (excepting lead in foliage). Lead concentrations were translated to relative bioavailability using published relationships from soil-based validations; uncertainties in this approach were assessed based on the forms of lead known to occur in tissues versus soil. For other metals, IVBA results were used directly as a proxy for relative bioavailability, as available data indicate this approach is generally conservative for the specific IVBA method used. We also review our results in the context of published data on metal bioaccessibility in biota tissue.

3.07.P-Th-222 Degradation Rates of Algal Toxins in Response to Various Environmental Conditions

Joshua Garber, Virginia Institute of Marine Science

Harmful algal bloom (HAB) toxins, or phycotoxins, are generally viewed as stable due to their ability to resist degradation during cooking and digestion of shellfish and thus can cause human poisonings through consumption of contaminated seafood such as oysters, mussels, clams, etc. Except for a handful of marine and freshwater toxins, stability of HAB toxins within the aquatic environment has not been thoroughly investigated. Additionally, research up until now has largely focused on the stability of toxins in matrices used for storage or reference standards, e.g., shellfish meat. The current study aims to fill in existing knowledge gaps by determining degradation rates in aqueous media for four phycotoxin classes that impact human or animal health: okadaic acid, pectenotoxins, yessotoxins, and azaspiracids. Three of the four toxins degraded in buffered water at pH 8 with salinities ranging from 5 - 30, while one phycotoxin, okadaic acid, was stable over the experimental period of 60 h. Two of the four toxins demonstrated differences in degradation in response to salinity. Pectenotoxin-2 was most susceptible to changes in salinity, undergoing little degradation at salinity of 5 and exhibiting increasing degradation rates with higher salinity. Yessotoxin produced similar notable results with changing degradation rates in response to salinity but not as dramatic as pectenotoxin-2. Finally, azaspiracid-2 was the least susceptible to salinity changes resulting in little degradation across the varying salinities. Pectenotoxin-2, yessotoxin, and azaspiracid-2 also degraded in water (salinity = 30) buffered at either pH 6, 8, or 10 with no noticeable difference in rates between treatments. Okadaic acid was again stable throughout the 60 h experimental period. Measuring chemical aqueous stability under varying environmental conditions (i.e., salinity, temperature, pH, etc.) will help to elaborate phycotoxin persistence, distribution, and fate within aquatic systems and under future climate scenarios.

3.07.P-Th-223 Perfluoroalkyl Substances (PFASs) in Seabird Eggs from Canada's Pacific Coast: Temporal Trends (1973–2019) and Interspecific Patterns

Robert Kesic¹, John E. Elliott², Kyle Elliott³, Sandi Lee² and France Maisonneuve², (1)Simon Fraser University, (2)Environment and Climate Change Canada, (3)McGill University

Spatial and temporal trends of perfluoroalkyl substances (PFASs) have been well documented in marine food webs in the Atlantic, Indian, and Arctic oceans. However, much less is known about long-term temporal trends of PFASs in the eastern North Pacific Ocean, a region that remains largely understudied in coastal North America and is often subject to long-range transport (LRT) of PFASs and other contaminants from various North American and Asian sources. Here, we examined longer-term temporal trends (1973-2019) of 4 PFASs and 13 PFCAs, as well as stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), in the eggs of 4 seabird species sampled along a nearshore-offshore gradient; double-crested cormorants (*Nannopterum auritum*), pelagic cormorants (*Urile pelagicus*), rhinoceros auklets (*Cerorhinca monocerata*), and Leach's storm-petrels (*Hydrobates leucorhous*) from the Pacific coast of British Columbia, Canada. PFOS was the most abundant

PFSA (79-94%) detected, with the highest concentrations, on average, measured in auklet eggs (mean PFOS = 58 ng g⁻¹, range = 10.8-286 ng g⁻¹ ww) collected from relatively remote colonies off Vancouver Island. PFUdA and PFTriDA were the dominant long-chain PFCAs (≥30% combined). The majority of PFSAs (including PFOS) are significantly declining ($p < 0.001$) in the eggs of all 4 species with half-lives ranging from 2.6-7.8 years. Concentrations of long-chain PFCAs exhibited a trajectory comprised of linear increases and second-order declines in recent years, suggesting that the rate of uptake of these PFCAs is slowing or levelling off. These trends are consistent with the voluntary ceased production of PFSAs and their precursors by 3M circa 2000-2003 and are among the first from Western Canada to suggest a positive response to recent legislative restrictions on PFCAs from facility emissions and product content.

3.07.P-Th-225 Can Illicit Drugs be Detected in Predators? A Case Study for the Detection of the Contaminant Methamphetamine and its Metabolites in *Alligator mississippiensis*

Terri Cox, Sarah Nash, Brandon Gross, Rameshkumar Angappan, Seenivasan Subbiah, Jordan Crago and Lou Densmore, Texas Tech University

The Gulf of Mexico is a vast area that includes many different habitats such as mangroves, estuaries, and swamp lands. This area includes both highly urbanized areas and more remote areas. Many studies of environmental contaminants cover substances such as pesticides, pharmaceuticals, and personal care products. However, in more recent years a new class of contaminant is starting to surface: illicit drugs. This study focuses on the detection of such contaminants, specifically amphetamine, MDA, MDEA, MDMA, and methamphetamine, in the keystone species *Alligator mississippiensis* collected from the Houston, TX area and the Rockefeller Wildlife Refuge in Grand Chenier, LA. *A. mississippiensis* are of particular interest as they are not only apex predators, but they are considered environmental indicators as well as trophic regulators. Given that this species is a highly opportunistic predator, it is suggested that there may be a potential of this chemical transferred to this apex predator through environmental exposure and/or trophic transfer through contaminated prey items. This study utilizes tissues liver and scutes collected from alligators in the Houston, TX area and the Rockefeller Wildlife Refuge in Grand Chenier, LA. Tissues were homogenized and processed using QuECHERS salt extraction methods. Chemical analysis using liquid chromatography-mass spectrometry (LC-MS) indicates that amphetamine, methamphetamine, MDEA, MDA, and MDMA were found in alligator liver and scute tissue at both locations. Statistical analysis yielded significant differences between tissue types sampled from the Houston area for all contaminants analyzed in this study. This study highlights the utility of predators as sentinels for emerging contaminants such as amphetamine, methamphetamine, and associated metabolites as well as demonstrating that these contaminants can be detected in predators *A. mississippiensis*.

3.07.P-Th-226 Emerging Pollutants and Stress Biomarker Response in Stranded Cetaceans in the Western Mediterranean Sea

Emma Martínez-Lopez, Sara Salcedo, Gabriel López-Berenguer, José Peñalver, Pedro Marin, Elisa Escudero, Verónica Hernandis and Silvia Martínez-Subiela, University of Murcia

The quality of aquatic environments is currently compromised by the input of pollutants mainly from anthropogenic activities. Pharmaceuticals, such as non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics, have been considered as such in the last two decades due to their presence, abundance and potential effects on aquatic ecosystems. This has led to emerging concerns about possible harmful effects on the ecosystem, non-target organisms and human health. Fishes are among the most widely used bio-indicators in the aquatic ecosystem. However, from a ONE HEALTH perspective, marine mammals, including cetaceans, possess a number of particular characteristics that make them as well suitable indicators of change in the marine environment and sentinels for public health and the health of our oceans. During the detoxification process, some drugs can be bioactivated or unbalance the redox cycles, altering the antioxidant systems and thus producing an accumulation of dysfunctional proteins, products of lipid peroxidation. This study evaluates the presence of NSAIDs, such as ibuprofen and diclofenac, as well as different antibiotics, azithromycin,

ciprofloxacin, clarithromycin and erythromycin in internal tissue samples (liver, muscle and kidney) from 60 cetaceans stranded off the coast of Murcia (southeastern Spain) in the period 2009-2022. In addition, it has investigated the response of stress biomarkers to exposure to these compounds in these species. Specifically, the enzyme activities catalase, glutathione peroxidase and glutathione reductase, the degree of lipid peroxidation, paraoxonase 1 and cholinesterase together with certain relevant indicators of hepatic stress, and the enzyme lactate dehydrogenase, vital for muscle physiology under conditions of chemical stress. In addition to being pioneering, the data obtained allow us to detect possible subtle changes due to exposure to these emerging pollutants in endangered species and will add extraordinary value to the risk map of pollutants in the western Mediterranean sea.

3.07.P-Th-227 Temporal and Spatial Comparison of Mercury Bioaccumulation in the Lower Trophic Levels of a Post-Glacial Lake Food Web

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Total mercury (Hg) concentrations and carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes were quantified among aquatic invertebrate and sediment samples collected from Keuka Lake in New York's Finger Lakes region to evaluate temporal and spatial variability in Hg bioaccumulation and trophic ecology among these lower trophic levels. Hg concentrations ranged from 6.3 – 158.8 ng/g (dry wt) across dreissenid mussel, zooplankton, and juvenile (< 10 mm) and adult (\geq 10 mm) mysid shrimp (*Mysis diluviana*) samples. Hg concentrations were generally higher in 2015 collected samples relative to 2022 collections ($p < 0.001$) which may reflect high ice-cover conditions during the 2014-2015 winter. Trophic positions as inferred by $\delta^{15}\text{N}$ were represented by adult mysids > juvenile mysids > large zooplankton ($\geq 500 \mu\text{m}$) > dreissenid mussels \geq small zooplankton (64 – 500 μm). Significant differences were determined for the regression slopes describing the relationships between sample Hg concentrations and $\delta^{15}\text{N}$ values across the lake's three basins ($p = 0.028$). Biota sediment accumulation factors ranged from 0.2 – 2.3 and were highest for adult mysis and differed significantly across the basins and sampling years. These results demonstrate local differences in Hg bioaccumulation among Keuka Lake's invertebrate community that are likely a contributing factor to the variable nature of Hg bioaccumulation across freshwater food-webs.

3.07.P-Th-228 Comparative Reproductive and Developmental Effects in Mice Exposed to a Per- and Polyfluoroalkyl Substance Containing Aqueous Film Forming Foams and a PFAS-Free Firefighting Foam

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Although replacements for per- and polyfluoroalkyl substances (PFAS)-containing aqueous film-forming foams (AFFFs) should have reduced persistence and bioaccumulation by design, their toxicity has not been extensively evaluated. As such, a variety of studies with a suite of candidate products are underway, including combined repeated-dose toxicity studies and reproductive and developmental toxicity screening tests with CD-1 mice. Herein, developmental data are selectively presented from mice exposed gestationally, via lactation, and post-weaning to Buckeye Platinum Plus C6 MILSPEC 3% (PFAS-containing) and National Foam Avio^{F3} Green KHC 3% (PFAS-free). Prior to weaning at postnatal day 21 (PND21), endpoints such as litter weight, pinna unfolding, eye opening, and anogenital distance (AGD) were observed. Starting at PND22, selected F1 animals were dosed also via oral gavage, and body weight gain and attainment of puberty via vaginal opening (VO) and balanopreputial separation (PPS) were assessed. These endpoints are especially valuable for the comparison of replacement products and for informing decision-makers throughout the transition from use of PFAS-containing AFFFs, especially given that reproduction and development are impacted in some species exposed to PFAS.

3.07.P-Th-229 Using Growing Brood Patch Feathers to Measure The Exposure In Breeding Leach's Storm Petrels To Polycyclic Aromatic Compounds In Atlantic Canada: First Results and Current Perspectives

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In Atlantic Canada, the breeding population of Leach's storm-petrels (*Oceanodroma leucorhoa*) has declined by about 54% since the 1990s. This decline is likely due to the combined effects of climate change, community modifications, and light and chemical pollution. Concerning the latter, the exposure of these seabirds to polycyclic aromatic compounds (PACs) remains unknown. Exposure to PACs is associated with several deleterious effects in birds, and these compounds are continuously emitted in the marine environment, particularly through oil pollution and combustion. The objective of this study is to investigate local exposure of Leach's storm petrels to PACs by measuring concentrations in growing brood patch feathers collected from adults breeding at four colonies in Atlantic Canada. We expected concentrations to vary between colonies, based on their different foraging ranges. In 2022, we collected feathers from individuals breeding on Kent Is, NB (n = 11), Country Is, NS (8), Gull Is, NL (7), and Baccalieu Is, NL (6). PACs were extracted from feathers through pressurized fluid extraction, without prior rinsing, and quantified by gas chromatography-tandem mass spectrometry. While a total of 54 PACs were analysed, 40 were quantified in at least 50% of samples and 16 were quantified in all the samples. The mean concentration (\pm SD) was 25.3 ± 19.2 mg/g for the sum of the alkylated light weighted (LW) PACs, 14.2 ± 12.0 mg/g for the alkylated heavy weighted (HW) PACs, 1.0 ± 1.0 mg/g for the parent LW-PACs, and 0.1 ± 0.4 mg/g for the parent HW-PACs. We found no significant difference in the PAC concentrations (total and groups) among colonies. Interestingly, the proportion of alkylated LW-PACs was significantly greater in feathers sampled on Kent Is ($F = 3.95$, $p = 0.02$), whereas the proportion of alkylated HW-PACs was smaller there compared to the other colonies ($F = 7.51$, $p > 0.001$). This indicates a difference in local sources of emission between Kent Is and the other sites. Our data show wide interindividual variations in exposure to PACs, which suggests that individual foraging movements affect this exposure. Further analyses of PACs in brood patch feathers and liver collected on petrel carcasses in fall 2019 will allow assessment of the association between exposure to PACs and bioaccumulation. This study is a step toward the development of a minimally invasive biomonitoring tool for seabird species that are at risk of oil pollution in their breeding area.

3.07.P-Th-230 Non-testing Methods for Estimating Amphibian Toxicity Values

Lennart Weltje, BASF SE

This contribution describes non-testing approaches for estimating amphibian toxicity values that can be used in environmental risk assessment. The methods comprise quantitative structure-activity relationship (QSAR) and interspecies correlation estimation (ICE) models, and the use of surrogate values. These methods rely on available information and thus contribute to avoiding additional testing on vertebrate animals. QSAR models require a substance's molecular structure, ideally with an experimental K_{ow} value. Amphibian specific QSARs for certain groups of chemicals have been published, while many QSARs are available to predict a fish LC_{50} value, e.g., the ECOSAR module in EPIWIN. The fish LC_{50} values could be used as a surrogate for aquatic amphibian life stages, because fish and amphibian LC_{50} s are highly correlated. If experimental acute fish data are available, they can be either used directly for the reason mentioned above, or for calculating a tadpole LC_{50} by using ICE models (e.g., Web-ICE by US EPA). QSAR and ICE approaches are for aquatic life stages, but an ICE model was also developed for acute dermal toxicity in terrestrial life stages, based on fish toxicity and fish bioconcentration data. Finally, although not a robust predictive approach, the lowest acute bird or mammal oral acute LD_{50} value can be used as a surrogate for oral acute toxicity in amphibian terrestrial life stages. All currently available approaches are for acute toxicity and most focus on aquatic life stages. No methods are

available for chronic toxicity. However, aquatic data comparisons suggest that NOECs from chronic fish studies could be used as surrogate for chronic toxicity in aquatic amphibians.

3.07.P-Th-231 Considerations in Applying Avian Egg Injection Studies to Characterize Effects of Hydrophobic Organic Compounds on Wild Birds

Shuo Yu, Sydney Kruse and Phyllis C. Fuchsman, Ramboll

Egg injection has been used for decades to determine embryonic mortality and developmental effects of chemical exposures in birds. Specific egg injection methods affect how well these studies replicate the process of chemical delivery to the embryo via maternal deposition, yet few data are available to compare exposure-response relationships between egg injection and maternal transfer studies. This information gap creates uncertainty when considering egg injection studies for assessment of risks or injury in wild birds. Focusing on hydrophobic organic compounds, we evaluated avian egg injection methods and potential methodological artifacts, through critical literature review and analyses of egg injection and maternal transfer data. Parameters that can affect embryotoxicity outcomes in egg injection studies include injection site, incubation orientation, type of vehicle, injection timing, injection volume, and different combinations of these parameters. Data to quantitatively compare embryotoxicity between egg injection and maternal transfer studies in the same species were identified only for a few polychlorinated biphenyl (PCB) mixtures and only for two egg injection methods, while comparisons among additional egg injection methods (without comparable maternal transfer toxicity data) were possible for certain PCB and dioxin congeners. Additionally, chemical uptake patterns in eggs and embryos were reviewed for different study designs. Overall, one egg injection study demonstrably replicated the exposure-response relationship from a comparable maternal transfer study, while 50% lethal doses varied up to 8-fold for the same congener across egg injection methods. Risk assessors should carefully consider the potential for over- or underestimation of toxicity when applying egg injection toxicity data, and researchers should prioritize realistic representation of wild bird exposures when designing and standardizing egg injection protocols.

Track 4: Chemistry and Exposure Assessment

4.01.P-Mo Advanced Non-Target Analysis, Bioassays/Biosensors and Assessment Tools to Monitor and Respond to Emerging Threats in Wastewater Management

4.01.P-Mo-094 Interagency Coordination Team on Non-Targeted Analysis and Environmental Biomonitoring

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In alignment with the 2020 National Defense Authorization Act (NDAA), a robust multi-agency collaboration has been established to address the challenge of Contaminants of Emerging Concern (CECs) within the environmental spectrum. This led to the formation of an CEC interagency working group (IWG) subsequently restructured into the National Science and Technology Council (NSTC) Strategy Team (ST) under the Joint Subcommittee on Environment, Innovation, and Public Health (the “JEEP”) in 2021. The NDAA charged the Office of Science and Technology Policy to lead the ST and develop a National Emerging Contaminant Research Initiative (NECRI) - delineating a comprehensive federal research strategy to address CECs. The NECRI report published in August 2022 and outlines five key research objectives: (1) Decrease the time from identification to risk mitigation; (2) promote technological innovation in tools to discover, track, and mitigate; (3) develop and deploy tools and approaches for decision making; (4) coordinate transdisciplinary research activities among Federal and non-Federal partners; and (5) foster transparency and public trust when communicating about these issues. Advancement in these areas will bring us a step closer to the universal goal of clean water. To expedite the implementation of the NECRI research strategy, three coordination teams have

been established: 1) Non-targeted Analysis and Environmental Biomonitoring (NTA/EBM); 2) Risk Characterization; and 3) Joint Solicitations. These teams, comprising members from multiple Federal agencies are dedicated to documenting existing research, identifying best practices, and devising strategies for harmonizing future activities. Though the immediate objective is to optimize existing agency budgets and avoid duplication, these activities aim to foster novel research initiatives that significantly enhance our understanding of CEC impacts on human and environmental health. By doing so, we strive to create a more efficient, coordinated, and transparent approach to managing emerging environmental contaminants.

4.01.P-Mo-095 The Role of Sorption in the Fate of Organic Contaminants in Onsite Wastewater Treatment Systems

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Onsite wastewater treatment systems introduce trace organic contaminants (TOrcs) to the subsurface and other water resources including drinking water. Widespread nutrient pollution of groundwater and surface waters has urged the development of alternative onsite wastewater treatment systems, particularly in coastal areas like Long Island, NY. Despite new developments, attention is rarely focused on the fate of TOrcs in these systems. Nitrogen removing biofilters (NRBs) are passive sand- and woodchip-based treatment systems that have shown promise not only in mitigating nutrient loading but also in removing some pharmaceuticals and personal care products (PPCPs) from domestic wastewater. While environmental and physicochemical properties can inform TOrc fate, predicting the fate of TOrcs in NRBs is difficult due to variability of wastewater and the complex biogeochemical environment. TOrcs can be transformed by biological or abiotic transformation or may be retained via adsorption, in which case TOrcs may accumulate over time if recalcitrant to degradation. In this work, the fate of sixteen well-removed PPCPs was studied by extracting aged NRB media from both a full-scale system and column-scale experiment to determine the extent of sorption and make interpretations about the role of TOrc biotransformation in NRBs. Cores were collected from a full-scale NRB that was operational for four years at the Massachusetts Alternative Septic System Testing Center. Column samples were collected from a prior experiment where sand columns were dosed with PPCPs in tap, synthetic, and raw wastewater. Ten PPCPs were detected in full-scale NRB cores. Depth profiles of PPCP concentrations show that sorption plays a key role in the removal of compounds like diphenhydramine, but less so for compounds such as acetaminophen and caffeine. Mass balance estimates suggest that biotransformation plays a greater role when columns are dosed with real and synthetic wastewater as opposed to PPCP-fortified tap water. This work provides essential information regarding the accumulation and transformation of PPCPs in NRBs. Future work aims to focus on identifying transformation products of well-removed compounds that were undetected or minimally detected in the sorbent samples described here.

4.01.P-Mo-096 Characterizing Variability in Wastewater Effluent and its Effects on Wastewater Reuse for Irrigation

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Increasing water scarcity and aging wastewater infrastructure are major challenges in the US and throughout the world. Water scarcity affects supplies for drinking water as well as irrigation for crops and landscaping. Wastewater reuse for irrigation is a commonly proposed strategy to combat water scarcity, and there is ongoing work to develop advanced water treatment methods for complete contaminant removal prior to reuse. However, these technologies are unlikely to be widely implemented in the near future. Most wastewater treatment plants in the US only provide secondary treatment, and many facilities treat a large amount of stormwater in addition to municipal wastewater, leading to high variability in the composition of the effluent. In this phase one of project, we examined the chemical fingerprint of wastewater effluent collected from a plant providing secondary treatment in a catchment with a partially combined sewer system, and compared the organic and

inorganic contaminants present in samples from wet and dry weather. In phase two, we are using wastewater effluent collected in wet and dry weather to irrigate zucchini plants and are assessing the effects of irrigation treatment on plant health, zucchini production, and contaminant accumulation. Additionally, we are investigating the relationship between the chemical fingerprints of the irrigation water and the zucchini. Our results work towards building an understanding of how variation in wastewater effluent quality affects its potential to be safely reused.

4.01.P-Mo-097 Evaluating Predictive Relationships Between Estimated Percent Accumulated Wastewater and Bioactive Contaminant Loading to Surface Waters Receiving Wastewater Treatment Effluents

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Although wastewater treatment facilities have dramatically improved water quality nationwide, wastewater effluents continue to be a significant source of anthropogenic contaminants to many aquatic ecosystems. The South Platte River near Denver, Colorado is representative of urban streams which, at least during parts of the year, are an effluent-dominated system. This can result in high concentrations of bioactive anthropogenic contaminants in certain reaches of the river as well as certain times of the year. Direct monitoring of chemical concentrations or bioactivity as a function of both time and space can be expensive and difficult to implement. Here we considered the use of concentration-response modeling of varying dilutions of final treated effluent, along with application of a hydrologic model to estimate stream reaches or seasons of the year when effluent contaminants or bioactivity may exceed pre-defined benchmarks. Final treated effluent was collected directly from the plant. The effluent was diluted (100%, 85%, 70%, 55%, 40%, 25%, 10%, 0% effluent) in corresponding upstream surface waters as well as reference deionized water. Each aqueous dilution was extracted via solid phase extraction, and then evaluated using an in vitro assay designed to detect estrogen-receptor agonist activity. A concentration-response curve based on the diluted wastewater effluent was established and preliminary results suggest the effluent would need to be diluted to less than 3% effluent to keep total concentrations of 17beta-estradiol (E2) equivalents in the receiving water less than 2 ng/L. However, ignoring the E2 present in upstream water, the effluent would only need to be to about 5% to stay below a benchmark concentration of 2 ng/L limit. Comparison to a hydrologic model of the system can now be used to identify reaches of the stream and periods of the year when percent accumulated wastewater in the stream is expected to exceed 3-5%. With appropriate caveats and assumptions, the approach may be viable for estimating impacts of changes in effluent quantity or quality or changing hydrologic conditions on the loading of bioactive contaminants to receiving waters. *The contents of this abstract neither constitute, nor necessarily reflect US EPA policy.*

4.01.P-Mo-098 Screening Water Quality in Mexico using the *Allium sp.* Bioassay

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Water quality is a globally important issue, considered among the UN Sustainable Development Goals (SDGs). Anthropogenic activities and poor management had led to surface and groundwater pollution, with the contribution of a great diversity of potentially toxic substances, which cannot be fully quantified due to limitations of time, budget and availability of specialized facilities and equipment. Given this scenario, the application of procedures that allow screening for the toxic potential of water samples is a good alternative for monitoring and detecting hot spots that require a complete contaminant analysis. The *Allium sp.* bioassay (toxicity tests using common onions) offers this possibility, due to its simplicity, low cost, and sensitivity to cytotoxic and genotoxic agents. In this study, the feasibility of applying the *Allium sp.* bioassay for monitoring water quality in Mexico was evaluated, carrying out tests on samples with different types and degrees of contamination. Water was collected at three sites in the upper course of the Lerma River, Estado de México: one with strong domestic

contamination, another after industrial discharges, and the third with water considered clean. Also, three groundwater samples from wells with saline intrusion in Sisal, Yucatán were included. Cytotoxic and genotoxic effect of the pollutants present in the water was assessed by recording changes in growth, mitotic index, and prevalence of nuclear aberrations in the roots of the onions selected for the bioassay. Additionally, levels of organic persistent pollutants, volatile organic compounds and caffeine (as a chemical tracer of urban wastewater inputs) in the water samples used for the bioassay were determined, in order to evaluate possible relationships between the content of contaminants and the effects on onions. The results obtained presented differences related to the sampling area and the presence of pollutants. The contaminated samples from the Lerma river presented the lowest mitotic indices and the highest prevalence of nuclear aberrations. The “clean” river water showed similarity to the saline-influenced well samples, but was significantly different from the negative control. The *Allium* sp. bioassay provides important toxicological information that allows the identification of sites that will require a deeper environmental study due to the presence of potentially genotoxic and cytotoxic compounds in the water that could represent an ecological and health risk.

4.01.T Advanced Non-Target Analysis, Bioassays/Biosensors and Assessment Tools to Monitor and Respond to Emerging Threats in Wastewater Management

4.01.T-01 Impact in Soil and Biosolids Extraction Methods on Nontarget Organic Contaminant Analysis

Younjeong Choi, Rodrigo Alvarez Ruiz and Linda S. Lee, Purdue University

Nontarget analysis for screening organic contaminants has garnered interest with the development of high-resolution mass spectrometry (HRMS), which enables the utilization of screening power for prioritization and facilitates information-rich data generation. This approach also helps overcome the challenge of limited reference chemicals for unknown compounds and transformation products. While significant progress has been made in instrumental and analytical workflows, data processing methods, and data assessments, less attention has been given to how the sample preparation method may impact the HRMS data acquired and subsequent screening results. In this study, we compared the effect of different sample preparation methods on chemical detection. Sample preparation variables included the use of different extracting solvents and subsequent matrix clean-up steps. For matrix removal after solvent extractions, we utilized Solid Phase Extraction (SPE) with a Hydrophilic Lipophilic Balanced (HLB) copolymer. Alternatively, we used a lipid-removing dispersive SPE method for matrix removal, which is known to be effective in fat-rich samples, making it applicable to biosolids. The detection of chemicals resulting from these diverse sample treatment approaches was evaluated in light of compound properties such as solubility and sorption affinity with classification into chemical groups or classes.

4.01.T-02 Data Science Enhanced Non-Targeted Analysis for Characterization of Exposome: Move Beyond Structural Elucidation

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Resolving the human and environmental exposome is an extremely challenging task due to the complexity of their chemical space (e.g. the number of potential structures). Liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS) is one of the most dominant and comprehensive techniques for non-targeted analysis (NTA). This combination has been widely employed for tackling the complexity of exposome. The NTA approaches, even though powerful, are limited to the identified portion of samples. For vast majority of the chemical constituents in the samples, no inferences regarding their toxicity and fate can be made as they remain structurally unknown (i.e., unidentified). Here we report the development of a set of data science and computational mass spectrometry tools that use the acquired MS/MS information to infer about the environmental fate and biological activity of structurally unknown chemicals. Both toxicity categories and the ionization efficiencies of the unknown chemicals were predicted based on the MS² spectra. This information

resulted in a quick and relatively accurate estimation of the risk for the chromatographic features, without the chemical identification bottleneck. Additionally, using the MS/MS information a model was built to predict the retention behavior of structurally unknown chemicals. This enabled the alignment of LC-HRMS chromatograms, thus providing the means to perform trend analysis across different chromatograms acquired using different methods. Finally, the network analysis enabled the clustering of the chemical structures that could potentially go through similar reaction mechanisms in the environment, thus providing insights into transformation products of structurally known and unknown chemicals.

4.01.T-03 Trace Organic Contaminant Removal from Municipal Wastewater by Styrenic β -Cyclodextrin Polymers

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Trace organic contaminants (TrOCs) present major removal challenges for conventional and advanced wastewater treatment. TrOCs such as pharmaceuticals, pesticides, personal care products, and per- and polyfluoroalkyl substances (PFASs) are associated with chronic toxicity at ng L⁻¹ exposure levels and should be removed from wastewater to enable safe reuse and release of treated effluents into the environment. Established adsorbents, such as granular activated carbon (GAC), exhibit variable and often insufficient TrOC removal, and are prone to fouling by wastewater constituents. These shortcomings motivate the development of novel adsorbents that are selective towards target contaminants and maintain robust performance in complex water matrices. Crosslinked β -cyclodextrin (β -CD) polymers are promising adsorbents with demonstrated removal efficacy for TrOCs from contaminated water sources. Here, we report a simplified and potentially scalable synthesis of a porous polymer comprised of styrene-linked β -CD and cationic ammonium groups through free radical polymerization. Batch adsorption experiments demonstrate that the β -CD polymer exhibits nearly complete removal of selected TrOCs with less adsorption inhibition than GAC in wastewater matrices. The styrene-linked β -CD polymer also exhibits fast adsorption kinetics, high adsorption affinity, and was regenerable by a simple solvent wash. Rapid small-scale column tests (RSSCTs) show that the β -CD polymer exhibits later breakthrough times compared to GAC and ion exchange resin. These results demonstrate the potential for β -CD polymers to remediate TrOCs from complex water matrices, and we anticipate their continued development and further scale-up and evaluation in larger engineered systems.

4.01.T-04 Operational Invertebrate Behaviour Videotracking for Chemical Identification and Real-Time Wastewater Surveillance and Management

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As availability of water resources decreases, the apparition of new contaminants of emerging concern identified in water networks continues to increase. Current wastewater monitoring of contaminants in treatment plants (WWTP) relies on interval based grab sampling measurements, which omit both a vast proportion of micropollutants and CECs as well as the high temporal variability associated with CEC discharge concentrations. The objectives of this communication, by means of our real-time approach to WWTP biomonitoring using the videotracking device ToxMate, are 1/ to show how we defined locomotor behavioural fingerprinting for micropollutants and emerging contaminants based on data accumulated for controlled exposure to 80 compounds ; 2/ to illustrate how this method has been applied at long-term WWTP monitoring sites to offer information on contaminant discharges to receiving water bodies and identify upstream sources of contamination. The tracking of organism movement, specifically avoidance behaviour, is carried out in the ToxMate device used for videotracking of locomotor behaviour of three species of invertebrate (Gammarus, Erpobdella, Radix). Organisms were exposed to chemicals either in a closed water circuit for laboratory experiments (for a range of classes of micropollutant) or a diluted bypass of the WWTP effluent (for urban and

industrial WWTPs across France and Switzerland). The behavioural time series data was effectively modelled with multivariate functional data analysis for fingerprint definition. Initially, good fingerprint discrimination was observed for a subset of contaminants with K-Means clustering, which has recently been broadened to the full dataset, showing distinct clusters of chemicals with similarity in behavioural response. This method was extended to the on-site WWTP data, using events collected at moments of naturally occurring micropollutants discharge, which in the case of a recently analysed industrial site showed how upstream pollution events were grouped to help identify sources of contamination. Laboratory results show that contaminants can be linked even in the case of novel emerging contaminants, while on-site use of fingerprinting to identify recurring and similar events at WWTP effluents has not only allowed clients to better understand sources of pollution, but also offers concrete indicators in critical moments of discharge for the suitability of relevant applications such as water re-use.

4.01.T-05 Non-targeted Analysis to Identify Unknowns and Prioritize Monitoring of Emerging Contaminants in San Francisco Bay

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Over the past decade, the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) has invested considerable resources in non-targeted analysis (NTA) of multiple Bay matrices as a tool to inform follow-up targeted analysis and risk screening studies. Via NTA, the RMP has tentatively identified hundreds of contaminants in Bay water, sediment, and biota, as well as municipal wastewater effluent and stormwater runoff. These efforts have helped to identify urgent science priorities and have directly led to targeted studies addressing the most readily identifiable compounds, such as tire-derived contaminants and ethoxylated surfactants. To maximize the utility of the information the RMP can obtain from NTA studies, several NTA datasets were further assessed to identify additional emerging contaminants that could be high priorities for further study in the Bay. As expected, many abundant compounds were observed in water and sediment that did not match curated mass spectral libraries, consistent with the presence of unanticipated, poorly-known organic compounds in highly urban environments. However, hundreds of compounds were annotated at the chemical structure level by high-confidence spectral matches to compounds present in libraries of high resolution tandem mass spectra. Many of the compounds thus annotated are already the subject of RMP targeted monitoring, confirming that current Bay monitoring sufficiently captures many priority contaminants. The remaining high-confidence annotated compounds were screened for information regarding potential sources and ecological concerns via comparison with NORMAN databases to identify compounds observed by other NTA practitioners around the world and to obtain available toxicity information for ecological risk screening. In addition, relative abundances, distribution patterns, potential pathway influences (i.e., wastewater vs. stormwater), and potential compound sources were assessed to inform prioritization. Overall, results indicate significant burdens of emerging organic contaminants in San Francisco Bay water and sediments, especially in south Bay coastal margin areas proximal to stormwater and municipal discharges.

4.01.T-06 Combination of Proteins, Small-Molecule and Microbiological Characterization for a Holistic Understanding of Wastewater Based Epidemiological Studies

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A fraction of the of the chemicals used in consumer products, industries, agriculture and/or livestock areas are collected by sewerage systems and end up in wastewater treatment plants (WWTP). Therefore, an efficient chemical monitoring in WWTPs influents can provide constant and up-to-date information on chemical usage rates. This approach, called sewage epidemiology, have been widely applied to assess drug consumption, to

evaluate exposure to contaminants, COVID-19 growth, Antibiotic Resistance Genes (ARGs) spread or animals and human proteins in WWTPs. The main goal of the present work was to combine all these data to validate usefulness of multiclass WW-based epidemiology. WWTP Influent samples were collected in 2022. 100 mL were treated for protein analysis, as described elsewhere [1], while other 150mL filtered with PTFE filters (0.2 µm) were treated as described elsewhere for small molecules [2], keeping the particulate in the filter for ARGs as described elsewhere [3]. HRMS instruments were used for both proteins and small molecule analysis, while qPCR were made for seven specific genes (*int11*, *qnrS1*, *tetM*, *mecA*, *bla_{TEM}*, *bla_{CTX-M-32}*, *bla_{OXA-5}*) which confers bacteria resistance against widely used antibiotics. A suspect analysis of antibiotics, as well as human and farm animal specific proteins (retrieved from proteomics analysis) were determined. ARGs were also analysed, showing that wastewater bacterial communities are exposed to antibiotics and subsequently, generating ARGs despite clear correlations could not be established. However, a decrease in the levels of antibiotic does not immediately affect ARGs presence. Influent wastewater was collected from WWTPs areas with clearly differentiated profiles in terms of economic sector of the population feeding it. Two of them serving primary sector towns, three serving areas heavily industrialized and one in the highly-populated area of Barcelona (mainly urban). Several correlations of different proteins with unknown small molecules were observed. Currently we are elucidating their identity to study potential links between the specific activities carried out in the studied area, to explore the high potential of this combined strategy. [1] Carrascal, M. et.al. 2020, Science of Total Environment [2] Gago-Ferrero, P. et.al. 2020, Journal of Hazardous Materials [3] Proia, L. et.al. 2018, Science of Total Environment [4] Ng, K. et.al. 2023, Antibiotics.

4.02.P-Tu Advances in Human Biomonitoring

4.02.P-Tu-133 Contamination Assessment and Potential Human Health Risks of Heavy Metals in Urban Soils from Grand Forks, North Dakota, USA

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Heavy metals (HMs) pollution of soil is an increasing serious problem worldwide. The current study assesses the metal levels and ecological and human health risk associated with HMs in Grand Forks urban soils. A total 40 composite surface soils samples were investigated for Mn, Fe, Co, Ni, Cu, Zn, As, Pb, Hg, Cr, Cd and Tl by using microwave-assisted HNO₃-HCl acid digestion and inductively coupled plasma mass spectrometry (ICP-MS) analysis. The enrichment factor (EF), contamination factor (CF), geo-accumulation index (I_{geo}), ecological risk and potential ecological risk index were used for ecological risk assessment. The metal levels revealed the following decreasing trend observed in park soils: Fe > Mn > Zn > Cr > Ni > Cu > Pb > As > Co > Cd > Tl > Hg. Based on mean levels, all studied HMs except As and Cr were lower than guideline limits set by the international agencies. Principal component analysis (PCA) indicated that Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb, Cr and Tl may be originating from natural sources, while Hg, Pb, As and Cd may be coming from anthropogenic/mixed sources. The I_{geo} results showed that the soil was moderately polluted by As and Cd, and based on EF results, As and Cd exhibited significant enrichment. Contamination factor revealed that Zn and Pb showed moderate contamination, Hg exhibited low to moderate contamination, while As and Cd showed high contamination in soil. Comparatively higher risk was noted in children than adults, and overall, As was the major contributor (> 50%) followed by Cr (> 13%) in non-carcinogenic risk assessment. Carcinogenic risk assessment revealed that As and Cr exhibited significant risk to populations associated with this urban soil. Lastly, this study showed that the soil was moderately contaminated by As, Cd, Pb and Hg and should be regularly monitored for metal contamination in soil.

4.02.P-Tu-134 Disproportionate Health Risks of PM_{2.5} in Bishkek, Kyrgyzstan

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Atmospheric concentrations of fine particulate matter (PM_{2.5}) have been surging in many low- and middle-income countries, such as Kyrgyzstan. One of the major concerns in public health management is the

disproportionate exposure of residents to PM_{2.5}. This study analyzed PM_{2.5} concentrations measured at 34 sites, covering all parts of Bishkek, Kyrgyzstan's capital, between March 2021 and February 2022 with a focus on disproportionate access to cleaner energy for space heating. The monitoring sites were divided into 3 groups based on accessibility to district heating and natural gas distribution infrastructure. A large fraction of low-income households in group 3 areas uses coal for space heating due to affordability and limited access to district heating and natural gas pipelines. Annual mean PM_{2.5} concentrations in group 1 (district heating; 23 µg/m³), group 2 (gas and/or electricity; 35 µg/m³), and group 3 (coal; 56 µg/m³) exceeded US Environmental Protection Agency (USEPA) ambient air quality standards (12 µg/m³) by 1.9 to 4.7 times and World Health Organization ambient air quality standards (5 µg/m³) by 4.6 to 11.2 times, showing highly elevated health risks, particularly for residents in group 3. The cancer risk of lifetime exposure to PM_{2.5} was calculated to estimate the probability of lung cancer development. Cancer risks were 2.3×10^{-4} , 3.5×10^{-4} , and 5.6×10^{-4} for residents in group 1, group 2, and group 3, respectively which is much higher than the USEPA guideline limit (1×10^{-4}) indicating a greater chance of developing lung cancer for the population in Bishkek. Although responsive actions are warranted to lower the risk of PM_{2.5} for residents in all three groups, more attention is required for residents in group 3. As the population in Bishkek is predicted to experience colder winters more frequently due to climate change, PM_{2.5} emissions from coal combustion for space heating are expected to increase, leading to worse health outcomes in vulnerable populations especially in low-income communities, unless clean heating solutions are implemented.

4.02.P-Tu-136 Assessing Aquatic and Human Health Risks Associated With Metal Occurrence in the Syr Darya and the Shardara Reservoir, Kazakhstan

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After the collapse of the Aral Sea fishery, Kazakhstan made efforts to support other fisheries in the region to meet the demands of local consumption and to expand global exports of fish protein. One of the largest fisheries in Kazakhstan is the Shardara Reservoir, which lies in the Syr Darya basin and supports multiple fish farms and fish processing plants. Land use in the Syr Darya watershed includes agriculture, mining and ore processing, all of which are associated with the release of metals and metalloids. Though metals have been shown to be present in the Syr Darya, their potential impacts on fishery and consumer health remain uncertain. The objectives of this work were to evaluate the potential impacts of metals on fish located in the Syr Darya basin and to discern potential health risks associated with the consumption of fish from the Shardara Reservoir. To achieve these objectives, surface water and fish muscle were collected at three sites along the Syr Darya: upstream, within, and downstream of the Shardara Reservoir. The risk to the fishery was assessed by comparing surface water metal concentrations to maximum permissible concentrations (MPCs) and calculating water quality index (WQI) and degree of contamination (C_d) values. To assess the risk to consumers, metal concentrations in roach (*Rutilus rutilus*) were used to calculate hazard quotients (HQs) and hazard indices (HIs). Water concentrations of Cu, Fe, Mn, Se, Sr and V were found to exceed MPCs, and all sampled sites were classified as highly polluted based upon derived WQI and C_d values. Though all HQs and HIs fell below the toxicity threshold suggesting no potential risk, Pb levels in roach exceeded MPCs for safe consumption indicating a potential risk to consumers. As such, metals in the Syr Darya basin pose a potential risk to both the fishery and to consumers. In light of these findings, future initiatives in this region aimed at the development of water quality monitoring and mitigation programs to address these risks are warranted.

4.02.P-Tu-137 The Environmental Health Burden of Pesticides: A National (United States) Assessment

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Environmental contaminants, such as pesticides, can elicit adverse health impacts in humans. Occupational exposure to agricultural pesticides, for example, has been found to be correlated with a variety of adverse health outcomes, including Parkinson's disease, Alzheimer's disease, and non-Hodgkin Lymphoma. In contrast, the influence of incidental (non-occupational) pesticide exposure and adverse human health impacts has not been thoroughly studied and remains poorly understood. One major challenge is that pesticide exposure pathways for non-occupational community members is difficult to ascertain, particularly at large geographic scales (i.e., state-wide, regional, or national). Fortunately, the USGS populates and maintains a website (<https://water.usgs.gov/nawqa/pnsp/usage/maps/>) that provides usage information for over 500 pesticides for every county in the United States from 1992 until 2017 on an annual basis. These data shed considerable light on geographic variation in pesticide use on the state, regional, and national levels. For example, the pesticide profile among the 11 states found in the western United States is divided into three distinct groups: the herbicide dominated states (CO, MT, NM, UT, WY), the fumigant dominated states (CA, ID, NV, OR, WA), and AZ, which uses the two pesticide groups at more or less the same amount. These differences in state and county pesticide profiles were correlated with total cancer and pediatric cancer incidence on both a state and county scale. Within Idaho, a principal component analysis found that pesticide usage, principally, but not exclusively, of fumigants was significantly correlated with pediatric cancer incidence. The research conducted for the 11 western states was so promising that we have begun to develop a national assessment of pesticide use on a state-by-state basis. It is interesting to note that across the United States, those states that specialize in the growth of animal feed (corn and soybeans) tend to have glyphosate as the single most heavily used pesticide within the state. States that grow produce for human consumption, tend to have a more diverse pesticide profile. Assessing the pesticide load that US residents are exposed to is a challenging and nuanced process. The USGS database provides invaluable information that can be used to develop a national assessment of pesticides and the environmental health burden that they may pose.

4.02.P-Tu-138 Risk Evaluation of Toxic Effects of Pool Water; Protein Oxidation in Experimental Rats and Extrapolation to Exposed Children

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Air pollution in Port Harcourt metropolis exacerbated by ambient air-borne black soot particles in the past 4 years has become a great concern especially for children who indulge in recreational pools' activities. This study was therefore carried out to evaluate the toxicities of recreational pools in Port Harcourt City and hence perform preliminary health risk assessment. Five recreational pools (4 outdoor and 1 indoor) were used out of the randomly selected 8, from 30 that were identified. Toxicity assessment was carried out using "Ostracods-linked mathematical model" while risk assessment was by analysing protein oxidation and hepatotoxicity in the hepatocytes of exposed rats. Questionnaire-based approach was used to elicit relevant information from children (10–13 years) who consistently engaged in recreational pool activities. The pH and temperature and bacteriological examination of the pools were also carried out. Results show that all the pools were in fairly good sanitary conditions based on the highest mean bacterial counts ($2.33 \pm 0.57/100$ ml), while all were slightly acidic and with normal temperature range. The toxicity indices of outdoor pools (SP 1) and (SP 5) are respectively 20.8 and 49.0 units, while that of outdoor pools (SP 2) and (SP 3) are the same (42.5 units). "No-observed- effect-toxicity" (NOET) was observed for the indoor pool (SP 4). Analysis of the structured questionnaires inferred that none of the children traced has or has had any of the recreational water illnesses. However, results of protein oxidation in rats and hepatotoxicity of rats' hepatocytes suggest that the exposed children may be at oxidative stress related risks in future, especially if the children continue, without adequate

precaution, in the use of these pools. It is recommended that further monitoring of these children be continued while measures such as frequent changes of the pool water are ensured.

4.02.T Advances in Human Biomonitoring

4.02.T-01 Standard Reference Materials for Quality Assurance Measurements of Contaminants of Emerging Concern

Jessica Lynn Reiner, National Institute of Standards and Technology

For over 25 years the National Institute of Standards and Technology (NIST) has developed a wide range of Standard Reference Materials (SRMs) that have values assigned for clinically important analytes, legacy organic pollutants, contaminants of emerging concern (CECs), and toxic metals. Examples of some SRMs include organic contaminants in human serum, human milk, and human urine, lead in caprine blood, elements in bovine liver and animal serum, arsenic species in human urine, and toxic elements in human urine. SRMs are useful to the global biomonitoring community by serving as materials for quality assurance measurements, method development, and cross laboratory comparability. In addition to producing SRMs, NIST has developed measurement methods for many compounds, and the results from the independent methods have been combined to provide certified values for contaminants in the different materials. This talk will describe how SRMs are used for quality assurance measurements, measuring chemicals of emerging concern, and how exposure science can benefit from SRM use.

4.02.T-02 Self-Collected PFAS Blood Test using Volumetric Microsampling Performs Well Compared to the Traditional Serum Approach in a Community with Elevated Exposures

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A remote sampling approach was developed at Eurofins for quantifying PFASs in whole blood samples collected using volumetric absorptive micro-samplers (VAMS), which allow for self-collection of blood using a finger prick. This study compares PFAS exposure measured by self-collection of blood using VAMS to the standard venous serum approach. Blood samples were collected from participants (n=53) in a community with prior PFAS drinking water contamination using a venous blood draw as well as participant self-collection using VAMS. Whole blood from the venous tubes were also loaded onto VAMS to compare differences in capillary vs venous whole blood PFAS levels. Samples were quantified for PFASs using liquid chromatography tandem mass spectrometry and online solid phase extraction. PFAS levels in serum were highly correlated with measurements in capillary VAMS ($r \geq 0.91$, $p < 0.05$). Serum PFAS levels were generally two-fold higher than whole blood, reflecting expected differences in their composition. Of interest, FOSA was detected in whole blood (both venous and capillary VAMS) but not in serum. Overall, these findings indicate that VAMS are useful self-collection tools for assessing elevated human exposure to PFASs.

4.02.T-03 Trace Analysis of PFAS in Dried Blood Spots and Liquid Whole Blood

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Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that have been widely used in consumer, personal care, and household products for their stain- and water-repellent properties. PFAS exposure has been linked to various adverse health outcomes. Such exposure has commonly been evaluated in venous blood samples. While this sample type can be obtained from healthy adults, a less invasive method of blood collection is required when evaluating vulnerable populations. Dried blood spots (DBS) have gained attention as a biomatrix for exposure assessment given the relative ease of collection, transport, and storage. The objective of this study was to develop and validate an analytical method to measure PFAS in DBS. A workflow

is presented for extracting PFAS from DBS, chemical analysis by liquid chromatography-high resolution mass spectrometry, normalization for blood mass, and blank correction to account for potential contamination. Over 80% recovery was achieved for the 22 PFAS measured with an average coefficient of variation of 14%. Comparison of PFAS concentrations detected in DBS and paired whole blood samples from six healthy adults was correlated ($R^2 > 0.9$). Findings demonstrate trace levels of a broad range of PFAS in DBS can be reproducibly measured and are comparable to liquid whole blood samples. DBS can offer novel insights to environmental exposures, including during critical windows of susceptibility (i.e., in utero, early life), which have been largely uncharacterized.

4.02.T-04 Screening and Quantitation of Environmental Pollutants in Serum Using Ultra-high Performance Liquid Chromatography/Tandem Mass Spectrometry

Shu-Yen Liu and Chia-Yang Chen, National Taiwan University

Target analysis could determine analytes at trace concentrations, but may miss crucial non-selected chemicals. Non-target analysis could be complementary for identifying crucial compounds. This study identified compounds in 186 serum samples, including colorectal and adenoma cases in Changhua County, Taiwan, with ultra-high performance/quadrupole time-of-flight mass spectrometry. The 296 Identified chemicals were prioritized using ToxPi software based on their detected frequencies, peak abundance, bioactivity data, acceptable daily intake, and carcinogenicity. Six chemicals were chosen for further quantitative analysis with ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS), which were tris(2-butoxyethyl)phosphate, tributylphosphate, diethyl phthalate, diethyl phthalate, methyltestosterone, ethotoin, and 8-hydroxyquinoline; eight carcinogenic polycyclic aromatic hydrocarbons (PAHs) and 1-hydroxypyrene (1-OHP) were also included. The parameters of the sample preparation and instrumental analysis were optimized. The prioritized chemicals and 1-OHP were separated on a Waters CORTECS C18 column (30×2.1 mm, $1.6 \mu\text{m}$) with mobile phases of (A) 0.1% formic acid_(aq) and (B) methanol at positive electrospray ionization; the eight PAHs were separated on an Agilent RRHD PAH column (50×3.0 mm, $1.8 \mu\text{m}$) with mobile phases of (A) 5-mM ammonium formate_(aq) and (B) acetonitrile at positive atmospheric pressure chemical ionization. 100 μL of serum samples were prepared by mixing with 250 μL of 1% formic acid in acetonitrile and 50 μL of isotope-labeled internal standards, extracted with Ostro 96-well plates, and the analytes were eluted with 100- μL methanol. The inter- and intra-day recoveries and coefficients of variation were 70-140% and below 20%, respectively. Tris(2-butoxyethyl)phosphate (mean 5.79 ng/mL), tributylphosphate (mean 6.37 ng/mL), diethyl phthalate (mean 7.31 ng/mL), and benzo[a]anthracene (mean 97.0 ng/mL) were detected in all serum samples. Still, these chemicals were not significantly different in serum samples among colorectal cancer, adenoma, and control groups. Multiple linear regression indicated that serum levels of tris(2-butoxyethyl)phosphate were positively associated with secondhand smoke and egg consumption; the levels of tributylphosphate were positively correlated with vegetable consumption; serum levels of benzo[a]anthracene were positively associated with tea consumption.

4.02.T-05 Non-Invasive Monitoring of Human Exposure to Environmental Contaminants – Combining Different Personal Passive Samplers with Indoor Air Measurements

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Non-invasive sampling methods to monitor human exposure to hydrophobic organic contaminants (HOCs) can provide a valuable alternative to conventional monitoring approaches that rely on sampling of human body fluids or tissues. Due to ethical considerations, these invasively taken samples can be challenging to obtain. In this study, we employed passive sampling approach to measure the personal HOC-exposure of 100 study participants. The participants were provided with two wearable passive sampler devices (PSDs): a well-established silicone wristband and a newly developed silicone skin patch. Due to their high affinity to HOCs, the silicone materials accumulate chemicals from their environment, sampling the immediate vicinity of the

participant. After an exposure time of 5 days, the PSDs were collected and the accumulated HOC-contents were evaluated after exhaustive solvent extraction, followed by a targeted GC-MS/MS analysis (>40 target analytes). The wristbands provided a simple design and straightforward application, but suffered from a high load of interfering compounds (such as personal care products) hindering the chemical analyses. Contrarily, the skin patches were covered with a medical gauze layer, which provided protection against the deposition of such interfering compounds. Due to their less exposed design, the accumulated amounts of HOCs in the skin patches tended to be lower than in the wristbands, which in some cases led to non-detects. Despite the differences in measured total concentrations, both PSD designs showed good a correlation in patterns shown by similar relative amounts for most of the tested HOCs. To supplement the collected data from the wearable PSDs with information on their individual indoor exposure, each participant was provided with an air sampler (consisting of a silicone sheet) to be attached to a window inside the participants' kitchen. However, only few of the tested analytes showed a good correlation between the indoor air and personalized exposure measurements. This discrepancy indicates that a large share of the compounds measured in the PSDs originated from other sources outside the participants' homes. All obtained results on HOC-exposure of the participants will be additionally compared to collected demographic data to identify particularly exposed groups. Altogether, the results of this study highlight the potential of non-invasive passive sampling methods for the exposure screening of larger population groups.

4.02.T-06 Implications of Climate Change for Dietary Mercury Exposure in High Latitude Subsistence Communities

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A growing number of studies report disproportionately high rates of warming in high latitude locations, leading to rapid glacial retreat, loss of permafrost, and more frequent and intense wildfires in these regions. All of these processes are known to release mercury (Hg) and other trace elements to nearby aquatic environments. The 2018 United Nations' Global Mercury Assessment report found that high latitude populations, Indigenous Peoples, and coastal communities who practice subsistence are at greatest risk for dietary methylmercury (MeHg) exposure. MeHg is an organic, highly neurotoxic form of Hg that is produced through microbial transformation processes that occur largely in aquatic sediments. MeHg is also highly bioavailable and can accumulate to potentially toxic levels in the muscle of long-lived, high-trophic level fish that are often preferred as a food source by humans. In addition to increasing export of previously sequestered Hg to aquatic environments, the rate at which Hg is converted to MeHg is also dependent on biochemical conditions that are coupled to climate. This suggests that the rate of conversion to MeHg and uptake into aquatic food webs may also be changing. Due to the aforementioned risk factors, lack of transport infrastructure in rural Alaska (USA), and availability of existing long-term datasets, rural Alaska was selected as representative study location to evaluate whether climate change may be influencing the degree to which high latitude subsistence communities may be exposed to MeHg via fish consumption. A multiple-lines-of-evidence approach was used to draw conclusions regarding the potential interaction between climate change and potential MeHg exposure via subsistence diets, through direct and indirect mechanisms. To evaluate the potential for glacial retreat to directly influence concentrations of MeHg in traditional foods, we measured Hg in mussels collected across a gradient of glacial influence in watersheds that support subsistence fisheries. To determine whether changes in resource availability may indirectly alter dietary MeHg exposure through compensatory dietary shifts, we examined long-term trends and potential interactions between subsistence harvest masses and water surface temperature anomalies. Finally, we investigated spatial and temporal trends in human hair Hg biomonitoring data, due to the known correlation between dietary MeHg exposure and concentrations in hair.

4.03.A.T Analysis of Pharmaceuticals, Pesticides, and Other Chemicals in Environmental Matrices to Support One Health

4.03.A.T-01 Combination of Targeted and Non-Targeted Screening for Bisphenol and Related Unknowns in Human Milk: A Method to Improve Current Human Milk Biomonitoring

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Breast milk is an essential source of infant nutrition but it can also contain environmental chemical contaminants that may pose health risks. Current human milk biomonitoring relies heavily on a targeted approach to detect levels of environmental contaminants and has limitations in detecting emerging contaminants, highlighting the need for novel tools to improve their detection. In this study, we conducted targeted and non-targeted screening on 194 human milk samples collected from the Vhembe district of Limpopo Province (South Africa), 204 samples collected from Pretoria (South Africa), and 207 samples from Montreal (Canada) to identify the presence of 9 selected bisphenols, and related unknowns. Liquid chromatography quadruple time-of-flight mass spectrometry from Agilent Technologies was used to detect and quantify bisphenol A, S and AF as well as the further identification of a total of 28 tentative bisphenol related unknowns using Profinder (Agilent technologies) with a customized database library derived from the Technical Consultation list of Health Canada. In total, five different bisphenol related unknowns were confirmed using available commercial standards highlighting the advantage of combining non-targeted with targeted analysis to improve current human milk biomonitoring. By using this method, we can assess exposure pathways and inform risk reduction strategies to protect the health of breastfeeding infants and promote global health. Supported by CIHR and McGill University.

4.03.A.T-03 Screening of Organic Corrosion Inhibitors, Industrial Antioxidants and their Transformation Products in Snow and Surface Water: Identification and Chemometric Evaluation

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The urban aquatic environment is a repository of a complex mixture of anthropogenic organic chemicals, many of which can enter the aquatic ecosystem via pathways such as snow melting, storm water runoff, and wastewater discharge. Environmental monitoring for organic pollutants along these pathways is limited to only a small set of chemicals. Thus, targeted and untargeted screening methods based on liquid chromatography coupled to an orbitrap mass spectrometer were developed and applied to provide a comprehensive insight on the occurrence, source influences and geospatial distribution of known and lesser-known contaminants and their transformation products (TPs) in Greater Montreal surface water and snow samples. Our results indicated that four industrial antioxidants (IAs) belonging to the p-Phenylenediamines (PPDs) class, and their corresponding TPs are widely present in urban snow samples. Similarly, four organic corrosion inhibitors (OCIs) of the benzotriazoles (BTs) and benzothiazoles (BTHs) classes are ubiquitously detected in snow and surface water. Our findings showed that N-(1,3-dimethylbutyl)-N-phenyl-p-phenylenediamine (6PPD) and its so highly toxic quinone variant, 6PPD-Q were the dominant IAs in the dissolved phase of the snow sample, with a concentration range of 5 – 740 ng/L and 4 – 1275 ng/L respectively. Also, 5-methyl-1-H-benzotriazole (MHBZ) and Hydroxybenzothiazole (HBZ) were the dominant BTs and BTHs present at concentration of 7 – 3917 ng/L and 25 - 2060 ng/L respectively. In contrast, 2-(methylthio)-benzothiazole (MTBZ) showed the least concentration range across all surface water sample sites (7-50 ng/L) with the exception of one site indicating a concentration of 315 ng/L. Notably, this sampling site also showed the highest reported HBZ concentration which is indicative of a point-source influence. Identification of lesser-known contaminants and their TPs using the untargeted screening approach was based on peak intensities, retention time, frequency of detection (DF) and substructural information. By applying this approach using the Compound Discoverer software, an initial

10,000 features were found in surface water and 30,000 features generated from snow samples, some of which are tentatively identified. Ongoing work is being conducted to prioritize the chemical features and confirm their identities using authentic standards based on retention time, MS1 and MS2 spectra.

4.03.A.T-04 Transition of Reference Methods for Dioxins, PCBs and Pesticides to Triple Quadrupole Mass Spectrometry (GC-MS/MS) for Better Exposure Measurement

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The measurement of non-polar persistent organic pollutants such as dioxins, PCBs and pesticides in environmental matrices is complex due to their relatively low concentrations of health concerns and the need to measure such low concentrations in complex (tissue, exposure media, effluents) and sample-limited (serum, tissue) conditions. The use of Gas Chromatography-High Resolution Mass Spectrometry (GC-HRMS) using magnetic sector mass spectrometers has been common since the late 1980's for analysis of these halogenated environmental contaminants with high sensitivity and specificity and demonstrated accuracy. For instance, reference EPA Method 1613B, promulgated for monitoring chlorinated dioxins and furans in regulatory applications, requires the use of HRMS. However, magnetic sector instrument options have decreased significantly as manufacturers focus their attention on newer technology such as the "Triple Quadrupole" MS/MS systems. These instruments have the potential to be much more sensitive and relevant for use in exposure monitoring and One Health measurements, enabling their use in sample-limited conditions and in every application that previously required the use of GC-HRMS. This study describes how critical components of the MS/MS method for each analytical group were developed and validated and will highlight the advantages of MS/MS in each application, such as enhanced calibration ranges, improved sensitivity (especially for pesticides) and more. The improved robustness of MS/MS methods compared to HRMS methods for analyzing field samples with complex background matrix will be described. Comparative data for HRMS versus MS/MS on complex samples will be presented. Many of the results from our ongoing work over the last 5 years have resulted in the production of an alternate test method ATM 16130 that is currently in the process of promulgation as an approved test method by the US EPA. The EPA review showed that the method validation passed all criteria for equivalence to the reference method EPA 1613B. We will also detail upon our work to extend GC-MS/MS methods to PCBs (EPA 1668) pesticides (EPA 1699) and brominated diphenyl ethers (EPA 1614).

4.03.A.T-05 Method Development for the Extraction and Analysis of Unregulated Organic Compounds in Soil and Biosolids

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Unregulated organic compounds (UOCs) include the many organic chemicals that improve our quality of life in some way such as pharmaceuticals, chemicals in personal care products (PCPs) and/or consumer goods, agrochemicals, industrial chemicals, and fire retardants among others. UOCs enter the environment through multiple direct and indirect ways. Methods to correctly detect and quantify their occurrence is critical for assessing their fate and potential risk to the environment and human health. During the treatment of wastes entering the water resource recovery facilities, UOCs may undergo some degradation, but most are minimally removed, thus UOCs can accumulate in biosolids. Biosolids land applied as a soil amendment or a waste management strategy is one pathway for UOCs to enter the environment. Biosolids have particularly complex matrices that can greatly impact recovery and detection of UOCs. The matrix complexities and that UOCs include chemicals with a wide range of properties presents significant challenges in achieving performance acceptance criteria. Here we evaluated the occurrence of UOCs in biosolids and soils from a biosolids-applied field using two approaches for a total of 124 UOCs. The first approach was a modified version of the EPA 1694 method that uses ultrasound assisted extraction followed by solid phase extraction (SPE). The second approach consisted of ultrasound assisted extraction followed by an Enhanced Matrix Removal-Lipid (EMR-Lipid) dispersive SPE as a specific clean-up for complex matrices. In both approaches, graphitized carbon black

cleanup was used for the final step prior to analysis. The extracts were analyzed by liquid chromatography high resolution mass spectrometry (MS) using a quadruple time of flight MS in both positive and negative electrospray ionization modes with SWATH data acquisition. The use of different extracting solvents allowed the recovery of a broader range of UOCs. Graphitized carbon black cleanup was critical in producing clean extracts, especially for biosolids. The EPA modified method generally showed better results than the EMR method, in terms of compounds extracted, recoveries and limits of quantification. Results showed that the method was generally able to extract pharmaceuticals, PCPs, opioids or phthalates, among others within acceptable criteria. While some UOC classes such as tetracyclines and hormones were more challenging and yielded mixed results.

4.03.A.T-06 Targeted Analysis of a Complex Mixture of Unregulated Organic Chemicals (UOCs) in Biosolids

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Biosolids (i.e., treated sewage sludge) have been recognized in the United States for nearly 50 years as a highly underutilized resource for agricultural purposes. The land application of biosolids (i.e., treated sewage sludge) offers multiple benefits over the hazards of landfilling and incineration. These benefits include improvements to soil health and plant nutrition. However, biosolids contain numerous unregulated organic chemicals (UOCs) leading to concerns that may impede a positive public perception of the benefits gained by land application, hamper expansion of its beneficial use to food crops, and delay the realization of a circular economy. Therefore, a significant research need is to determine which UOCs pose a high risk to ecosystem and human health when biosolids are land applied to agricultural crops at environmentally realistic rates. It is further necessary to develop methods of extraction and chemical analysis that can be used to determine the occurrence, fate, and transport of such concerning biosolids-borne UOCs once land-applied. To address these imminent challenges, several universities were collaborating on an EPA team project to undertake a multi-year study utilizing a combination of field, laboratory, and modeling approaches. We carried out laboratory experiments to develop a rapid, biomimetic chemical assay using thin-film passive samplers to predict the bioavailability of ‘priority’ UOCs in soil and further assess probable bioaccumulation. Field validation data were collected from passive samplers, earthworms, vegetables, and fruits grown in to evaluate the bioavailability, uptake, and presence of the priority UOCs in the edible portions of food and feed crops and in earthworms resulting from land-application of Class A biosolids. These results are expected to allow for rapid assessment of chemical bioavailability and prediction of bioaccumulation potential for an extremely diverse chemical mixture and contribute to a holistic human health and ecological risk assessment. Here we present our finalized instrument and biosolids extraction methods, and UOC residue results from Class A biosolids samples.

4.03.B.T Analysis of Pharmaceuticals, Pesticides, and Other Chemicals in Environmental Matrices to Support One Health

4.03.B.T-01 Assessing Manatee Exposure to Current-Use Pesticides

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Florida manatees (*Trichechus manatus latirostris*) are exposed to pesticides through a variety of pathways including their habitat and diet (e.g., water, sediment, and vegetation contaminated from agricultural and urban runoff or direct applications to aquatic vegetation). Pesticides may have direct adverse effects on manatee health or may indirectly affect manatees through alterations in food sources. This study assessed potential exposures of Florida manatees to pesticides through the analysis of water and aquatic vegetation from known manatee habitats (November 2022 and March 2023) and examined pesticide uptake through the analysis of archived manatee plasma samples (February 2013 to February 2019). All samples were analyzed for 183 pesticides and pesticide transformation products using both liquid chromatography and gas chromatography tandem mass

spectrometry (LC-MS/MS and GC-MS/MS). In surface water, 13 pesticide and pesticide transformation products were detected with at least one compound detected in 89% of water samples. Atrazine was the most frequently detected pesticide, found in 74% of surface waters. Individual pesticide concentrations ranged from 0.7 to 39.0 ng/L, and summed concentrations ranged from 0.7 to 51.0 ng/L. In aquatic vegetation, seven pesticides were detected with at least one pesticide detected in 90% of samples. Pesticide transformation products were not detected in aquatic vegetation. Chlorfenapyr was the most frequently detected pesticide, found in 70% of vegetation samples. Individual concentrations ranged from 0.9 to 29.6 ng/g dry weight and summed concentrations ranged from 1.9 to 74.9 ng/g dry weight. In plasma samples, eight pesticides and transformation products were detected with at least one analyte detected in 20% of samples. Bifenthrin was the most frequently detected pesticide, found in 11% of plasma samples. Individual concentrations ranged from 0.4 to 4.8 ng/mL, and summed concentrations ranged from 0.4 to 6.1 ng/mL. Assessing recent and long-term pesticide exposures of Florida manatees will improve the understanding regarding the consequences of environmental stressors towards this threatened species.

4.03.B.T-02 Investigating Micropollutant Partitioning in Five Environmental and Biological Matrices Collected in Replicate Artificial Streams

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The physico-chemical properties of the vast majority of micropollutants render them susceptible to various mass transfer and transformation processes such as biodegradation, photodegradation, and sorption. If appropriate environmental conditions exist (e.g., biomass, sunlight, and particulate matter), these mechanisms can be highly effective in reducing the concentrations of micropollutants in the aquatic environment. Whole-system studies that assess fate and transport are important to develop an understanding of micropollutant behaviour. Unlike lab-based studies, these systems incorporate the natural conditions that are associated with contaminant attenuation. The Advancing Canadian Wastewater Assets facility in Calgary (Canada) is equipped with 12 naturalized artificial streams (320 m long each) integrated with a fully operating municipal wastewater treatment plant and 2 pilot wastewater treatment plants. The streams have hydraulic and ecological parameters that mimic natural local systems. This study investigates the partitioning of a diverse group of micropollutants, including pharmaceutical and personal care products in replicated ACWA streams in five environmental compartments: water, sediments, invertebrates, biofilm, and fish. In addition, it aims to evaluate the impact of different levels of wastewater treatments (e.g., ultrafiltration, ozonation, reverse osmosis) on the occurrence and partitioning of these compounds in the environment. To address the study questions, two methods were used to extract the environmental samples: Solid Phase Extraction (SPE) for stream and wastewater treatment effluent samples and QuEChERS for sediment, biofilm, invertebrate, and fish tissues. The extracts were analyzed via liquid chromatography, triple quadrupole mass spectrometry (LC-QQQ). Preliminary results of this study show that 18 of the 22 chemicals assessed were found in the streams, including analgesics, antidepressants, neuroinhibitors, cardiovascular, antibiotics, and personal care products. On the other hand, fewer chemicals were observed in other matrices, suggesting that further sample clean-up is necessary for other substances. Carbamazepine (anti-epileptic) and venlafaxine (antidepressant) are present at low concentrations in biofilm, fish, and invertebrates (<0.1 ng/g -0.7 ng/g), while higher amounts can be found in sediments (<0.1 ng/g-12.6 ng/g).

4.03.B.T-03 Occurrence of Seed Treatment Pesticides in Tadpoles and Songbird Eggs

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Seeds treated (coated) with insecticides and fungicides are regularly planted across the United States, especially

in the midwestern United States. However, the fate, transport, and effects of these pesticides to non-target organisms is understudied. We evaluated the potential uptake and accumulation of pesticides in amphibians and songbird eggs collected from sites surrounding the AltEn ethanol plant in Mead, Nebraska, a plant which had previously received pesticide treated (coated) seeds. American Bullfrog (*Lithobates catesbeianus*) tadpoles were collected from surface-water sites (four ponds and one reservoir), and Red-Winged Blackbird (*Agelaius phoeniceus*) and Brown-headed Cowbird (*Molothrus ater*) eggs were collected from seven sites. Tadpoles hatched in June 2021 and were collected in November 2021, and songbird eggs were collected in June 2021. Collected samples were analyzed for more than 150 pesticides and transformation products using gas and liquid chromatography-tandem mass spectrometry. Overall, 22 compounds were detected in sampled tadpoles and songbird eggs. Pesticides detected included neonicotinoid insecticides (clothianidin, thiamethoxam) and their metabolites, other insecticides (bifenthrin, chlorantraniliprole), fungicides (fluoxastrobin, ipconazole, metconazole, propiconazole, pyraclostrobin, tebuconazole), herbicides (atrazine), and the historically used insecticide p,p'-DDT and its degradates (p,p'-DDE and p,p'-DDD). Study results can help determine which pesticides may accumulate in amphibians and songbirds for further risk analysis.

4.03.B.T-04 Outdoor Law Enforcement Training Exposure Assessment

Samuel Cole Smith and Courtney Roper, University of Mississippi

Law enforcement officers are routinely exposed to gunshot residue (GSR), but there is limited research on the exposure levels of GSR in environmental matrices, preventing health effects research. Forensic science research has established that GSR released after discharging a firearm consists of both inorganic (e.g., Pb, Ba, Sb, Cu) and organic (e.g., Nitrotoluenes, Diphenylamine, Ethyl centralite) components. Released GSR particles range in size, but fine particulate matter (PM_{2.5}) is an important health relevant size fraction. Ambient PM_{2.5} is associated with cardiorespiratory diseases, so it is crucial to understand GSR in this size fraction for an accurate exposure assessment. Additionally, few studies have investigated GSR concentrations outdoors where the influence of temperature, humidity, and wind direction may alter officer exposure. This project explores daily differences between pistol (n=3) and rifle (n=3) qualifications of the University of Mississippi Police Department's outdoor trainings. A PM_{2.5} air sampler was worn on the right arm of officers at each training to collect released GSR (totaling 9 samples per firearm type). Throughout each sampling period, meteorological parameters (e.g., temperature, humidity, wind direction) and shooter information (e.g., height, position in the firing line, number of rounds fired) were collected. Nondestructive methods of analysis were used to analyze for black carbon (sootscan transmissometer) and inorganic components (X-ray fluorescence). All collected samples were blank corrected to calculate the average concentrations for the shooting qualifications. Black carbon concentrations were significantly different between sampling days (two-way ANOVA, $p \leq 0.05$). Lead concentrations were significantly higher for the pistol (240.58 ± 51.90 ppm) qualifications than the rifle qualifications (105.44 ± 23.73 ppm) (two-way ANOVA, $p \leq 0.05$). Method development for organic analysis via micro-Fourier transform infrared spectroscopy is underway to nondestructively identify organic GSR components collected on filters. Associations between meteorological parameters and concentrations of all measured GSR components will be conducted using Pearson correlations. The completion of this project will determine the daily exposure to inorganic and organic GSR during pistol and rifle firearm trainings and the impact of meteorological parameters on the concentrations.

4.03.B.T-05 Target and Suspect Per- and Polyfluoroalkyl Substances in Fish from an AFFF-impacted Waterway

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We investigated the presence and distribution of per- and polyfluoroalkyl substances (PFAS) in fish tissues from the Columbia Slough. The Columbia Slough is a slow-moving waterway (~30 km) that provides critical

wetlands and riparian habitats in the Portland metropolitan area of Oregon, United States. Several PFAS bioaccumulate in aquatic organisms, posing potential health threats to piscivorous wildlife and humans. Our objectives were to 1) quantify the differences in PFAS composition in fish tissues across three reaches within the Columbia Slough, 2) identify drivers of PFAS clustering across the dataset including fish tissues, fish species, reaches, and years, and 3) develop a fish consumption health advisory based on the PFAS concentrations in fish tissue and evaluate potential risk to piscivorous wildlife using the US EPA's draft ambient water quality criteria. We screened for 50 targets and over 4,700 suspect PFAS in 107 fish samples. Overall, 31 target PFAS and 6 suspect compounds were detected ranging in concentrations from <LOD - 856 ng/g. Compounds most frequently detected in all tissue types included seven carboxylic acids (PFOA, PFNA, PFDA, PFUDA, PFDoA, PFTrDA, PFTeDA), three sulfonates (PFHxS, PFOS, PFDS), three electrofluorination-based compounds (FBSA, FHxSA, FOSA), and two fluorotelomer-based compounds (8:2 FTS, 10:2 FTS) and were consistent with the documented use of AFFF adjacent to the study area. PFOS and FHxSA were detected at concentrations 1-3 orders of magnitude greater than the other PFAS detected. This study also identified Cl-PFOS, FPeSA, and FHpSA in fish tissue for the first time. Tissue type explained greatest variability in PFAS concentrations, highest in blood, followed by liver and fillet. Some clustering occurred based on fish species, but little to none by reach or year. This study raises concerns about PFAS consumption through fish and its impact on human health. PFOS was the only compound detected frequently enough for which we have toxicity information to calculate meal recommendations. Based on PFOS results, the Oregon Health Authority modified the fish advisory, advising against whole-body fish consumption to avoid elevated liver PFOS levels. Although other compounds were detected, their toxicity information is lacking for meal recommendations, indicating further research is needed on toxicological effects of perfluoroalkyl sulfonamides and fluorotelomer sulfonates detected.

4.03.B.T-06 Safe Use of Treated Wastewater: Effects of Irrigation Alternation on Contaminants of Emerging Concern Accumulation in Vegetables

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The reuse of treated wastewater (TWW) has become widespread; however, concerns persist regarding the potential human exposure risk associated with the accumulation of contaminants of emerging concern (CECs) in plant tissues. Currently, there is a lack of effective mitigation strategies for the safe reuse of TWW, particularly in realistic field condition. This study aims to address this gap by investigating CEC accumulation in three common vegetables (carrot, lettuce, and tomato) under field conditions with two treatments: the first group was irrigated with TWW throughout the entire growth period, while the second group received TWW during the early half of the growing season and switched to freshwater for the later half. Additionally, to assess the impact of irrigation alternation, another two parallel treatments were also conducted, where TWW was fortified with individual CEC at concentrations of 250 ng/L. A total of 33 targeted CECs, encompassing tire wire particle-derived compounds, per- and polyfluorinated substances, and pharmaceutical and personal care products, were analyzed. Regular sampling of irrigation water, soil, and edible tissues of the plant samples was conducted, which were subsequently analyzed for the presence of CECs. It is anticipated that switching to freshwater for the second half of the growing season will significantly reduce CEC accumulation in the final edible produce. This alternation strategy allows for extensive soil degradation, promoted plant metabolism and growth dilution, thereby reducing CECs in the edible parts of the vegetables. By providing evidence-based insights into the risks and benefits of water reuse, this study has significant implications for minimizing human exposure risk, increasing public awareness of water reuse practices, and promoting the safe reuse of nontraditional waters.

4.03.P-Mo-100 Oxidative Stress Induced in *Hyalella azteca* Exposed to Non-Steroidal Anti-Inflammatory Drugs

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Non-steroidal anti-inflammatory drugs (NSAIDs) are common pharmaceutical drugs used to alleviate pain and inflammation; however, these pharmaceutical drugs lack regulation for their use and disposal. NSAIDs are emerging sources of contamination of freshwater bodies near industrial sites and pose serious risks to the aquatic ecosystem. Additionally, toxicity can be increased as a result of mixture of NSAIDs. This study investigated the effects of acetaminophen, ibuprofen, and naproxen on freshwater amphipods, *Hyalella azteca*. The amphipods were exposed to different concentrations of the NSAIDs for 96 hours using isolated and combination form. Lipid peroxidation (LPX) and superoxide dismutase (SOD) biomarkers were examined to determine the amphipod response to oxidative stress caused by NSAID exposure. All the NSAIDs decreased the survival of the amphipods. The order of LC₅₀ for ibuprofen, acetaminophen, and naproxen was 1.01 mg/L, 4.89 mg/L, and 5.09 mg/L, respectively. The results showed a significant increase in SOD activity ($p < 0.05$) in both NSAID isolated and combination forms while LPX activity was not different from that of the control. This study demonstrates that NSAIDs could induce oxidative stress in *H. azteca*.

4.03.P-Mo-101 Soil Sorption of Biosolids-borne Unregulated Organic Chemicals

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Biosolids are the primary product of municipal wastewater treatment. Land applications of biosolids in agricultural fields represent a beneficial use that contributes to environmental and agricultural sustainability, but the presence of numerous unregulated organic chemicals (UOCs) in biosolids is a human and environmental health concern. Understanding the broad human and environmental health risks of biosolids-borne UOCs is imperative for the safe use of biosolids in agriculture. The adsorption or distribution coefficient (K_d) is a measure of the strength of chemical adsorption to soil and hence an indication of its environmental mobility. K_d values vary greatly as a function of physicochemical properties of chemicals as well as soil types. In the current study, K_d values were obtained for a large number of UOCs of known and potential environmental concern that are commonly present in biosolids that are destined for land applications. A sandy loam soil from southern California was used in batch equilibration experiments to derive the K_d values. The relationship of K_d values with physicochemical properties such as hydrophobicity (K_{ow}), pKa, and pH adjusted K_{ow} was evaluated. The derived K_d values will be used to predict the soil porewater concentrations of UOCs at different biosolids amendment rates, and further off-site transport potential such as leaching and runoff. The derived K_d values will be also used for describing uptake and accumulation of UOCs by vegetables grown in biosolids-amended field plots, as well as the potential bioaccumulation into earthworms.

4.03.P-Mo-102 Simultaneous Determination of Spirotetramat, Clethodim, and Their Metabolites Using Liquid Chromatography-Tandem Mass Spectrometry

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Spirotetramat is an insecticide that effectively targets sucking insects infesting crops, while clethodim acts as an herbicide specifically designed to control the growth of both annual and perennial grasses. The two pesticide components have been evaluated to possess intricate pretreatment procedures, complex analysis methods, and limited analysis efficiency. In this study, we have developed a method capable of simultaneously analyzing two pesticide components and their metabolites, namely spirotetramat-enol, clethodim-sulfone, and clethodim-sulfoxide, using the QuEChERS method. This method serves as a straightforward and efficient sample pretreatment technique. A comparison was conducted between the extraction methods of acetonitrile and acidified acetonitrile, using the QuEChERS EN method. The objective was to establish the optimal analysis conditions for the target substances using LC-MS/MS and to identify the most efficient extraction and purification methods. Various purification methods, such as PSA, C18, and Z-Sep, were employed to determine

the optimal purification method. For the verification of the application of agricultural products, five representative crops (mandarin, potatoes, peppers, beans, and rice) were selected to confirm the specificity, linearity, accuracy, and precision. The quantitative limit for all target compounds was set at 0.01 mg/kg or lower, and the coefficient of determination (r^2) for the calibration curve was more than 0.999. Furthermore, accuracy and precision were evaluated at three concentration levels (LOQ, 2×LOQ, 10×LOQ), and the results demonstrated values within the range of 70-120% with a relative standard deviation (RSD) below 20%. This ensures that the standards set by international guidelines, such as the EU and OECD guidelines, have been met. Therefore, the LC-MS/MS-based simultaneous analysis method developed for spirotetramat, clethodim, and their metabolites can be effectively employed for rapid and accurate determination of residual concentrations in agricultural products.

4.03.P-Mo-103 Future Requirements for Regulatory Testing of Mixtures and UVCBs

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Aquatic testing of complex mixtures and UVCBs (Unknown Variable Composition and Biological) can be challenging for industry, CRO's and regulators alike. It is difficult to design tests that fulfil everyone's requirements especially as the requests are often contradictory to one received the week before. They often rely on the use of Water Accommodated Fractions (WAFs) and as such, any toxicity observed cannot be attributed to an individual component therefore nominal values are used. Analytical techniques vary from identifying key markers, identifying and quantifying as many components as possible or considering the entire UVCB as a single component. The expectation is also that Environmental Fate studies are conducted on mixtures and UVCBs and that traditional endpoints and validity criteria are met despite our inability to radiolabel the entire substance and the complexities in the interpretation of the metabolite formation in complex mixtures is no simple task. A scientifically robust testing strategy is needed to fully support the testing and registration of complex mixtures and UVCBs. Following on from a lunchtime seminar we held on this topic in SETAC Dublin 2023, we would like to continue the discussions and start identifying solutions for a harmonized approach for mixtures and UVCBs.

4.03.P-Mo-104 Occurrence, Distribution, and Ecological Risk Assessment of Phthalate Esters in the Surface Water of the St. Lawrence River and Estuary in Canada

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Phthalate esters (PAEs) are high-production volume chemicals widely used as plasticizers and are added to many consumer products such as cosmetics, medical devices, and toys. PAEs are known to be ubiquitous in various environmental matrices. They are endocrine disruptors and can lead to multiple health concerns, such as toxicities in reproduction, metabolism, and growth of organisms, including humans. As a result, the United States Environmental Protection Agency (US EPA) has designated six PAEs as priority pollutants due to their harmful impacts on human health and the environment. However, there is very little information on the fate of PAEs in the aquatic environment of Canada, which makes it difficult to assess the risks of these contaminants in the context of plastic pollution. The St. Lawrence River and Estuary (SLRE) host a suite of unique ecosystems and constitute a vector of contaminants to the Atlantic Ocean. Given that the SLRE is critical to the resident organisms and to more than 5 million Canadians living on or near its coast, the current lack of data on PAEs contamination in the region impedes understanding their effects on this important ecosystem and prioritizing management strategies. To this end, this study aims to (i) analyze the spatial distributions of nine PAEs in the surface waters of the SLRE; (ii) elucidate their field-based partitioning coefficients between the aqueous phase and suspended particulate matter (SPM); and (iii) assess their ecological risks in SLRE water. Surface water samples were collected from 30 coastal sites and 15 offshore sites in the SLRE in 2022. The aqueous dissolved phase was extracted via solid-phase extraction, while the SPM was treated by ultrasound-assisted solvent

extraction. Samples were analyzed by GC-MS/MS. All target PAEs were detected in the aqueous dissolved phase, and the total PAEs ranged from 6.3 to 565 ng/L. Dicyclohexyl phthalate (DCHP; 100% detection frequency, median 14.8 ng/L), Diisobutyl phthalate (DiBP; 88%, median: 10.6 ng/L), and di-n-octyl phthalate (DnOP; 86%, median 10.6 ng/L) were the dominant PAEs, and DnOP is one of the six priority PAEs classified by the US EPA. The SPM results will also be presented. An ecological risk study will be conducted for the water using the risk quotient method. This study establishes baseline concentrations for PAEs in the SLRE and helps to better elucidate plastics-associated pollution in this region so future trends can be assessed.

4.03.P-Mo-105 Analysis of Contaminant Residues in Honey Bee Hive Matrices

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Pollinators provide ecological services essential to maintaining our food supply and propagating natural habitats. However, populations are in serious decline due to causative environmental stressors including pesticides, pathogens, poor nutrition, and habitat loss. To better understand the impacts of pesticide exposures on colony health, a field survey in central Ohio, USA was conducted to monitor the potential contamination of honey bee colonies by agricultural use pesticide residues. Apiaries (n=10) were situated across an agricultural gradient, and samples were collected over a 4-week period encompassing corn planting. Dead bees from entrance traps (DBT), pollen, and in-hive (IH) matrices including bee bread, honey, larvae, and nurse bees were analyzed for organophosphates, organochlorines, organonitrogens, neonicotinoids, and pyrethroids using GCxGC-ToF/MS, GC-QTOF/MS, and LC-MS/MS. Out of ~200 pesticides and their degradates targeted, over 60 residues were detected and quantified across 306 samples. Neonicotinoids and fungicides were the dominant pesticide classes identified throughout all the matrix types. Neonicotinoids were detected at both higher concentrations and frequencies compared to fungicides, specifically in field pollen samples. Dead bee traps also contained high concentrations of these two contaminant classes, although detection frequencies for neonicotinoids were typically lower. Overall, the herbicides and non-neonicotinoid insecticides were found with low frequency and at low concentrations. For most pesticide classes, trends for the mean concentration were DBT > IH nurse bees > field pollen > IH larvae > IH honey. Pesticides were detected in 100% of the pollen, dead bee trap, nurse bees, larvae, bee bread and honey samples analyzed with concentrations ranging from 0.01 ppb (diphenylamine) to 2790 ppb (clothianidin). Among all targeted analytes, N-(2,4-dimethylphenyl)formamide (DMF; amitraz metabolite, a miticide commonly used by beekeepers) was detected at the highest frequencies in dead bee traps, nurse bees, larvae, bee bread, and honey. All samples analyzed were contaminated with at least two pesticide residues, while 19 samples presented over ten detects and a maximum detect of 20 in a dead bee trap sample. Pesticide residues were positively correlated with agricultural gradients across sites and sampling periods. Ultimately, these data will inform how the foraging nature of honey bees permits colony-wide exposure to pesticides.

4.03.P-Mo-106 Assessment of Baseline Bioaccessible Pesticide Loads in the Sacramento Deep Water Ship Channel

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The Sacramento Deep Water Ship Channel (SDWSC) in the San Francisco Estuary is critical habitat to the threatened pelagic fish species *Hypomesus transpacificus* (Delta smelt). Their population decline has been attributed to covarying factors such as manipulation of their habitat, introduction of invasive species, decrease in food production, and pesticide contamination. Despite pesticide contamination being a potential factor, prior work in the SDWSC on quantification of bioaccessible contamination loads is limited. To characterize pesticide contamination and to inform a potential future action of reintroducing the Sacramento River to the SDWSC,

sediment, zooplankton, and suspended solid samples were collected seasonally from three different sites along the channel as well as one larger longitudinal event across six sites. An additional zooplankton sample was collected from each location for identification, with calanoid copepods being ubiquitous across all sites and sampling periods. In the assessment of boat traffic's effect on bioaccessible pesticide contamination, samples were collected before and after a boat passed through a single point in the system. A subsample of the surficial sediment was extracted using Tenax to measure the bioaccessible fraction of sediment-associated contaminants. Zooplankton, suspended solids, and sediment underwent total exhaustive chemical extraction via accelerated solvent extraction. All sample matrices were analyzed for 16 organochlorine pesticides (OCP) and 6 current-use pyrethroid insecticides using gas chromatography-mass spectrometry. Sediment characteristics including particle size distribution and total organic carbon were assessed to show the influence of physical characteristics on pesticide partitioning. Using Tenax extractions, bioaccessible OCP concentrations were found at nearly all sites, particularly p,p'-dichlorodiphenyldichloroethylene, alongside bifenthrin at multiple locations. Total pesticide concentrations extracted from zooplankton samples tended to be higher than both the bioaccessible and total sediment pesticide concentrations on a dry weight basis. These findings confirm the presence of bioaccessible pesticide concentrations in the SDWSC, which are indicative of the potential uptake of hydrophobic organic contamination by Delta smelt and other biota residing in the system.

4.03.P-Mo-107 Transformation and Metabolism of 6PPD and 6PPD-quinone in Different Soils Conditions

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Large amounts of tire wear particles (TWPS) are released into the environment through friction between the vehicle tire and the road surface, which provides a pathway for tire additives to enter the environment. Studies have shown that 6PPD-quinone (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone), an ozonated derivative of the common tire rubber antioxidant 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine), caused acute mortality in coho salmon after exposure to urban run-off and has highly toxic to aquatic organisms. 6PPD and 6PPD-quinone have been found to be prevalent in the environment, but little is known about the environmental behavior and fate of 6PPD and 6PPD-quinone in soils. The study was conducted to investigate the environmental behavior of 6PPD and 6PPD-quinone in different soil conditions. Two different roadside soils and a sediment were used to study the metabolism of 6PPD and 6PPD-quinone under aerobic and anaerobic conditions, respectively. Differences in the degradation of 6PPD and 6PPD-quinone in sterilized and unsterilized soils were compared to assess the role of microorganisms in soil degradation. In addition, three different electron acceptors (NO_3^- , SO_4^{2-} and Fe^{3+}) were added to the sediments to further study the transformation kinetics and transformation products of 6PPD and 6PPD-quinone under different redox potential of soils. This study comprehensively analyzed the transformation and metabolism of 6PPD and 6PPD-quinone under different soil conditions, providing a reference for scientific evaluation of environmental risk and pollution remediation of 6PPD and 6PPD-quinone in soils.

4.03.P-Mo-110 Using Lampricide and Sea Lamprey (*Petromyzon marinus*) to Study the Effects of Temperature on Phenol Toxicity

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The persistent presence of organic aromatic phenol (hydroxyl derivative of benzene) contaminants in the environment attributed to industrial waste is a continually growing concern. Phenol toxicity leads to rapid and extensive compound accumulation in the tissues, attributed to its dual hydrophilic and lipophilic characteristics, which in turn will cause the breakdown of cell membranes and impairment of ATP production, leading to death. Most of what is known about phenol toxicity comes from past medical uses and accidental workplace exposures where high concentrations quickly lead to organ failure and death, so toxicity studies and sublethal effects are hard to extrapolate. One exception to this is the extensive implementation spanning the last 60 years of lampricide (lamprey-specific pesticide) TFM (3-trifluoromethyl-4'-nitrophenol) applications to successfully

control parasitic sea lamprey populations in the Great Lakes. TFM was chosen from over 4000 chemicals tested because, when compared to non-target fishes, larval sea lamprey are more sensitive to TFM due to their limited ability to detoxify and excrete the lampricide. This results in faster TFM accumulation within the tissues leading to impaired mitochondrial ATP production, followed by the depletion of anaerobic energy stores and eventual death. In the aquatic environment, TFM behaves like a typical phenol where environmental factors, such as pH and alkalinity, can significantly influence TFM effectiveness by altering its bioavailability. Recent work suggests that water temperature may also influence TFM toxicity, which may explain why sea lamprey TFM tolerance increases in the summer. In the present study, we tested the hypothesis that this greater TFM tolerance is due to a greater capacity to detoxify TFM due to their higher metabolic rates. LC-MS/MS was used to measure TFM within the liver and muscle of lamprey acclimated to one of three temperatures (6, 12 or 24°C) and exposed to 12-h LC₂₅ TFM concentration measured in 12°C acclimated fish. Lower TFM accumulation was observed in the tissues of sea lamprey at warmer temperatures, supporting our hypothesis that sea lamprey's ability to survive TFM treatments at warmer temperatures was due to an enhanced capacity to detoxify TFM. These findings suggest that TFM and sea lamprey could be a good model system for studying phenol contaminants as they can provide answers to phenol fate within the environment and the organisms found in those environments.

4.03.V Analysis of Pharmaceuticals, Pesticides, and Other Chemicals in Environmental Matrices to Support One Health

4.03.V-007 Spatial Distribution, Temporal Trend, and Risk Assessment of Cyclic Volatile Methylsiloxanes in Tokyo Bay Catchment Basin, Japan

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Methylsiloxanes are high-performance materials used in diverse applications, such as transportation, construction, electronics, industrial processes, health care, and personal care. However, a part of cyclic volatile methylsiloxanes (cVMSs) has recently been identified as priority chemicals for environmental risk assessment due to their persistence in the environment and bioaccumulative potency. In this study, we investigated the spatial distribution, temporal trends, and aquatic risk assessment of cVMSs, including octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5), and dodecamethylcyclohexasiloxane (D6) in surface water from the Tokyo Bay catchment basin, Japan. River water samples (n=359) collected from more than 100 locations were measured for D4, D5, and D6. We observed widespread distribution of D4, D5, and D6 in the water samples, with concentrations of <0.4–160 ng/L, <1–1510 ng/L, and <0.2–209 ng/L, respectively. Mean concentrations of cVMS from 39 fixed-locations during 2013–2021 ranged from 9.8–14.4 ng/L for D4, 138–193 ng/L for D5, and 12.3–20 ng/L for D6, with the detection frequencies of cVMSs greater than 84% for all survey years. It appears that the annual mean concentrations of cVMS found in river water have been gradually decreasing since 2013, with levels varying from 38% to 56% on a year-2013 basis. This could potentially be connected to a decrease in the usage of cVMS and related compounds in rinse-off type cosmetics due to the regulations implemented in the European Union. In risk assessment of the adverse effects of D4 and D5 in aquatic organisms, the respective distributions indicated no overlap between the 95th percentile field water concentration and the 5th percentile chronic no-effect concentration in aquatic organisms. The results of probabilistic risk assessment for D4 and D5 were not high enough to indicate a threat to aquatic organisms in the study area. For D6, the field water concentrations were far low compared with the predicted no-observed effect concentration. As cVMS concentrations have shown a decreasing trend during 2013–2021, the future aquatic environmental risk is expected to be even lower. However, cVMS has a high affinity to

organic phases, and elevated concentrations of cVMSs in the Tokyo Bay sediment were reported. Further research will be conducted to elucidate the accumulation behavior of cVMSs through sediment and benthic food webs.

4.04.P-We Can You See What I See: Taking A Look at Nanoparticle Environmental Interactions

4.04.P-We-069 Antimicrobial Resistance in Surface Water Pathogens Induced by Silver Nanoparticles

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Antimicrobial resistance is estimated to cause 700,000 deaths globally and 23,000 deaths in the United States annually. The widespread use of silver nanoparticles (AgNPs) as antimicrobial agents in antibiotics and consumer products to overcome antimicrobial resistance has raised concerns about their release into the environment and ability to induce the development of antimicrobial resistance (AMR). This study examined the potential for AgNPs to induce AMR in *Escherichia coli* O157:H7 and *Pseudomonas aeruginosa* isolated from Horseshoe Lake located in a State Park in Illinois. Citrate-coated AgNP and uncoated AgNP were synthesized and characterized for size, surface plasmon resonance, and zeta potential. Water samples collected from the lake were processed using heterotrophic plate count to determine AgNP effects on microbial populations. Selective media were used to isolate *E. coli* O157:H7 and *P. aeruginosa* followed by biochemical and molecular techniques for identification. Isolated colonies were treated with different concentrations of citrate-coated AgNP and uncoated AgNP in a nutrient broth in triplicates and incubated for 24hrs. Aliquots of the broth were inoculated into Mueller-Hinton agar containing ampicillin, ciprofloxacin, doxycycline, gentamicin, levofloxacin, streptomycin, tetracycline for susceptibility testing using Kirby-Bauer method. Both citrate-coated AgNP and uncoated AgNP reduced microbial populations, but citrate-coated AgNP exerted a greater antimicrobial effect. Eight out of 56 bacterial isolates were resistant to one or two antibiotics compared to the controls. One *E. coli* O157:H7 and two *P. aeruginosa* isolates treated with uncoated AgNP were resistant to ampicillin and gentamicin, respectively. Five *P. aeruginosa* isolates treated with citrate-coated AgNP were resistant to gentamicin and ciprofloxacin only. The findings of this study suggest that contaminants of emerging concern may be part of the driving forces behind the development of antimicrobial resistance in aquatic systems among other resultant effects of their mechanisms.

4.04.P-We-071 Examining Toxicity of 2D Nanomaterials, Nanocomposite Membranes and Their Potential for Removal of Per-And Polyfluoroalkyl Substances

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An inorganic 2D nanomaterial, phosphorene, due to its unique electronic and photocatalytic properties has a potential for the development of the nanocomposite membranes with the purpose of removal and degradation of the persistent organic pollutants, such as per- and polyfluoroalkyl substances (PFAS). PFAS are recognized as contaminants of global concerns with a significant risk to human health. The aim of this research is to apply toxicity evaluations to govern development of safe nanocomposite phosphorene membranes for PFAS removal from drinking water. The toxicity assessment of the polymeric membranes alone and phosphorene, in, free-standing form and after it has been embedded into the membrane, was conducted using a nematode *Caenorhabditis elegans*, which serves as a powerful toxicity model of various contaminants, including nanomaterials. When *C. elegans* were exposed to different pHs (pH 3, 5, 7, 9) in a moderately hard reconstituted water (MHRW) at different filtration orders (unfiltered, 1x, 5x, and 10x filtration) through the membranes without nanomaterials, there was no significant difference in mortality at pH 5, 7, and 9. For the free phosphorene, the concentration-dependent relationship was established for mortality and reproduction endpoints with exposure in MHRW at neutral pH. However, significant mortality was only observed at 45 mg/L of phosphorene concentration while reproduction was more sensitive with inhibition at 2mg/L and above. After phosphorene was embedded into the membrane, the release of phosphorene from the nanocomposite membrane was below detection limit and no toxicity was detected after multiple filtrations of the MHRW at neutral pH.

Further research is required for the toxicity assessment using more sensitive endpoints, low and high pHs and filtration orders when nanomaterials are embedded in the membranes. The nanofiltration membranes were also tested for removal and degradation of Perfluorooctanoic acid (PFOA) and on average, there was 99% removal of the PFOA accumulated on the surface of the membrane. The SEM images and the atomic profile scans of the membrane surface via X-ray photo electron spectroscopy (XPS) provided some evidence for potential breakdown of PFOA into smaller fluorine compounds. These results are promising for potential application of the phosphorene membranes for removal and breakdown of PFAS in drinking water.

4.04.P-We-072 Resilience of Two 3D Printed Polymer Nanocomposites to UV-Degradation in Environmental Applications

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The photocatalytic properties on nano-scale TiO₂ are well studied for degrading organic contaminants in water. However, in certain surface water applications dispersal of free TiO₂ nanoparticles may be undesirable. In previous research we demonstrated that TiO₂ can be successfully immobilized and 3D printed using the thermoplastic polymer polylactic acid (PLA). PLA was selected since it is derived from natural sources (corn, sugarcane, etc.) and is thus more biocompatible and sustainable than hydrocarbon-based polymers for environmental studies. The 3-dimensional PLA-TiO₂ structures showed similar photocatalytic efficacy to that of free TiO₂ particles based on methylene blue dye studies. Additional studies demonstrated the PLA-TiO₂ composite structures degraded polycyclic aromatic hydrocarbons (PAHs), per- and polyfluoroalkyl substances (PFAS) and harmful algal bloom toxins. The current study investigates the resilience of the PLA-TiO₂ composites to UV light and aqueous challenges designed using the previously developed and freely available NanoGRID/AdMGRID environmental health and safety tools. The accelerated UV-weathering studies were prioritized since free radicals formed during UV-light irradiation were hypothesized to degrade PLA polymer chains in repeated use applications based on earlier onset of thermal degradation of the composites relative to neat PLA in thermogravimetric analysis. The polymer-TiO₂ composite structures were subjected to accelerated weathering using the ATLAS SUNTEST XL+ cabinet employing ISO method 4892-2 followed by the Toxicity Characteristic Leaching Procedure (TCLP) in moderately hard reconstituted water. Two days of intense accelerated weathering resulted in negligible increases in TiO₂ in the water while 14 days intense accelerated weathering resulted in a two order of magnitude increase in aqueous TiO₂ concentrations. Polyethylene terephthalate glycol (PETG) TiO₂ printed composites were also studied to determine the relative durability of a potentially more UV-resilient thermoplastic polymer in repeated use applications. Results will be presented on the relative rates of TiO₂ release from the different polymer matrices for un-weathered samples, samples weathered under environmentally relevant solar simulation and after intense accelerated weathering. The findings will inform the useful lifetime of printable photocatalytic composites in surface water applications.

4.04.P-We-076 Adsorption of CuO-based Nanopesticide on Chilean Volcanic Soils

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The purpose of present research is to predict the environmental fate of engineered nanomaterials. A key aspect to accomplish this is to study the adsorption processes over relevant adsorbents such as complex soils. However, there are several experimental parameters that influence the obtainment of repeatable results of nanoparticle adsorption process. Here, the classical batch adsorption characterization methods, (kinetics and isothermal) of homogeneous solute over solid adsorbents are adapted for non-conventional heterogeneous nanoparticle suspension adsorption over complex adsorbents such a Chilean Ultisol (Metrenco) and Andisol (Ralun) samples. The developed methodology enables the obtainment of repeatable outcomes for adsorption points with low standard deviations. Adsorption kinetics, are adequately fitted by the pseudo second order adsorption model, thus describing an interaction of NORDOX nanoparticles at two adsorption sites over the soil

sample. On the other hand, adsorption isotherm, displays S-class shape curve possibly meaning that activation energy for solute removal of NORDOX from soil is concentration-dependent. Also, isotherm experimental points are well fitted by the Langmuir-Freundlich model, meaning that the adsorption process consist on a combination between physical and chemical interactions. In conclusion, the outcomes agree well with the known dependence between nanoparticle suspension stability with concentration for analogous reported systems. Classic batch-adsorption method can be adapted for nanoparticles as solute. Finally, the intensity of the interaction between the nanoparticle and the soils allows to infer that the particles will tend to be retained by the soil with low risk for mobilization following aquifer contamination. But, this particles could then interact with plant, considering that these soils have high agronomic potential.

4.04.T Can You See What I See: Taking A Look at Nanoparticle Environmental Interactions

4.04.T-01 The Effect of Nanoparticle Surface Charge on Microalgal Growth and Morphology

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As use of engineered nanoparticles increases, there is a need to understand the relationship between nanoparticle properties and their interactions with biological systems in order to reduce environmental and health risks posed by nanotechnology. Although the impact of nanoparticle surface charge on nano-bio interactions with algae has been studied, we lack studies about the toxicity of differently charged nanoparticles to microalgae, key species in aquatic ecosystems. In this study, the impact of nanoparticle surface charge on nano-algae interactions was investigated using functionalized carbon dots (CDs) and the green freshwater algae *Raphidocelis subcapitata* as a model system. In addition to growth inhibition, a traditional measure in algal toxicology, high-content imaging was utilized to determine the morphological impacts of carbon dot exposure on cells and organelles of *R. subcapitata*. Results indicate that positively charged polyethylene amine (PEI) functionalized CDs were the only particles to induce significant toxicity. However, exposure to carboxylated PEI (CP) and polypropylene vinyl (PVP) CDs with zeta potentials below 0 was associated with sublethal, morphological impacts. These included changes in cell size and increased lipid droplet accumulation. Future work should investigate the mechanisms by which these impacts occur, thereby supporting environmental protection and sustainable nanoparticle design efforts.

4.04.T-02 Aquatic Toxicity of Nano Tin Oxide to *Ceriodaphnia dubia*, *Daphnia pulex*, *Hyalella azteca*, and *Chironomus dilutus*

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Nano tin oxide (SnO₂) is a material used in sensors for its high sensitivity, stability, and affordability. With expanded use, the potential impact of the nano enabled products have on aquatic organisms must be considered. The objective of this study was to determine the toxicity of nano tin oxide to a variety of standard aquatic toxicity organisms across several points of exposure. *Ceriodaphnia dubia* and *Daphnia pulex* were chosen as they are pelagic invertebrates exposed to nano material in the water column. *C. dubia* and *D. pulex* are also a model species for toxicity determination, with *D. pulex* having a developed oxidative stress gene panel. *Hyalella azteca* and *Chironomus dilutus* were chosen as epibenthic to benthic organisms that interact with the nano material as it settles out of the water column. The cladocerans, *C. dubia* and *D. pulex*, were exposed to nano-SnO₂ at 0, 6.3, 12.5, 25, 50 and 100 mg/L (nominal), with no significant impacts on survival or transcriptional expression of oxidative stress-related genes compared to after an acute (48 hr) exposure. The results indicate there is no toxicity to organisms in the water column. However, it was observed nano-SnO₂ settles out of the water column and remains in the benthic zone potentially leading to a higher rate of exposure. Therefore, toxicity tests were conducted using *H. azteca* and *C. dilutus*. After a 10-day exposure to nano-SnO₂ at 0, 6.3, 12.5, 25, 50 and 100 mg/L (nominal), it was determined there is no significant impact on survival or

growth of the organisms from the control or lower concentrations. As a result of these tests, we can conclude there is no acute toxicity occurring with the nano tin oxide within the water column at the benthic level up to 100 mg/L spiking levels.

4.04.T-03 Transcriptomic Response in *Caenorhabditis elegans* Exposed to the Multiple Stressors Zinc Oxide Nanoparticles and Soil-borne Pathogen, *Klebsiella pneumoniae*

Jarad Cochran, Jason Unrine and Olga V. Tsyusko, University of Kentucky

Nanoparticles, such as Zinc-oxide nanoparticles (ZnO-NP) are rapidly being incorporated into agricultural practices as precision fertilizers. To assess their potential environmental risks, we examined the toxicogenomic response of the model soil nematode, *Caenorhabditis elegans*, exposed to ZnO-NP. Additionally, because organisms in nature are often exposed to multiple sources of stress, we assessed nematodes infected with *Klebsiella pneumoniae*, a common soil-borne pathogen. A ZnSO₄ treatment was included to account for the toxicity associated with dissolved Zn ions. To assess the combined stress, nematodes were exposed to EC₃₀ concentrations of Zn with the pathogen for 8 hours. When exposed to the stressors individually, reproduction decreased for all treatments. However, combined exposures resulted in reproduction that was not significantly different from controls. Transcriptomic responses at the EC₃₀ for reproduction resulted in a total of 7,967 significantly differentially expressed genes (DEGs) at fold change of ± 1.5 compared to controls. Analysis of transcriptomic data shows that there are only 10 unique DEGs in response to ZnSO₄ while the other DEGs are shared with the individual or combined ZnO-NP treatments, which indicates that some of these responses are due to NP dissolution. The gene expression profile is much stronger in response to ZnO-NP compared to ZnSO₄, with 1154 unique DEGs, likely due to particulate Zn precipitating on *C. elegans* surfaces. Among the genes induced at 10-fold in Zn exposed treatments are the metal response genes (*mtl-1*, *mtl-2*, *numr-1*, and *numr-2*). In individual *K. pneumoniae* exposures, the gene encoding metabolizing enzyme, *fmo-2*, had a 100-fold increase in expression. Despite individual Zn exposure not affecting *fmo-2* expression, in the combined treatments, its expression is above 200-fold. The decrease in reproduction from Zn exposure can partially be explained by drastic reduction in expression of vitellogenin associated genes (*vit-1*, *vit-3*, *vit-5*, and *vit-6*). Interestingly, *vit-1* and *vit-3* are overly expressed only in *K. pneumoniae* exposures. Continuing analysis of the transcriptomic data and validation in respective mutant and reporter strains are being conducted to elucidate the potential pathways associated with these genes and how they may affect antagonistic response of *C. elegans* to ZnO-NP and *K. pneumoniae*.

4.04.T-04 Field Scale Agricultural Applications of Nanopesticides for Fate, Transport, and Impact to Nutrient Cycling

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Pesticide applications are a necessary part to increasing agricultural production for the growing world population, but bulk pesticide application has resulted in environmental contamination, human toxicity, and endangerment to non-target species (i.e, honeybees, monarch butterflies). Nanoengineered particles (ENP) are a potential solution with increased efficiency, longer duration, and enhanced stability of pesticides. However, the potential long-term impact to agroecosystems is still unknown. Therefore, the goal of this project was to evaluate the fate, transport, and persistence of two nanopesticides (copper(II) hydroxide and nano-imidacloprid) within soils and runoff water of agricultural systems in Central Kentucky. It was hypothesized the nanopesticides would remain in the soil with the potential for toxic byproducts produced from interaction with the soil carried by surface runoff. The study has been accomplished with a field scale application of the pesticides using 2.4 m by 6.1 m plots enclosed with metal boundaries. Surface water and soil samples were collected following rainfall events and throughout the growing season. Samples were analyzed for nutrients, pesticide, and pesticide byproducts along with physiochemical soil/water characteristics (i.e., pH, specific conductivity, temperature). This data has shown that these pesticides do not have a significant impact on

nutrients that are leaving the systems, and currently are impacting the systems. Further, findings will provide guidance for nanopesticide regulation and application standards to prevent ecological disasters.

4.04.T-05 Impacts of Functionalized Polystyrene Nanoplastics on Morphology of Rainbow Trout Gill Epithelial Cells

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Plastic production in 2020 has reached a total of 367 million tons (Mt) with 6.1% of this being the polymer polystyrene (PS). Due to the abundance of plastics in aquatic ecosystems, their breakdown into nanoplastics (NPs), and their adverse impacts to aquatic organisms at the cellular and molecular level, NPs have become an emerging contaminant of concern. Previous studies have shown that plastic particles can be ingested and assimilated by aquatic organisms resulting in the buildup of NPs in fish gill tissues. Furthermore, the surface chemistry of NPs plays a major role in the interactions at the nano-bio interface. In this study, an epithelial cell line from rainbow trout gills (RTgill-W1) was used as an *in-vitro* ecotoxicological model to investigate the impacts of NP surface chemistry using (Plain-PSNPs), carboxyl-functionalized (COOH-PSNPs), and amino-functionalized (NH₂-PSNPs) polystyrene nanoplastics. More specifically, AlamarBlue™ were used to assess viability in response to PSNPs, respectively. Additionally, fluorescence microscopy was utilized to determine morphological impacts on the RTgill-W1 cells. This study demonstrated that relative to the uncharged and negatively charged PSNPs, the positively charged PSNP negatively impacted cell viability the most. Furthermore, cells exposed to the positive PSNPs suggest an increase in nuclear DNA, decrease in cell area, and cell count via bio-imaging analysis. Future work will focus on further morphological profiling of cells after nanoparticle exposure.

4.04.T-06 Epigenetic Changes in *Caenorhabditis elegans* after Multigenerational Exposure to Pristine and Transformed Silver Nanoparticles

Olga V. Tsyusko, Anye Wamuch, Jason Unrine and John May, University of Kentucky

Multigenerational and transgenerational reproductive toxicity in a model organism, a nematode *Caenorhabditis elegans*, was observed previously after exposure to silver nanoparticles (Ag-NPs) and silver ions (AgNO₃). To gain a better understanding of the transfer mechanism of the increased reproductive sensitivity to subsequent generations, in this study we examined epigenetic changes in histone and DNA global methylation levels after multigenerational exposure of *C. elegans* to pristine Ag-NPs and transformed via sulfidation sAg-NPs and AgNO₃. Among the selected histone methylation marks were H3K4me₂ and H3K9me₃, while changes in DNA methylation were examined using N⁶-methyl-2'-deoxyadenosine (6mdA). The nematodes were exposed at equitoxic Ag concentrations at EC₃₀ for three generations with subsequent rescue for three more generations. The histone methylation levels and 6mdA levels were measured prior and after the exposure as well as after rescue using ELISA and high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS), respectively. Histone methylation levels at H3K4me₂ increased in response to pristine Ag-NPs and did not recover after rescue from the exposure, suggesting transgenerational inheritance. Compared to pristine Ag-NPs, exposure to transformed sAg-NPs significantly decreased H3K4me₂ and H3K9me₃ levels. For DNA adenine methylation, only AgNO₃ exposure caused a significant increase in global 6mdA level after exposures. However, after rescue, the 6mdA level were not maintained and did not significantly differ from those in Controls. Pristine and sulfidized Ag-NP treatments did not induce significant changes in global DNA adenine methylation. Overall, our results demonstrate that multigenerational exposure of *C. elegans* to Ag-NPs and not AgNO₃ induces epigenetic changes at histone methylation marks that are inherited by unexposed offspring. Exposure to AgNO₃ affects DNA methylation levels but without transgenerational epigenetic inheritance. Thus, despite both pristine Ag-NPs and AgNO₃ causing multigenerational toxicity, our epigenetic results suggest different toxicity mechanisms for these treatments. Additionally, as indicated by the decrease in histone methylation levels in response to sAg-NPs, the environmental transformation of Ag-NPs decreases this multigenerational reproductive toxicity potentially via epigenetic mechanisms.

4.05.P-We-078 Per- and Polyfluoroalkyl Substances (PFAS) in South Africa: A Survey of Variable Feedstock Composts

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Per- and polyfluoroalkyl substances (PFAS) pose serious toxicological concerns due to their persistence in the environment and potential adverse health effects. Their extensive presence has been measured in natural environments where they are theorized to permeate into animal food streams and compost derived from animal products. Thus, the presence of PFAS in compost is of significant concern, as compost is applied to agricultural products for human use, possibly leading to their subsequent incorporation into human food streams. PFAS have been observed in composted biosolids, food-waste compost, and commercially-available potting mixes, contributing to an overall pervasion of PFAS into agricultural products that use compost as organic fertilizer. However, the differences in concentrations and profiles of PFAS among the large variety of compost types are not well understood. In this study, 18 South African composts were obtained from different compost facilities and commercial sources and were compared to 10 commercially-available composts in the United States. A diverse range of composts were selected for this preliminary survey, including a range of facilities, feedstock materials (food waste, yard waste, frass, manure, compostable paper and other plant-fiber products), and composting methods (windrow, lasagna beds, vermicompost, bokashi). Samples were extracted in triplicate using a solids rotation extraction and analyzed for >90 PFAS via ultra-high performance liquid chromatography tandem mass spectrometry. Since South African industries do not, to our knowledge, manufacture PFAS, their detection in locally produced composts could indicate the migration of PFAS from imported products, feedstock materials, or general environmental contamination into the final compost product. Analyzing PFAS concentrations in South African composts in comparison to U.S. composts provides valuable information regarding the global dispersion of these compounds and the understanding of their persistence in waste streams, while also beginning the discussion of navigating the PFAS issue in the composting process.

4.05.P-We-079 Tracing the Potential Nutrient Pollution Sources in Urban Watersheds: Utilizing Organic Chemical Tracers for Source Apportionment to Biscayne Bay (Florida)

Kassidy Troxell, Milena Ceccopieri and Piero Gardinali, Florida International University

Tracing nutrient pollution back to its source in environmental surface water can be challenging in complex urban water systems due to nutrients themselves not being source specific. Phosphorus is a naturally occurring element and an essential nutrient for algae and aquatic plant growth. However, excess amounts of phosphorus entering water bodies can lead to harmful algal blooms and other negative environmental impacts. Identifying the specific sources of phosphorus pollution is crucial for implementing effective mitigation strategies especially within a phosphorus-limited and water quality declining Biscayne Bay watershed located in southeastern Florida. In this study, three different highly urban impacted canals in Miami, Florida were assessed for a variety of water quality parameters including nutrients, metals, and organic chemical tracers. Water samples were systematically collected along the canals, starting from their origin, and extending all the way to the point where they discharge into Biscayne Bay. This sampling approach aims to provide a comprehensive assessment of phosphorus at different sections of the canals, providing insights into the spatial distribution. In efforts to source apportion the phosphorus inputs, a set of organic chemical tracers was established, specifically designed to identify and differentiate the sources of phosphorus within the canals. These tracers are chemical compounds such as pharmaceuticals, hormones, flame retardants, herbicides, and artificial sweeteners associated with various sources including septic tanks, wastewater from sewage leaks and treatment plants, and agricultural activities. The chemical tracers were analyzed by online solid phase extraction high pressure liquid chromatography coupled to a TSQ Altis™ Plus triple quadrupole mass spectrometer. This approach is aimed to

provide a more detailed understanding of the relative importance of different pollution sources along the canals, helping prioritize mitigation efforts and implement targeted measures to address specific sources of phosphorus pollution.

4.05.P-We-080 Detection of PFAS in Water Repellents in the Japanese Market and Estimation of their Environmental Impact

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PFOS, PFOA, and PFHxS are regulated under the Stockholm Convention, and PFAS, such as PFOS and PFOA, have various effects on living organisms. It is speculated that PFAS is also used in household products such as waterproofing sprays; however, the actual situation is unknown. Therefore, it is necessary to understand the PFAS present in recent waterproofing sprays to evaluate the environmental impact of their use. Accordingly, an investigation was conducted on 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 43 PFAS that can be analyzed using LC-MS/MS. N-MeFBSA and N-MeFBSE were detected in six of the past samples. The detected concentrations were 0.12–3.8 µg/g for N-MeFBSA and 1.6–53 µg/g for N-MeFBSE. It is speculated that these two PFAS are used as part of the polymers in the waterproofing sprays. In addition, PFBS, PFBA, PFHxA, PFOA, PFDA, and PFDoA were detected in eight samples. However, their detected concentrations were lower than those of N-MeFBSA and others, ranging from 0.027–0.16 µg/g. Based on the volume of waterproofing sprays surveyed, the PFAS load released into the environment when one bottle of waterproofing sprayer is used was calculated. The results showed that 9.5–7767 µg of PFAS are released into the environment. Therefore, an evaluation of the environmental persistence and ecological effects of N-MeFBSA and N-MeFBSE is required. Conversely, no targeted PFAS were detected in any of the samples purchased in 2022. Therefore, it was determined that water repellents currently distributed in Japan had been counteracted, and we have no exposure to PFOS, PFOA, and PFHxS due to the use of repellents. The behavior of current water repellents due to degradation requires investigation in the future.

4.05.P-We-081 Industrial Antioxidants and Their Transformation Products in Snow from Urban Roads in Greater Montreal, Canada: Identification and Chemometric Evaluation

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Industrial Antioxidants (IAs), particularly the *N,N'*-Substituted *p*-phenylenediamines (PPDs) class are high-production volume chemicals widely occurring in different environmental matrices. Current monitoring studies of these chemicals in snow from urban roads is not only limited to a few sampling sites but lacks chemometric evaluation of the influences from varying road traffic levels. In this study, we present a comprehensive, mass spectrometry-based insight on the geospatial distribution and road traffic influences on the occurrence of PPDs and their TPs in the urban environment. Our findings demonstrated that four PPDs and their corresponding TPs are widely detected in snow. All identified contaminants are related to vehicular emissions from roads of varying traffic levels. Amongst contaminants identified, N-(1,3-dimethylbutyl)-N-phenyl-*p*-phenylenediamine (6PPD) and its so highly toxic quinone variant, 6PPD-Q were dominant in the dissolved phase of the snow sample, with a concentration range of 5 – 740 ng/L and 4 – 1275 ng/L respectively. Identification of lesser-known PPDs and their TPs using the untargeted screening approach was based on peak intensities, retention time, frequency of detection (DF) and substructural information. By applying this approach using the Compound Discoverer software, an initial 30,000 features were generated, some of which are tentatively identified. Ongoing work is being conducted to prioritize the chemical features and confirm their identities using authentic standards based on retention time, MS1 and MS2 spectra.

4.05.P-We-082 Characterization and Treatment of Effluent from the Galvanization Industry in Vanderbijlpark, South Africa

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The discharge of untreated industrial effluent is a global environmental and health concern. High concentrations of toxic chemicals from a Galvanization Industry in Vanderbijlpark, South Africa have been discharged into receiving water bodies. Noncompliance from this industrial institution has continuously resulted in high effluent penalties from the local municipality. The present study aimed to characterize and treat galvanizing effluent using lime-anionic polyacrylamide. Effluent samples were collected from two sampling points in the galvanizing industry between September 2020 and February 2021. Sampling points were as follows: (1) raw sample from the plant and (2) treated sample from the treatment plant. Both samples were characterized for conductivity, chlorides (Cl⁻), pH, and heavy metals: Iron (Fe), Lead (Pb), Zinc (Zn), Manganese (Mn), Copper (Cu), Chromium (Cr), Cobalt (Co) and Nickel (Ni). Conductivity and pH were determined using a conductivity meter and pH meter. Inductively coupled plasma (ICP) and the Mohr method were used to measure heavy metals and chlorides. Before treatment, conductivity, (Cl⁻), and pH ranged between 9741 -16580mS/m, 8997 - 22470ppm and 0.53 -1.78, respectively. The following were ranges for heavy metals: Fe (224.75-481.82ppm), Pb (93.82-309.42ppm), Zn (36.45-1112.86ppm), Mn (7.40-29.00ppm), Cu (0.00-1.46ppm), Cr (2.12-4.72ppm), Co (0.00-0.135ppm) and Ni (0.00-0.41ppm). According to the South African discharges and local municipal by-laws, the parameters were within acceptable limits except for pH, conductivity, Cl⁻, Fe, Pb, Zn, and Mn. After effluent treatment with lime-anionic polyacrylamide, 100% removal efficiency was observed for Fe, Zn, and Mn. As for Pb, Conductivity, and Cl⁻, the removal efficiency was 92.2%, 78%, and 67.4%, respectively. The use of lime-anionic polyacrylamide was effective in treating heavy metals. However, lower % removal efficiency was observed for Conductivity and Cl⁻. These parameters should undergo further treatment such as electrocoagulation and membrane before the release of effluent into receiving water bodies.

4.05.T Chemicals in Domestic, Agricultural and Industrial Waste: Occurrence, Fate, and Use as Tracers

4.05.T-01 Discussion - Chemicals in Domestic, Agricultural and Industrial Waste: Occurrence, Fate, and Use as Tracers

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4.05.T-02 Investigating Per- and Polyfluoroalkyl Substances (PFAS) in Food-Waste Compost: Sources, Fate, and Implications for Land Application

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An impediment in the progress of food-waste (FW) composting across the US is the growing concern that land-applied compost can cycle per- and polyfluoroalkyl substances (PFAS) back into the food supply and the environment. Previous studies suggest that most compost is likely to contain PFAS, that land-applied PFAS can migrate into the edible parts of plants, and that FW compost from facilities that incorporate compostable food-contact materials (FCMs) contain more PFAS than compost from facilities that do not accept FCMs. In this study, we partnered with a pilot-scale windrow composting facility to further investigate the sources and fate of PFAS in FW compost. First, the relative contributions of PFAS from feedstocks (mulch, FW, and separated used FCMs) were compared. Then, composite samples of compost, collected at five intervals along the windrow over a period of three months to simulate a timeline of compost maturation, were used to observe potential PFAS profile transformation throughout the composting process. A combination of leach tests and methanol rotation extractions were performed and resulting extracts were analyzed for 40 PFAS via ultra-high

performance liquid chromatography tandem mass spectrometry. Preliminary data indicate that compostable food contact materials leached PFAS within the parts-per-million range, while mulch and food waste yielded fewer than one part-per-billion concentrations of PFAS. Interestingly, short-chain perfluorocarboxylic acids (PFCAs) made up the majority of total measured PFAS concentrations in FCM feedstock leachate, compost extracts and compost leachates. This pattern suggests that FCMs are typically the greatest contributor of PFAS to food-waste compost, since we know that short chain PFCAs can be both directly applied to FCMs and emerge as degradation products of precursor PFAS also commonly applied to FCMs. Furthermore, concentrations of measured PFAS consistently increased as compost matured along the windrow. This increase was primarily driven by PFCAs, which suggests precursor PFAS transformation into the more recalcitrant PFCAs. PFCAs have been more extensively measured and studied than precursor PFAS, and several PFCAs measured in this study are potential candidates for future PFAS regulations. In terms of best management practices, this data suggests that excluding FCMs from the composting waste stream could significantly reduce the load of PFAS in mature compost that is intended for land application.

4.05.T-03 Per- and Polyfluoroalkyl Substances (PFAS) Fate and Transport Following Long-term Application of Biosolids

Lynda Godwin Peter and Linda S. Lee, Purdue University

Beneficial reuse of biosolids for agricultural production concurrently boost biomass productivity, minimizes chemical fertilizer input, and improve physiochemical properties of the soil. However, the presence of PFAS in biosolids can result in redistribution and elevated levels of PFAS in the environment following land application of biosolids. This study aims to investigate the impact of soil properties on PFAS transport in a site that is currently receiving biosolids after a six-year pause on land-application. The study site is dominated by three soil series: a deep and slowly permeable Acredale silt loam, a deep and moderately permeable Bojac sandy loam, and Tomotley loam. There are also seven groundwater monitoring wells generally located along the borders of the site. Prior to resuming biosolid application, groundwater, soils, and runoff were sampled to establish the extent of legacy PFAS contamination within the site. PFAS concentrations were determined using isotope dilution with appropriate clean up steps and analysis was done using uPLC Zeno Time of Flight mass spectrometry (uPLC/ZenoTOF). Concentrations of quantifiable PFAS in groundwater varied greatly among wells depending on their spatial location and depth. Total PFAS detected ranged from 0.9 to 620 ng/L which were predominantly perfluoroalkyl acids (PFAA). PFOA had the highest maximum concentration (224.4 ng/L), followed by PFPeA (92.9 ng/L), PFHxA (78.2 ng/L) and PFBS (71.1 ng/L). Extraction and analysis of pre-application soil and runoff samples are ongoing. Additionally, surface soil composites, soil cores and groundwater are being collected periodically post biosolid application to evaluate temporal trends in PFAS. Available pre- and post-application PFAS data will be coupled with hydrologic and soil factors in identifying key factors impacting PFAS transport in the vadose zone.

4.05.T-04 Fate and Transport of Emerging Contaminants Entering, Leaving, and Flowing Past Wastewater Treatment Plants in Central Kentucky

Tiffany Messer, Kyra Sigler, Bill Ford and Wayne Sanderson, University of Kentucky

Per- and polyfluoroalkyl substances (PFAS) and pesticides are two emerging contaminant classes that have been recently detected ubiquitously throughout the environment. Wastewater treatment plants (WWTPs) have been identified as likely hotspots for introduction of PFAS and pesticides into the environment; however, there is limited information addressing the fate of PFAS and pesticides into downstream surface water and sediment following treatment. Additionally, a better understanding of the effects of unique WWTP characteristics, such as disinfection method, have on PFAS and pesticide transformation is still needed. Thus, the aim of this study was to investigate the occurrence, transformation, and fate of 18 PFAS species and 20 neonicotinoids and strobilurin fungicides and their metabolites in two WWTPs with varying disinfection processes, prevailing land uses, and during two distinct time periods. Polar organic chemical integrative samplers were deployed in

WWTP influent and effluent and placed upstream and downstream of the effluent mixing zone (EMZ) for two weeks in April and July 2022. Analytes were analyzed using an Acquity ultrahigh pressure liquid chromatography (UPLC). PFAS concentrations typically increased from influent to effluent at both WWTPs, regardless of disinfection method, but changes in surface water concentrations from upstream to downstream of the effluent mixing zones varied, likely due to upstream sources of PFAS. Nearly all PFAS persisted in aqueous compartments rather than sediment or biosolids. In contrast, neonicotinoid insecticides and strobilurin fungicides were frequently detected in aqueous samples at WWTPs and in surface water, while they were detected less frequently and in much lower amounts in sediment and biosolids, and as a result, were primarily discharged from WWTPs through treated effluent rather than in biosolids. Imidacloprid desnitro was the most prominent analyte detected in aqueous samples, indicating high usage of imidacloprid across both sampling periods and landscapes. Results from this study highlight the importance of developing effective PFAS and pesticide treatment for WWTPs as the load being discharged by WWTPs will potentially put an increasing strain on downstream drinking water source waters. Further, findings emphasize the need for consideration of additional finishing treatments (i.e., treatment wetlands) for WWTP effluent.

4.05.T-05 Per-and Polyfluoroalkyl Substances in Onsite Wastewater Treatment Systems

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Per and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants that pose risks to human health and vital drinking water resources. PFAS are detected in municipal wastewater treatment plants, though their fate is uncertain in onsite wastewater treatment systems. Many alternative onsite wastewater treatment systems rely on soil biological treatment units like fixed sand beds and leaching galleys, but the fate of known and novel PFAS, and the transformation of PFAA precursors, have not been closely investigated in these systems. In this study, influent and effluent grab samples were collected from eleven onsite wastewater treatment systems and analyzed for PFAS via targeted and untargeted high-resolution approaches. Microporous polyethylene passive samplers were also deployed to incorporate a time-integrated approach. Samples were analyzed by liquid chromatography coupled to a quadrupole time of flight mass spectrometry (LC-QTOF-MS) in data-independent mode and screened using Fluoromatch software in parallel with extensive database screening to maximize coverage and minimize false positives. Quantitative targeted analysis of passive samplers was also performed to monitor PFAS uptake over time. Thirteen targeted PFAS were confirmed with reference standards, and more than forty additional PFAS were tentatively identified across samples (level 3-4 suspects on the PFAS Confidence Scale). Results of suspect and target screening exhibit both upregulation and downregulation of target PFAAs at various onsite wastewater treatment locations. Quantitative results from passive samplers show that over time, concentrations of perfluoroalkyl acids (PFAAs) are consistently higher in effluent relative to influent, suggesting transformation of precursors is occurring. This work highlights suspect screening workflows of PFAS in onsite wastewater and showcases passive sampling of residential wastewater to describe time-averaged relative abundances of PFAS in highly variable samples. This effort ultimately provides a first look at utilizing high resolution mass spectrometry, nontarget analysis, and suspect screening for the identification of PFAS in onsite wastewater and provides essential information to inform optimization of alternative onsite wastewater treatment systems for the removal of PFAS and other organic contaminants in the future.

4.05.T-06 PFAS in Canadian Municipal Wastewater Treatment Systems: Results from 12 Years of Monitoring by Environment and Climate Change Canada

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Environment and Climate Change Canada's (ECCC) wastewater monitoring program was initiated in 2008 to monitor concentrations of chemical substances in wastewater systems as potential environmental exposure

pathways in support of risk assessment and risk management activities. We have collected samples from over 80 wastewater treatment plants (WWTPs) across Canada which have been analyzed for a multitude of substances including per- and polyfluoroalkyl substances (PFAS). The objectives of this study were to evaluate the fate of PFAS through the liquid and solids trains of typical treatment process types used in Canada, and to assess time trends of PFAS in wastewater influent, effluent, and biosolids between 2009 and 2021. PFAS were consistently detected in wastewater influent, effluent, and biosolids at WWTPs across Canada. Recently collected (2018 to 2021) data indicate both negative and positive removals of PFAS. Negative removals are attributable to transformation of unmeasured PFAS precursors during wastewater treatment. Concentrations of short-chain PFAS in wastewater influent and effluent showed consistent significant increases between 2009 and 2021. Such increasing patterns reflect the use of short-chain PFAS as replacements for phased-out and regulated longer-chained PFAS. Short-chain PFAS were not consistently detected in biosolids. Concentrations of consistently detected long-chain PFAS such as perfluorooctanoate (PFOA) and perfluorononanoate (PFNA) generally decreased over time in influent, effluent, and biosolids, which is attributable to industrial phase-outs and regulations. Concentrations of perfluorooctanesulfonate (PFOS) increased significantly in influent and did not change over time in effluent and biosolids. Several currently used PFAS replacement chemicals, such as per- and polyfluoroether carboxylates (including HFPO-DA and ADONA) and ether sulfonates (e.g., 9Cl-PF3ONS and 11Cl-PF3OUdS) were not detected in influent, effluent, or biosolids. Our results show that regulatory action and industrial phase-outs of PFOS are slow to be reflected in wastewater media, perhaps due in part to the continued presence of this substance in household products or to the complexity of these matrices leading to more uncertainty in the measurements and lower power to detect trends. These data will be useful as a baseline as the Government of Canada moves forward to address the broad class of PFAS.

4.06.P-Tu-141 Degradation Kinetics of Veterinary Antibiotics and Estrogenic Hormones in a Claypan Soil

Adam Moody, U.S. Geological Survey

Veterinary antibiotic (VA) application and estrogenic hormones are excreted with livestock waste that is then applied to agricultural lands as fertilizer, contaminating soil and adjacent waterways. The objectives of this study were 1) to investigate the degradation kinetics of the VAs sulfamethazine (SMZ) and lincomycin (LIN) and the estrogens estrone (E1) and 17 β -estradiol (E2 β) in soil mesocosms, and 2) to assess the effect of the benzoxazinoid phytochemical DIBOA-Glu (DBG), secreted in eastern gamagrass (*Tripsacum dactyloides*) roots, on VA degradation due to the ability of DBG to facilitate hydrolysis of atrazine in solution assays. Mesocosm soil was a Mexico Silt Loam collected at 0-8 cm depth; this soil represents a typical claypan soil in Missouri and portions of the Central United States. Soil mesocosms (N = 133) were treated with a single target compound (VA concentrations at 125 ng g⁻¹ dry weight, estrogen concentrations at 1,250 ng g⁻¹ dry weight), a subset of mesocosms treated with VAs were also treated with DBG (12,500 ng g⁻¹ dry weight), all mesocosms were kept at 60% water-filled pore space and incubated at 25°C in darkness. Randomly chosen mesocosms were destructively sampled in triplicate for up to 96 d (0, 1, 3, 7, 21, 48, and 96 d). All target compounds followed pseudo first-order degradation kinetics in soil. For the VAs, the soil half-life ($t_{0.5}$) of SMZ ranged between 17.8-30.1 d, and the $t_{0.5}$ of LIN ranged between 9.37-9.90 d. The VA results showed no significant differences in degradation kinetics between treatments with or without DBG, indicating that DBG facilitated hydrolysis did not affect VA degradation in soil. For the estrogens, degradation rates of E1 and E2 β were very similar (E1, $t_{0.5}$ = 4.71-6.08 d; E2 β , $t_{0.5}$ = 5.59-6.03 d); however, results showed that E1 was present as a metabolite in the E2 β treated mesocosms and vice-versa within 24 h. The VA results showed that LIN soil degradation was 2-3 times faster than SMZ, suggesting that SMZ has a greater potential to persist in soil than LIN. The interconversion of E2 β and E1 in soil increased overall persistence of these estrogenic compounds, leading to sustained soil estrogenicity. The persistence of these compounds in soil demonstrates the environmental risks associated with land application of livestock waste.

4.06.P-Tu-142 Advances in PFAS Analysis: Exploring the use of DLLME Cleanup and LC HRAM for the Analysis of EPA 1633 PFAS Target List in Clam Tissue, Mulch, and Sediment Extracts

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In this study, we report efforts made in the application of a miniaturized DLLME (dispersive liquid-liquid microextraction) cleanup approach prior to performing LC HRAM analysis of Native PFAS standard (mix) PFAC-MXG, MXJ, MXI, MXF and MXH; Extraction Internal Standards, (EIS) – MPFAC-HIF-ES and Non-extraction Internal Standards (NIS) – MPFAC-HIF-IS. Prosects of TOPSIS (Technique for Order of Preference by Similarity to Ideal Solution) method was explored in optimizing the dispersive-extraction solvent pairs and experimental parameters for effective cleanup, and concentration of PFAS in clam tissue, mulch, and sediment extracts. The results showed that the DLLME approach successfully achieved recoveries within the desired range of 50 to 150%. Notably, the DLLME approach yielded a significant improvement in the recovery of M2PFTeDA, increasing it from 58% with the SPE cartridge method to 80% with DLLME. The majority of the EIS compounds (14) displayed recoveries ranging from 58% to 149%, with an average recovery of 97%. However, five isotopic labeled compounds used as EIS namely d7-N-MeFOSE, d-N-EtFOSA, d-N-MeFOSA, d9-N-EtFOSE, and M4PFHp, had low recoveries ranging from 58% to 66%. Additionally, four EIS compounds, namely d3-N-MeFOSAA, d5-N-EtFOSAA, and M3HFPO-DA, showed recoveries between 67% and 82%. Furthermore, two compounds, M2-8:2FTS and M5PFPeA, exhibit recoveries above 150%, specifically 152% and 169% respectively. PFD_oA had the lowest LOD at 0.34 ng/g, while 7:3FTCA has the highest LOD at 97.55 ng/g, with the rest of the EIS compounds falling in between. The LOQ were determined to be 1.02 and 295.60 ng/g for PFD_oA and 7:3FTCA, respectively. The extraction time per sample was determined to be 20 minutes for the SPE procedure, with 25 mL of methanol used per sample. In contrast, the DLLME approach required only 2 minutes and utilized 120 µL of perfluoro tert-butanol and 800 µL of acetonitrile per sample for the cleanup step. Overall, these findings highlight the miniaturized DLLME method as a rapid and cost-effective alternative to the SPE cleanup procedure outlined in the 2nd Draft Method EPA 1633 for analyzing PFAS in solid matrices.

4.06.P-Tu-144 Ion Mobility Enabled Workflow for Standard-Free and Non-Targeted Identification of PFAS in Water Samples

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Per- and polyfluoroalkyl substances (PFAS) have been a health and environmental concern for more than 70 years, shortly after their development for use in many household and industrial products. At present, nearly 5000 compounds are included within the commercial PFAS family, all with at least one perfluorinated methyl group (-CF₃) or at least one perfluorinated methylene group (-CF₂-). Accurate and comprehensive screening for this ever-growing list of PFAS is challenged by many factors, including a lack of reference standards. A broad, standard-free approach to directly screen for PFAS in water samples was tested using the timsTOF Pro 2 system. The use of trapped ion mobility separation improved resolution quality within the MS and MS/MS data pools, increasing the number of features detected while providing characteristic collision cross-section (CCS) values for each. PFAS signals were filtered from the complex data sets by applying Kendrick mass analysis. Based on the molecular structures of an assembled list of well-known and characterized PFAS contaminants, in-silico fragmentation patterns and CCS values were predicted and matched against the observed data, and corresponded to those which could be matched based on analytical standards. The data collected also enabled rapid separation and putative identification of untargeted PFAS in spiked water samples, using MetaboScape tools to mine public databases for candidate compounds for comparisons of elemental compositions, fragmentation patterns, and CCS values. Separation by trapped ion mobility using data-dependent MS/MS provided a broad pool of high-quality MS and MS/MS data, with MS/MS coverage near 95%. Kendrick mass analysis successfully filtered the data due to shared CF₂ moieties and can be used for exploratory PFAS screening. Forty-six governmentally monitored PFAS were identified in the tested water samples from an

extended target list created using structural information derived from InChI coding, with no standards needed. Statistical analyses within MetaboScape permitted clear differentiation between the control and spiked water samples for the putative identification of additional PFAS. This workflow shows excellent potential to push the analytical limits for standard-free PFAS screening to support improved environmental protection.

4.06.T Environmental Fate of Organic Contaminants: Kinetics, Mechanisms, Transformation Products, and Application of High-Resolution-Accurate Mass Methods

4.06.T-01 Natural and Bio-Stimulated Removal of Nitrotriazolone in Contrasting Freshwater Sediment Systems Using Stable Isotope Tracers

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The environmental fate of insensitive high explosive compound nitrotriazolone (NTO) has not been adequately identified in surface freshwater environments. The goal of this study is to evaluate and quantify removal of NTO in freshwater sediment systems under natural and bio-stimulated conditions. We conducted aquaria-scale experiments using three freshwater sediment types: low organic carbon (OC) river sand, high OC wetland sediment, high OC pond silt with and without submerged macroalgae to investigate natural attenuation of NTO. Similarly aquaria-scale bio-stimulation experiments were also conducted using high OC pond silt mixed with two types of organic matter: leaf litter (high C:N) and algae (low C:N) to compare the differences in NTO processing rates over natural attenuation. 50 L aquaria maintaining oxic water column and hypoxic/anoxic sediment layer were spiked with isotopically labelled NTO (both ¹³C and ¹⁵N labelled NTO for natural experiments while only ¹⁵N labelled NTO for bio-stimulation experiment) as a single pulse input with a target concentration of 1.5 mg L⁻¹. Experiments were conducted over three weeks, and time series aqueous samples and sediment samples were collected. Dissolved NTO and inorganic mineralization products including ¹⁵NO_{2,3}, ¹⁵NH₄⁺, ¹⁵N₂O, ¹⁵N₂, and ¹³C-DIC (dissolved inorganic carbon) and total ¹³C and ¹⁵N isotopic compositions of solids in the aquaria were quantified. Adsorption of NTO or its derivatives onto solids was not a significant path of environmental fate of NTO. However, mineralization of NTO was seen in all the treatments and the quantity of mineralization products formed decreased from wetland > pond silt + submerged macrophyta > pond silt > river sand treatments under natural environmental conditions. We observed the highest rates of NTO loss and mineralization in the organic matter addition experiments compared to all other natural treatments. Two isotope tracers, ¹³C and ¹⁵N based total mineralization kinetics and mass balances of NTO in each of these treatments will be modelled separately and validated to get a robust estimate for NTO mineralization. The results of this study will propose mineralization pathways, identify possible factors affecting mineralization and therefore have the potential to aid in parameterization of fate and transport models and contaminant management schemes.

4.06.T-02 Improving the Robustness of Persistence Assessments in Surface Water – Sources of Variability and New Reference Substances in the OECD 309 Test Guideline

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For many environmental organic contaminants, degradation is a major determinant of their environmental exposure and risk. The OECD 309 test guideline describes a standard method for assessing degradation in surface waters, and is the preferred method for generating data for persistence assessments under EU REACH. However, experience with the method is still relatively limited. The guideline describes certain conditions that must be adhered to. However, there are also discretionary options (e.g., inoculum characteristics and storage, test temperature, experimental set-up), which potentially lead to variation in observed degradation. Reference substances are used to evidence an active inoculum. However, it is suggested that currently recommended reference compounds (sodium benzoate and aniline) do not provide a good indicator of inoculum performance due to their high biodegradability. The use of less easily degraded reference substances has thus been suggested.

Further, the application of benchmarking using substances of known persistence is another potential option for improving robustness of persistence assessments. Existing validity criteria within the OECD 309 test guideline are also somewhat vague and have not been verified for running the tests at lower temperatures, as is now the practice for persistence assessments under certain regulatory regimes. The Cefic-LRI ECO55 project has sought to address these issues by identifying potential new reference substances for use in OECD 309, and to explore sources of variability that may affect the robustness of these tests. A list of candidate reference substances was identified by first searching the literature, and then screening the list based on biodegradability, commercial availability and physical-chemical properties. The shortlist of candidates underwent biodegradation screening testing to select the two candidate reference substances: caffeine (positive) and 2,4-D (negative/benchmark). Experimental sources of variability were also investigated. Aniline, caffeine and 2,4-D were tested according to OECD 309 to assess their performance under various conditions. An interlaboratory ring trial is planned to validate proposed candidates for use as reference substances under OECD 309. The results of this work are intended to support an update to the guideline to improve its robustness and support reaching reliable persistence conclusions in regulatory processes.

4.06.T-03 Evaluating Droplet-Accelerated Chemistry for the UV-Photooxidative Remediation of PFAS

Riley Madison Weatherholt, Bailey Bowers, Kaitlyn L Chung and Ryan C. Sullivan, Carnegie Mellon University

Given their toxicity, bioaccumulative potential, recalcitrance, and ubiquity in the environment, Per- and Polyfluorinated Alkyl Substances (PFAS) are a threat to human health and the environment. The US EPA recently proposed maximum contaminant levels of 4 ppt for legacy PFAS like perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), which would require effective and scalable remediation strategies to achieve. However, the high strength of carbon-fluorine bonds makes this process energy-intensive, and the low concentration of most PFAS compounds in water makes this chemically challenging. In addition, complete mineralization is rarely achieved, and many approaches are not scalable. UV-photooxidation, which involves the use of ultraviolet (UV) light to produce oxidants like the hydroxyl radical and ozone in situ from gas-phase photochemistry, is a promising technology for breaking down partially fluorinated substrates such as 6:2 fluorotelomer carboxylic acid (6:2 FTCA). Though, in bulk solution the main transformation products are typically shorter-chain PFAS, which are often still persistent and toxic. However, the high surface activity of PFAS means their concentration is enhanced through aerosolization. This property could afford a concentrated micro-environment in the droplet phase, which has the potential to accelerate the rate of oxidation and further transformation, particularly with gas phase oxidants at the air-water interface. This work investigates the rate of UV-photooxidation of 6:2 FTCA, a perfluoroalkyl acid precursor, in droplets and bulk solution, with the goal of determining whether this process is droplet-accelerated, and identifying the transformation products via non-target analysis. We determined the rate constant for aqueous photo-oxidation of 6:2 FTCA in bulk solution and submicron aerosol droplets. Gas content and relative humidity were varied to determine the role of direct photolysis at these wavelengths. Understanding the role that different oxidants, UV wavelengths, and interfacial reactions play in the remediation of PFAS is crucial to developing scalable and successful approaches at a large scale. Droplet-accelerated PFAS degradation has the potential to be a low-cost, reagent-free means of enhancing the remediation of PFAS from drinking water sources.

4.06.T-04 Identification of Biotransformation Products in Rats Exposed to 6:1 Fluorotelomer Alcohol

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Fluorotelomer alcohols (FTOHs) are precursors and intermediates to the toxic, persistent, and bioaccumulative perfluorocarboxylic acids and are of concern to human health and the environment. To better understand the toxicity and risk from FTOHs, male and female rats were orally dosed with 6:1 FTOH for five days at dose-

levels ranging from 0.1 to 300 mg/kg/day. Plasma was collected 24 hours after the last dose at study termination (or on day 6). Preliminary internal dose results in plasma suggest that rats quickly metabolize 6:1 FTOH. Therefore, the identification of biotransformation products is useful for understanding the adverse effects observed (decreased body weight and mortality at the highest dose levels). A SCIEX X500R liquid chromatography-quadrupole time-of-flight in negative electrospray ionization mode was used to detect and identify biotransformation products based on high-resolution accurate mass and fragmentation fingerprints collected using data-independent and data-dependent acquisition scans. Chemical transformation predictions were used to build suspect screening lists and guide identification. SCIEX Analytics Software was used to initially screen data based on comparison to vendor and in-house spectral libraries. SCIEX MarkerView Software was used to identify features that were unique and dose-dependent across treatment groups using statistical analyses. The use of t-tests and principal component analysis greatly reduced the features of interest. Preliminary results in plasma showed a tentative match of a predicted biotransformation product, perfluoroheptanoic acid (PFHpA), to an in-house spectral library with a library hit score of 100% and mass error of less than 1 ppm. The feature tentatively identified as PFHpA was also observed to be one of the most abundant peaks with significant differences ($p < 0.001$) observed across treatment groups. The Environmental Protection Agency's Hazard Comparison Dashboard classifies PFHpA hazard scores as very high for genotoxic mutagenicity and high for both endocrine disruption and oral acute toxicity, suggesting potential mammalian health effects from 6:1 FTOH (and likely PFHpA) exposure. *The abstract does not reflect EPA policies.*

4.06.T-05 LC-MS/MS Characterization and Automated Data Analysis of Biological Transformation Products of Per- and Polyfluoroalkyl Substances (PFAS)

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Per- and polyfluoroalkyl substances (PFAS) are persistent and widely used synthetic chemicals. They have been linked to a wide range of adverse human health effects, including delayed metacognition and behavioral problems in children, impaired kidney function and kidney cancer, testicular cancer, obesity, and lipid dysregulation. Assessment of PFAS exposure has focused on the parent PFAS compounds and transformation products from naturally occurring microbial pathways in waste waters and landfills. Our understanding, however, of mammalian PFAS transformation is limited. This study aimed to identify biological PFAS transformation products and generate mass spectral libraries to facilitate automated search and identification of PFAS transformation products from raw data files. Biological transformation of 27 PFAS of various chemical classes, including alcohols, sulfonamides, carboxylic acids, ethers, and esters, etc., were evaluated using mouse liver S9 fractions with an *in vitro* assay, where PFAS standards were incubated with mouse liver S9 fractions. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to analyze the supernatant after incubation and identify potential PFAS transformation products. Four major PFAS transformation pathways were proposed, including glucuronidation; sulfation; dealkylation and oxidation. A total of 13 transformation products were identified and their MS/MS spectra were obtained. Glucuronidation products were found to be the most common terminal transformation products among PFAS alcohols and class-specific fragment peaks were annotated and common fragmentation peaks across glucuronide conjugates including [C₂H₃O₂]⁻, [C₂H₃O₃]⁻, [C₅H₅O₃]⁻, were incorporated into FluoroMatch 3.0 to enable future identifications of products from the same chemical series. Time-course experiments of varying incubation times showed different formation rates for different Phase I and Phase II transformation products. Non-targeted analysis further revealed additional molecular features that were associated with PFAS transformation. Our study provided a clearer picture of PFAS biological transformation and extended the FluoroMatch library to enable future identification of PFAS transformation products from non-targeted data sets.

4.06.T-06 A Portable Database Infrastructure for Per- And Polyfluorinated Alkyl Substances High Resolution Accurate Mass Data

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There are a broad set of tools and techniques used for non-targeted characterization of chemical composition within a material, which can include high resolution accurate mass (HRAM) spectrometry. Confident identification of components relies heavily on mass spectral libraries and analytical standards; NIST provides several in wide use, yet research needs in new or expanding areas often run ahead of availability. Additionally, mass spectrometer settings can result in differences in fragmentation spectra used to identify unknown compounds, which can further complicate compound matching. Research groups often then build internal libraries based on their needs and methods. Sharing such libraries can often be difficult, and making them compliant with the FAIR principles is often even harder. The NIST Chemical Sciences Division has developed a toolkit to create portable, self-contained databases to capture HRAM data and associated metadata regarding sample and instrument properties. The Database Infrastructure for Mass Spectrometry (DIMSpec) project uses only open source tools to capture and evaluate data for such projects. Capabilities include storage of MSⁿ data, mass spectral uncertainty evaluation, controlled vocabulary, QA/QC evaluation, and a toolkit facilitating identification of compounds and automatic annotation of known fragments. A consistent schema allows for easier sharing and reuse, with import and export routines able to be constructed to meet any data workflow need; one example is exporting to the .msp format used in the NIST mass spectral library search program, but data should be exportable to any data repository format. The associated toolkit allows for rapidly establishing new databases supporting new research areas and provides a consistent framework around which to build and use them. As distributed, the toolkit is populated with HRAM PFAS data and provides to users by default (a) chemometrics support through integration of the RDKit library, (b) an extensible application programming interface with predefined endpoints for use, and (3) web applications built in R for understanding the database schema, performing quality assurance evaluation, and - perhaps of most interest - matching of experimental data to known compounds and fragments. Use of only open-source products ensures the project is available to any research group. Inclusion of existing workflows is encouraged, as DIMSpec is designed to be easily extensible.

4.07.P-We Environmental Fate of Polymers

4.07.P-We-083 Applying Colorimetric Methods to Predict the Biodegradation of Polymers

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Polymers are used in a wide range of products, resulting in high environmental exposure upon their disposal, however, their complexity and variability make it challenging to assess their fate in the range of environments that they reach. Traditional screening biodegradation take weeks to months to conduct and are labour intensive. To address this, we have developed a colorimetric test for assessing the biodegradability of polymers, focusing specifically on polysaccharides. The test allows downstream biodegradation products of the test polymer to be converted into a coloured compound and then measured according to light absorbance. Since the analytical endpoint is absorbance, this method utilises 96-well plates, making it a high-throughput method with potentially hundreds or thousands of replicates for each polymer tested. Once optimised, this method was applied to measure the biodegradation of two polysaccharides – Pullulan and Dextran. The method was further used to investigate the effect of differing molecular weight and Degree of Substitution on the test polymer biodegradation potential. The results of this suggest that the test can reliably measure biodegradation when tested with enzymes alone, further research is currently underway to assess its reliability with microbial inocula and to link the results to traditional biodegradation screening test results.

4.07.P-We-084 Evaluating CO₂ Evolution Test Designs using Natural Polymers

Sean P. McLaughlin and Roxanne Brackett, Smithers

Assessing the persistence of organic chemicals is a requirement for product registration worldwide. Several standardized test methods are used for this assessment, to provide evidence of whether a chemical will remain intact once exposed to the environment, through a sewer system, land application, or disposal. One of the most straightforward ways of determining the ultimate biodegradation of an organic chemical is by measuring the amount of CO₂ produced after it is in contact with an inoculated matrix. Some chemicals, especially polymers, may be challenging to work with due to their chemical and physical properties (e.g., low water solubility, adsorptive nature, etc.) and are often not 'readily' biodegradable in an aqueous test system. To further complicate the situation, these molecules might not be amenable to radiolabeling typically used to track the chemical due to the complexity of their structure, and/or the process of radiolabeling might be cost-prohibitive. Therefore, this study investigates at least three natural polymers representing a disaccharide, protein, and fat in both an aqueous standard OECD 301B CO₂ evolution test and a soil CO₂ evolution test. This experiment compares the efficiency of the soil CO₂ evolution test to the standard aqueous test to see if a soil CO₂ evolution test containing a higher density of microbial biomass is a plausible option for assessing the biodegradability of challenging compounds such as polymers.

4.07.T Environmental Fate of Polymers

4.07.T-01 Withdrawn - Do's & Do Not's for Assessing Biodegradability of Plastics in the Environment

Ramani Narayan, Michigan State University

Plastic wastes on land and in oceans have become major societal issues. Articles in print, television, and social media about plastics waste issues and bans on plastic items are on the rise everywhere in the world. Most serious, is the issue relating to plastics persistence and microplastics contamination of the environment. Against this backdrop, the lecture reviews the science around biodegradability and compostability of polymers and its value contribution towards an environmentally responsible end-of-life (EoL). 77% of the 454 million tons of plastic polymers manufactured world-wide (2018 numbers) are carbon-carbon backbone polymers like polyethylene/s, polypropylene/s and polystyrene/s. Ninety percent of packaging polymers are carbon-carbon backbone polymers. These lightweight, recalcitrant, non-biodegradable packaging plastics are difficult to recover from waste streams. This is particularly true for plastic packaging associated with food and paper. The difficult to recover, recalcitrant plastics fragment and leak into the ocean (ocean micro plastics pollution) and on land Recent, EPA's MSW data analysis shows that recycling of non-durable plastics as percent of generation is only 2.4%. There is many misleading claims in the market place centered on using additives (oxo, enzyme, microbe, and organic molecules) to degrade, and completely biodegrade persistent, carbon-carbon hydrocarbon plastics in natural environments and landfills under ambient temperatures. This lecture reviews necessary requirements for assessing and reporting plastic biodegradation in the environment. We discuss applicable ASTM/ISO/EN standards for measuring biodegradability and compostability of plastics. Terms like "biodegradable" vs "compostable" & "industrial vs home composting is increasingly used to differentiate and market products. However, the fundamental science on the use and applicability of these terms is lacking resulting in misleading claims and creating confusion.

4.07.T-02 Multi-laboratory Investigation on Intra and Interlaboratory Reproducibility of Polymer Biodegradation Assessments Applying Respirometric Methods

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Regulations world-wide are rapidly developing for polymeric materials. One of the key concerns underpinning the use of polymers are their uncertain environmental persistency (or biodegradability). This has put an increasing emphasis on the need to identify, and critically evaluate testing guidelines for assessing biodegradability to determine their applicability and limitations for polymeric materials. This research focused on understanding the intra and inter-laboratory variability when applying OECD 301F and OECD 301B Ready Biodegradation test methods to evaluate polymer biodegradation as well as evaluating the impact of method modifications in test duration, inocula level and test substance concentration on results. The mineralization of 5 different polymers of varying structural components, molecular weight (MW), charge, and solubility was evaluated at 8 different laboratories in 4 different countries providing significant geographic variability in inocula source as well as lab to lab variations in test setup. Across all laboratories intralaboratory variability was low indicating the reproducibility of results between replicates and uniformity of test setup in each laboratory. Interlaboratory variation was also low for all 5 polymers with extent of mineralization being remarkably close in all OECD 301F&B studies even when test methods were modified. Across all studies average mineralization was $89 \pm 6.9\%$ for polyethylene glycol 35,000, $86 \pm 9.5\%$ for polyvinyl alcohol 18-88, $42 \pm 9.4\%$ for carboxymethyl cellulose (DS 0.6), $49 \pm 4.5\%$ for modified guar gum, and $84 \pm 10\%$ for microcrystalline cellulose (MCC). MCC is proposed as a possible suitable reference material for polymer biodegradation studies. Additional work was conducted evaluating mineralization of the 5 polymers in river water respirometry studies to evaluate the relationship with OECD 301 results. A similar extent of mineralization was observed for all 5 polymers in the OECD 301 and river water studies but time to reach complete mineralization was longer in the river water studies likely due to the lower microbial counts (10^6 CFU/L) in the study. Future work will include evaluating poorly soluble polymers, further exploring inoculum effect and identifying suitable reference materials. Modifications of the existing standards to improve method deficiencies is an expected product of this effort.

4.07.T-03 Accelerating Polymer Biodegradation Through the Use of Adapted Sludge in an OECD 302B Inherent Biodegradability Test

Vurtice C Albright III, Nathalie Vallotton, Aaron M Williams, Lyndsay Leal, Scott Backer and Yunzhou Joe Chai, The Dow Chemical Company

The demand for biodegradable polymeric materials has increased dramatically in recent years. However, limited research has been performed to adapt screening testing methods for the assessment of the biodegradability of polymeric substances. The existing testing guidelines are not fully applicable to polymers, but they are currently recognized and accepted by regulators and industry for the assessment of discrete chemicals. Thus, these guidelines provide a solid foundation that can be adapted to study polymer biodegradation. A unique challenge for testing polymer biodegradability, is that polymers degrade much more slowly than the discrete organic materials for which the standardized tests were designed. One approach for addressing this challenge is extending the duration of these guideline tests. However, this raises additional challenges, such as the viability of the microbial inoculum after extended periods and the acceptability of such extensions by regulators or industry clients. Thus, developing accelerated test methods that are within the scope of the standardized guidelines is seen as a possible approach to bridge the gap until new, polymer-specific methods are approved. The OECD 302B Zahn-Wellens/EMPA Test for inherent biodegradability is an ideal candidate method for developing an accelerated method. Sludge adaptation, exposing the microbial inoculum to the test material prior to the start of the test, is one method that can be used to accelerate the degradation rate. This adaptation is currently accepted within the OECD testing framework. In this study, microbial inoculum aliquots were individually pre-exposed to increasing concentrations of six polymers over weeks. Then, six separate OECD 302B tests were conducted using the adapted inoculums to determine the degradation rate for each polymer. Another OECD 302B study was conducted for all six polymers using fresh, non-adapted inoculum. Data analysis of the results are still on-going. However, for two of the materials the use of adapted sludge appears to have not a significant effect on the rate of degradation. For the remaining four materials, the use of adapted

sludge appears to have led to an increased degradation rate. Thus, the use of an adapted sludge inoculum could be an acceptable method for accelerating biodegradation screening tests. This approach could lead to faster screening of materials for biodegradability, and to a decrease in the time needed to bring new products to market.

4.07.T-04 A Novel Approach to Analysis of Water-Soluble Polymer Degradation Chemistry by High Resolution Mass Spectrometry: Understanding Polyacrylamide Degradation via Enhanced Non-Target Workflow

Phoebe Keyes, Noor Halimah and Boya Xiong, University of Minnesota

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 a commercial software has very low accuracy in predicting formula of MS¹ 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 24% 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 hinderance of sulfate radicals. We also employed theoretical fragmentation of aldehyde and ketone end groups to confirm this result with the MS² data. Eleven structures with confidence level 2b-3 were identified using MS² information. Additionally, a concentration dependent crosslinking and precipitation of degraded polymer were observed which also impact the fate of polymers. The mechanism of this phenomena will be explored further. This is a first attempt to resolve complex polymer degradation chemistry using of HRMS that can advance our understanding of the environmental impact of overlooked water-soluble polymer pollutants.

4.07.T-05 Environmental Fate of Water-Soluble Polyvinyl Alcohol (PVOH)

Jennifer Menzies, Ashley Wilcox, Maura Hall, Ken Casteel and Kathleen McDonough, Procter & Gamble

Within the class of polyvinyl alcohols (PVOH), materials with a degree of hydrolysis between 85-90 and molecular weight 44,000-190,000 Da are of interest due to their water solubility and use as an ingredient in detergent films. The end-of-life disposal of detergent films is down the drain and the fate of this subset of PVOH polymers was assessed accordingly. A variety of tests following OECD guidelines and modifying these guidelines for application to polymers was conducted. These included OECD 301, 302, 303A, and adsorption studies. Activated sludge (AS) from domestic wastewater treatment plant and river water (RW) were used as inoculum sources. The data show that the water soluble PVOH materials were fully mineralized in respirometric tests by both AS and RW inoculum. Removal of PVOH under simulated wastewater treatment conditions was > 90% by dissolved organic carbon with no adsorption evident. This data was combined with industry volume estimates to predict environmental concentrations and compared to ecotoxicity data. This comparison 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.

4.07.T-06 Speeding it Up: Biodegradation of Poly(lactic acid) at Mesophilic Conditions by Biostimulation

Pooja Mayekar, Anibal Bher and Rafael Auras, Michigan State University

Societal benefits obtained from plastics due to their unique properties increase yearly. However, the mismanagement of plastic waste from different regions worldwide has created omnipresent plastic pollution. Several solutions are being sought to overcome this challenge. One of the solutions is the development of bio-

based and biodegradable polymers, to increase the amount of plastic recovered through organic recycling with food waste and to reduce the amount of waste ending up in landfill/incineration facilities. Since these polymers are becoming part of the future circular economy and are promoted heavily, ensuring their complete biodegradation without any traces of microplastics is crucial. One such polymer is poly(lactic acid) - PLA, which has garnered attention due to its compostable nature in industrial composting facilities. PLA is industrial compostable when subjected to thermophilic aerobic conditions for an extended period. The dependence of PLA to first undergo chemical hydrolysis enhanced by high temperatures (~60 °C) makes it difficult for PLA to degrade in a mesophilic environment, such as poorly managed industrial composting operation and backyard composting. Furthermore, compared to readily biodegradable organic waste fraction, the slower rate and longer timeframes associated with PLA biodegradation adversely affect its acceptance in industrial composting facilities. These reasons force us to find new, more targeted solutions for PLA degradation, especially at mild temperatures. The current study aims to evaluate the biodegradation of PLA at mesophilic conditions (37 °C) by biostimulating the compost environment for 180 days with selected compounds. Fe₃O₄ nanopowder, skim milk, gelatin, and ethyl lactate were added to the compost to target the chemical and enzymatic hydrolysis and lactate stimulation at different stages of biodegradation. The CO₂ evolution was measured using an in-house direct measurement respirometer system, the reduction of molecular weight (M_w) was tracked by size exclusion chromatography, and the crystallinity was measured by differential scanning calorimetry. PLA in biostimulated compost showed faster M_w reduction when compared to PLA degraded without the addition of biostimulants. Combining skim milk and gelatin with Fe₃O₄ showed the largest reduction in M_w by ~ 54% and 59%, respectively. This work opens new pathways to accelerate PLA degradation at mesophilic conditions, such as home/backyard composting.

4.07.V Environmental Fate of Polymers

4.07.V-009 Biodegradability of Polymeric Compounds under Controlled Composting Conditions According to ISO 14855-2

Jean-Rene Thelusmond, Yunzhou Joe Chai, Vurtice C Albright III, Matthew LeBaron, Jing Hu, Gary Kozerski, Nathalie Vallotton, Sue Marty and Yinzong Guo, The Dow Chemical Company

The biodegradability of eight polymeric materials was assessed in controlled composting conditions according to the International Organization for Standardization (ISO) 14855-2 test method. These test materials consisted of two natural biopolymers i.e., cellulose and walnut shell powder, three polyurethanes identified as PU1, PU2, and PU3, and two polyvinyl alcohols with 40 or 100% degree of hydrolysis, and one polyvinyl acetate. An open flow respirometer was used to measure carbon dioxide (CO₂) evolution, which in turn was used to calculate the percent of biodegradation for the polymers. In a previous composting experiment, the impact of polymer biodegradation was evaluated on the microbial community structure of three compost sources using 16S rRNA amplicon sequencing. Aerobic biodegradation test mixture was prepared using compost inoculum from the West Madison Ag Research Station. The compost and test material mixtures (6:1 dry wt.) were incubated in 1-L glass vessels placed in an incubator with temperature set at 58±2 °C. The moisture content of the mixture was adjusted to be approximately 60% (on a wet weight basis) before incubation was started. The test vessels were supplied with a constant air flow of 250 mL/min. The CO₂ production in each vessel was measured approximately every 6 h. The biodegradation data showed that cellulose, walnut shell powder, PU2, and PU3 reached 100% biodegradation. However, the biodegradation kinetics varied among these compounds and the biodegradation rates decreased in the following order: cellulose > walnut powder > PU2 > PU3. While polyvinyl alcohol with low degree of hydrolysis biodegraded slowly, fully hydrolyzed polyvinyl alcohol, PU1, and polyvinyl acetate did not biodegrade. Data on the microbial community analysis and efforts to accelerate biodegradation under composting conditions are still being processed and will be presented.

4.07.V-036 Speeding it up: Biodegradation of Poly(lactic acid) at Mesophilic Conditions by Biostimulation

Pooja Mayekar, Anibal Bher and Rafael Auras, Michigan State University

Societal benefits obtained from plastics due to their unique properties increase yearly. However, the mismanagement of plastic waste from different regions worldwide has created omnipresent plastic pollution. Several solutions are being sought to overcome this challenge. One of the solutions is the development of bio-based and biodegradable polymers, to increase the amount of plastic recovered through organic recycling with food waste and to reduce the amount of waste ending up in landfill/incineration facilities. Since these polymers are becoming part of the future circular economy and are promoted heavily, ensuring their complete biodegradation without any traces of microplastics is crucial. One such polymer is poly(lactic acid) - PLA, which has garnered attention due to its compostable nature in industrial composting facilities. PLA is industrial compostable when subjected to thermophilic aerobic conditions for an extended period. The dependence of PLA to first undergo chemical hydrolysis enhanced by high temperatures (~60 °C) makes it difficult for PLA to degrade in a mesophilic environment, such as poorly managed industrial composting operation and backyard composting. Furthermore, compared to readily biodegradable organic waste fraction, the slower rate and longer timeframes associated with PLA biodegradation adversely affect its acceptance in industrial composting facilities. These reasons force us to find new, more targeted solutions for PLA degradation, especially at mild temperatures. The current study aims to evaluate the biodegradation of PLA at mesophilic conditions (37 °C) by biostimulating the compost environment for 180 days with selected compounds. Fe₃O₄ nanopowder, skim milk, gelatin, and ethyl lactate were added to the compost to target the chemical and enzymatic hydrolysis and lactate stimulation at different stages of biodegradation. The CO₂ evolution was measured using an in-house direct measurement respirometer system, the reduction of molecular weight (Mw) was tracked by size exclusion chromatography, and the crystallinity was measured by differential scanning calorimetry. PLA in biostimulated compost showed faster Mw reduction when compared to PLA degraded without the addition of biostimulants. Combining skim milk and gelatin with Fe₃O₄ showed the largest reduction in Mw by ~ 54% and 59%, respectively. This work opens new pathways to accelerate PLA degradation at mesophilic conditions, such as home/backyard composting.

4.08.P-We Environmental Forensics

4.08.P-We-086 Evaluation of Polytopic Vector Analysis Sensitivity to Overlapping PCDD/F Source Signatures

Nicholas Rose and Michael J Bock, TIG Environmental

Chemometric methods, also called mixing models or receptor models, are an important tool in environmental forensics. Mixing models can be used to identify the sources contributing to a set of environmental samples and develop an estimate of the relative contribution of each source to a given sample. Sources of environmental contaminants often share common analytes that can potentially confound mixing model results. This problem could be particularly acute for polychlorinated dioxins and dibenzofurans (PCDD/Fs) because only 17 analytes are typically measured and common sources often have the same dominant analytes and/or are composed of a single analyte. To investigate this, we developed a series of artificially generated datasets that were representative of realistic environmental dataset, including noise and left-censored results. These datasets were generated utilizing literature values for the PCDD/F sources of background soil, aroclor 1260, copper smelting slag, smelter kiln, and TCP. These sources were chosen because aroclor 1260 and copper smelting slag both have the same two dominant PCDD/Fs, the TCP fingerprint is dominated entirely by a single analyte that is also present in the smelter kiln, and background PCDD/Fs are ubiquitous in the environment. These source signatures were used to generate populations of all the potential mixing combinations of these five sources with varying levels of noise. Then 100 samples were collected from these populations to approximate the environmental dataset. These datasets were evaluated with polytopic vector analysis (PVA), one of the common

mixing models used in environmental forensics. Because the datasets were artificially generated, the PVA predicted values could be compared to the true values. With low noise, PVA is very accurate at separating out the source signature and predicting their contributions to each sample even with the overlapping signatures. With higher noise, PVA still does a reasonable job of identifying the source signatures and the relative contribution. It also identifies a source signature that consists of just noise in the dataset.

4.08.P-We-087 Comprehensive Fingerprinting of Polycyclic Aromatic Compounds (PACs) In Samples From The Great Lakes

Ifeoluwa Grace Idowu¹, Gregg Tomy¹, Paul Helm², Chris Marvin³, Sonya Kleywegt⁴ and Courtney Sandau⁵, (1)University of Manitoba, (2)Ontario Ministry of the Environment, Conservation and Parks, (3)Environment Canada, (4)Ministry of the Environment, Conservation and Parks, Canada, (5)Chemistry Matters Inc.

Polycyclic aromatic compounds (PACs) are ubiquitous environmental contaminants used as geological markers for fingerprinting in environmental forensics. This is due to their persistence and resistance to degradation in the environment. The most common of this group is polycyclic aromatic hydrocarbons (PAH) and their alkylated homologs. Gas chromatography coupled with mass spectrometry has been the primary technique for separating, identifying, and quantifying these compound classes in environmental samples. Pavement seal coats are black, viscous liquids applied to driveways and parking lots in North America to improve the appearance and increase the longevity of asphalt surfaces. The two most common products are (a) coal tar-based sealant (CTSC) and (b) asphalt-based seal coat (ASC). Studies indicate that certain polycyclic aromatic hydrocarbons (PAHs) concentrations are approximately 1000 times higher in CTSC than in ASC. Polycyclic aromatic compounds (PACs) are diverse compounds, including PAHs, alkylated PAHs, sulfur-, nitrogen-, and oxygen-containing PACs. Some PACs exhibit greater mobility and bioavailability in the environment than PAHs due to their increased solubility in water, which increases the risk of exposure to aquatic biota and potentially human exposure through groundwater contamination. This study aims to comprehensively identify and measure PACs in environmental samples and assess what proportion of PACs are from coal tar-based products compared to other sources, including asphalt sealants, roofing material tires, and road diesel rubbers particulate. Sediments and biota (fish muscles) samples around the Great Lakes, specifically from Toronto and Hamilton Harbour, were evaluated for the input of these coal-tar-based products. Profiles have been obtained for different source-based materials using two-dimensional gas chromatography high-resolution-mass spectrometry (GCxGC HRTofMS). The native PAHs were higher than the alkylated PACs for most CTSC samples analyzed. Statistical tools and source apportionment models were also applied to these samples to assess the source contributions of different source-based materials in these environmental samples.

4.08.P-We-088 Comparing sources of Polychlorinated Dibenzo-p-dioxins and -Furans to the Newtown Creek and Passaic River/Newark Bay

Mahdi Chitsaz¹, Saranya S Anantapantula², Mohson Al Hello¹, Kelly L Francisco¹ and Lisa A. Rodenburg¹, (1) Rutgers University, (2)Spring-Ford Area High School

Polychlorinated Dibenzo-p-dioxins and -Furans (PCDDFs) are contaminants of concern in the New York/New Jersey Harbor, which includes or borders several contaminated sites, including the Passaic River/Newark Bay complex and Newtown Creek. Sources of PCDDFs are very different between these locations, suggesting that they export different PCDDF fingerprints with differing toxicities to the harbor. The sources of PCDDFs to these systems were investigated by analyzing large data sets on concentrations of both PCDDFs and polychlorinated biphenyls (PCBs) in sediment using Positive Matrix Factorization (PMF). The Newtown Creek data was obtained from the Remedial Investigation databases. Analysis of this data demonstrated that the refineries that once lined the creek and PCDDFs present as impurities in Aroclors are major sources of toxicity, with each responsible for about 40% of the Toxic Equivalency Quotient (TEQ) in the sediment. PCDDFs entering Newtown Creek from the harbor, which may include PCDDF exported from the Passaic River, contribute about 5% of TEQ, and Combined Sewer Outfalls contribute about 7%. The data analyzed from the

Passaic River/Newark Bay complex was taken from studies conducted in 2012-2019 to focus on newer sources. In contrast to Newtown Creek, about 90% of TEQ in the Passaic River and Newark Bay data set comes from 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is associated with historical production of 2,4-dichloro phenoxy acetic acid and other industrial products. 2,3,7,8-tetrachlorofuran (TCDF) is also an important source of TEQ in the Passaic River/Newark Bay system. This presentation will discuss the sources of TCDF.

4.08.P-We-089 An Artificial Intelligence Approach to Characterizing Three Decades of Polycyclic Aromatic Hydrocarbon Data

Kimani Kimbrough¹, Erik Davenport¹, Neil Fuller², Felipe Arzayus¹ and Dennis Apeti¹, (1)National Oceanic and Atmospheric Administration, (2)National Centers for Coastal Ocean Science

The National Oceanic and Atmospheric Administration, National Status and Trends Program contaminant dataset includes over 35 years of data from the U.S. and territories. Temporal contaminant monitoring data from the Mussel Watch Program, placed based assessment contaminant data from the Bioeffects Program, and contaminant data collected in response to events such as hurricanes, oil spills, and the attack on the World Trade Center comprise the dataset. Artificial intelligence and Bayesian models were used to address the unbalanced nature of the combined dataset derived from studies with different sampling designs. Polycyclic aromatic hydrocarbons (PAHs), the largest group of contaminants, are the focus of this presentation. Unsupervised techniques, such as cluster analysis, and Random Forest were used to identify patterns in relative concentration data. Supervised approaches, such as Bayesian linear regression, and machine learning were used to successfully predict concentration based on spatial data such as land use, and assess associations between PAH concentration and PAH patterns. A strong relationship with impervious surfaces was identified between PAH concentration and urbanization. The artificial intelligence and Bayesian approaches identified new information that was used to identify data gaps to drive sampling efforts, and more efficiently address national contaminant threats.

4.08.P-We-090 Identification of Ventilation Equipment, Adhesives, Caulking, and Structural Insulation as Sources of Airborne PCBs in Vermont Schools

Jason Hua, Michael P Jones, Rachel Marek and Keri Hornbuckle, University of Iowa

Airborne polychlorinated biphenyls (PCBs) in schools are emitted from building materials, modern paints, and surface treatments, posing significant risk of exposure to occupants. We hypothesize there are local sources in school rooms that can contain mixtures of Aroclors, a known historic source of PCBs. We also hypothesize that PCB sources can be predicted based on materials found in schools. To test our hypothesis, we conducted measurements of emissions and concentrations of airborne PCBs in five Vermont schools. We deployed polyurethane foam passive air samplers (PUF-PAS), passive emission samplers (PUF-PES), and field blanks throughout schools for three to five weeks. We analyzed for all 209 congeners represented in 173 chromatographic separations. Surfaces sampled include carpet, dry wall, cove base, brick walls, cinderblock walls, structural insulation, and floor tile. Using cosine theta similarity, we identified different Aroclor sources as well as modern sources in these schools. Aroclor 1254 was the most common source identified. We also measured Aroclor 1221 in one school, which has not yet been reported in schools. This study illustrates the benefit of direct measurement of PCB emissions to identify major sources for remediation.

4.08.P-We-091 Non-Target Chemical Composition of Surface Waters May Reflect Ecosystem Processes More Than Discrete Source Contributions

Cheng Shi, Gouri Mahadwar, Emmanuel Dávila-Santiago, Ted Bambakidis, Byron C. Crump and Gerrad Jones, Oregon State University

We investigated environmental, landscape, and microbial factors that could structure the spatiotemporal variability in the non-target chemical composition of four riverine systems in the Oregon Coast Range, USA.

We hypothesized that the non-target chemical composition in river water would be structured by broad-scale landscape gradients in each watershed. Instead, only a weak relationship existed between the non-target chemical composition and land cover gradients. Overall, the effects of microbial communities and environmental variables on chemical composition were nearly twice as large as those of landscape, and much of the influence of environmental variables on the chemical composition was mediated through the microbial community (i.e., environment affects microbes, which affect chemicals). Therefore, we found little evidence to support our hypothesis that chemical spatiotemporal variability was related to broad-scale landscape gradients. Instead, we found qualitative and quantitative evidence to suggest that chemical spatiotemporal variability of these rivers is controlled by changes in microbial and seasonal hydrologic processes. While the contributions of discrete chemical sources are undeniable, water chemistry is undoubtedly impacted by broad-scale continuous sources. Our results suggest that diagnostic chemical signatures can be developed to monitor ecosystem processes, which are otherwise challenging or impossible to study with existing off-the-shelf sensors.

4.08.P-We-092 Source Apportionment of Polycyclic Aromatic Hydrocarbons (PAHs) Throughout Playground Soils in Oklahoma City Using Various Analyses

Sarah Hileman and Jason B. Belden, Oklahoma State University

The Greater Oklahoma City Urban Area showcases an intersection of human diversity, varying architectural landscape, and a central city hub surrounded by urban sprawl, smattered with industrialization. As expected in such an urban setting, there are elevated anthropogenic activities leading to environmental contamination. Polycyclic aromatic hydrocarbons (PAHs) are generally ubiquitous in the environment and are often observed at higher levels in urban areas. These persistent organic pollutants may correspond with atmospheric deposition, and therefore in urban soils these contaminants tend to be found in elevated levels due to proximity to sources such as vehicular traffic. Sampling in the Greater Oklahoma City Urban Area has indicated that PAH accumulation (and especially carcinogenic PAHs or cPAH concentration) has been significant, and in many cases cPAH soil load has been measured above the USEPA's residential soil screening level of 110ppb. This occurrence has been noted across the city, as well as within public elementary school playgrounds: 138 public elementary schools across 15 school districts were examined for levels of PAHs. Schools in this metropolitan range tend to have a high degree of concern, and there is the potential for oral exposure to these contaminants in sensitive age groups by way of hand-to-mouth actions involving soil. By using hotspot analysis and other techniques such as source apportionment diagnostic ratios, it is possible to make inferences about PAH origins throughout the study area. This type of analysis is important to inform urban development and placement of community infrastructure to protect vulnerable populations.

4.08.T Environmental Forensics

4.08.T-01 Untargeted Mass Spectrometry for Analysis of Chemical Trends in Municipal Wastewater Before, During, and After the 2022 World Athletic Championships

Sam Bassett¹, Luke Marney¹, Jeff Morre¹, Corey De La Cruz¹, Thando Mawasha¹, Michael Harry¹, Casey Kanalos¹, Daphne Guo¹, Lya Carini¹, Kyle Barber², Gerrad Jones¹, Christine Kelly¹, Tyler Radniecki¹ and Claudia Maier¹, (1)Oregon State University, (2)Athletics Integrity Unit, Monaco

A population's consumption and elimination of chemical compounds end up in municipal wastewater, providing a unique opportunity to view the day-to-day use of chemical substances from a molecular epidemiological viewpoint. The goal of our study is to see if unique chemical signatures in wastewater can be associated with a Western Athletic Conference (WAC) event in Corvallis, Oregon using a combination of chemical analysis and machine learning techniques. Association of chemical features were based on sample location and collection time relative to the event. We applied solid-phase extraction and subsequent size-exclusion membrane filtration followed by UPLC-MS E evaluation to detect changes in chemical features in wastewater during the 2022 World Athletic Championships, a prestigious track and field event with athletes

from 179 nations attending. At nine sampling sites an average of three, nine, and six wastewater samples were collected before, during, and after the event; resulting in 162 samples. Samples were subjected to SPE, dried under nitrogen, reconstituted, and centrifuged. The supernatant was passed through 1kDa size-exclusion membrane filters. UPLC-MS E data was collected over a mass range of 50-1500 m/z with lock mass correction. A QC pooled sample and blank were run every ten samples. The LC method used a C18 2.1x100 mm 1.8 um UPLC column and a forty-five minute solvent gradient of water and acetonitrile, both containing 0.1% formic acid. Progenesis QI was used for data alignment, normalization, and potential compound identification. Based on non-metric multidimensional scaling (NMDS) ordination, which summarized the chemical variation within the dataset, spatial variability in sample location (e.g., upstream vs downstream of the event location) better explained differences in chemical composition than the timing of the sample collection (pre, during, and post event). Nevertheless, we used six machine learning classification tools were used to determine which chemical features best distinguish samples collected both at and during the WAC event vs all other samples. On average, hold-out classification performance was ~75% suggesting that there diagnostic chemicals associated with the differences distinguishing WAC activities. Initial tentative chemical identifications included medications, common environmental pollutants, dietary metabolites, and more.

4.08.T-02 Time Series Analysis of Non-target Chemicals and Related Environmental Processes in a Small Stream

Cheng Shi¹, Damian Helbling² and Gerrad Jones¹, (1)Oregon State University, (2)Cornell University

Non-target chemical composition in surface water represents the sum of all ecosystem processes. Previous studies have identified temporal variations in non-target chemical composition and linked them to potentially related seasonal events. However, it is challenging to attribute temporal changes in overall non-target chemical composition to different ecosystem processes. In this study, we propose a combined unsupervised and supervised machine learning approach to determine how ecosystem processes structure the time-series in non-target chemical composition. Weekly water samples were collected from a small stream for 78 weeks and non-target chemicals were extracted and analyzed with high-resolution mass spectrometry. After data pre-processing, we retained non-target chemicals present for at least 4 weeks for further analysis. Non-negative matrix factorization (NMF) was used to summarize the holistic non-target chemical profiles to different numbers of time series. Watershed characteristics (e.g., hydrology, soil, climate, etc.) and water quality data were used to predict each extracted time series with support vector classifiers. The classifiers with good performance were selected to determine the important ecosystem variables that are contributing to the corresponding time series of chemical composition. Suspect screening was performed on the indicator chemicals with higher weights for each extracted time series to find further evidence to explain their existence in that time series. The results suggest that water temperature, evapotranspiration, soil moisture, stream flow rates, baseflow, and sewage discharge are the most important watershed variables that contribute to temporal variations in non-target chemical composition. Water quality variables were less important, except for turbidity. This workflow shows that the time series of non-target chemical composition can be attributed to ecosystem processes and inform temporal changes in watersheds.

4.08.T-03 Sources of Polychlorinated Biphenyls to Upper Hudson River Water Post-Dredging

Mohson Al Hello, Kelly L Francisco, Lisa A. Rodenburg and Tzu Ning Liu, Rutgers University

Polychlorinated biphenyls (PCBs) are persistent, bioaccumulative, and toxic chemicals that are the dominant contaminant in the Upper Hudson River (UHR) in New York State where two General Electric (GE) plants historically discharged PCBs to the river. Portions of the UHR were dredged from 2009-2015 to address PCB contamination. Previous studies analyzed PCB congener fingerprints in the sediment and fish collected post-dredging and concluded that sources of PCBs other than GE explained about 2% of PCBs in the sediment and about 13% in the fish. Data on concentrations of PCB congeners (from EPA method 1668) in the water column at several locations during 2017-2021 was analyzed using Positive Matrix Factorization (PMF) to determine the

fraction of non-GE PCBs in the water column. Data from (from north to south) Thompson Island Dam, Schuylerville, Waterford, and Albany were analyzed. PMF analysis isolated eight factors (denoted UHRw1 through UHRw8), most of which contained high contributions from PCB dechlorination products and/or resembled PCB formulations used by GE, suggesting that they arise from GE. UHRw8 was higher in molecular weight than the formulations used by GE and was more abundant downstream of the GE plants, suggesting that it represents non-GE PCBs. This fingerprint explained 4% of PCBs in the data set, and its contribution increased from 1% at the northernmost Thompson Island Dam location to 7% at Albany.

4.08.T-04 Exploring the Effectiveness of PCA, t-SNE and UMAP for Analyzing PCB Fingerprints: A Case Study on Portland Harbor Superfund Site, Oregon, USA

Mike Dereviankin, Dereviankin Consulting Inc.

Techniques in multivariate statistics are used by a wide variety of investigations in environmental forensics. Many of these investigations only emphasize the outcome of a single multivariate statistic with which the expert is familiar. However, it is surprisingly rare for studies to show a detailed understanding of the uncertainties created by multiple multivariate statistical techniques or how uncertainties in chemical analysis impact statistical modelling outputs. These models are most often untrained, and the forensic investigation does not involve a “data exploration”, step. The outcome of different statistical models may vary and create contention between experts. The fact that a single model can provide different results is rarely acknowledged. In this study, the efficacy of three specific dimensionality reduction techniques, principal component analysis (PCA), t-distributed stochastic neighbour embedding (t-SNE), and uniform manifold approximation and projection (UMAP), was explored for analyzing PCB fingerprints in sediment samples collected from the Portland Harbor Superfund Site in Oregon, USA. Silhouette scores were applied to the clustering output of each of the 3 dimensionality reduction techniques to assess the quality of the separation between unique clusters of PCB fingerprints. Among the 3 dimensionality reduction techniques examined in this study, UMAP performed better in characterizing the chemical profiles of PCB fingerprints in sediment samples. UMAP can maintain better consistency between clustered data points compared to t-SNE and PCA, which is useful in preserving the overall structure of the data. This suggests that UMAP could be a more effective technique for accurately representing the chemical profiles found in sediment samples and may be useful in further applications such as source apportionment. Although UMAP performed better in characterizing the chemical profiles of PCB fingerprints in sediment samples in this study, it is important to note that there is no one-size-fits-all model in environmental forensics. Variables like sample size, data quality, and unknown sources of variability can influence the performance of dimensionality reduction techniques. Therefore, running multiple techniques and assessing their results is essential to identify the most suitable technique in each situation.

4.08.T-05 Differentiating Between Three OCDD-Dominant Dioxin/furan Fingerprints in Soil and Sediment of the Lower Roanoke River.

Glenn W Johnson, University of Utah

Distinguishing between multiple comingled sources, all with profiles dominated by octachlorinated dibenzo-p-dioxin (OCDD) is a common challenge forensics investigations. In that regard, the Lower Roanoke River in eastern North Carolina is of interest to the dioxin forensics community because (1) three OCDD-dominant profiles are observed there; and (2) it was the case-study used to demonstrate a data analysis method known as FALCON (EPA, 2004). The town of Plymouth, situated on the banks of the Lower Roanoke River, was home to both a pulp and paper mill and a sawmill. Both sites were contaminated with polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), and both were subjects of remedial investigations under CERCLA (Superfund) in the late 1990s and early 2000s. While not identical, the congener profiles characteristic of each source are similar in that they are dominated by OCDD. Complicating matters, a third OCDD-dominant profile was also observed in upstream/background river sediments. FALCON was able to distinguish between two of the three OCDD dominant profiles (pulp mill and upriver) but that success was due in large part to two project-

specific decisions that are not generally advisable: (1) excluding OCDD from the dataset, and (2) excluding samples most representative of the sawmill/pentachlorophenol source, allowing the analysis to reduce to a two end-member solution. In this paper, we revisit the Plymouth/Lower Roanoke River data set in context of the limitations of FALCON, and present results of reanalysis of that data using other common forensic approaches.

4.08.T-06 Predicting the Concentrations and Temporal Changes of Volatile Methyl Siloxanes in Dense Urban Areas

Christopher Brunet, Rachel Marek and Keri Hornbuckle, University of Iowa

Volatile Methyl Siloxanes (VMS) are a class of organosilicon compounds widely found in personal care products (PCP) which have raised concern due to their high volatility, long atmospheric lifetimes, and ability to form secondary organic aerosols (SOA). Due to their association with PCP, VMS emissions are expected to be largest in cities with high population densities. However, our ability to accurately predict concentrations of VMS in these areas and their temporal changes is limited by a lack of robust measurements. To address this need, we carried out a 1-month long field sampling campaign in New York City, the most populous dense city in the United States. VMS samples were collected on SPE extraction cartridges 3-5 times per day and analyzed via GCMS. Measured concentrations were compared to results from a previously developed Community Multiscale Air Quality model of VMS and to meteorological factors such as temperature and planetary boundary layer height to determine their influence. Finally, the relationship between air mass back trajectories and VMS concentrations was examined to better understand the influence of population density as a predictive factor. Herein we present the average and temporally resolved concentrations of 6 VMS congeners and show statistical comparisons between measurements of 3 of these congeners and modeled predictions. Finally, we demonstrate the relationships between these VMS concentrations and the factors discussed above and discuss their relative importance as predictors of temporal changes in VMS concentrations.

4.09.P-Mo Fate and Effects of Metals: Biogeochemical Perspective

4.09.P-Mo-113 Distribution of Rare Earth Elements in the Food Web of Two French Estuaries

Julie Rétif¹, Aurore Zalouk¹, Nicolas Briant² and Laurence Poirier¹, (1)Nantes University, (3)IFREMER, RBE-BE-LBCM

Rare earth elements (REEs) regroup 17 metallic elements including the 15 lanthanoids (from lanthanum to lutetium), yttrium and scandium. Their increasing use in many industrial sectors causes an increase of discharges into the environment and particularly in estuarine areas subject to strong anthropogenic pressures. This study focused on assessing the distribution of REEs along the food web of two French estuaries: the Loire and the Seine estuaries, subject to different anthropogenic pressures. Several species representative of different levels of the estuaries food webs were sampled: 8 vertebrates (*T. luscus*, *O. eperlanus*, *P. flesus*, *D. labrax*, *C. ramada*, *S. Solea*, *A. anguilla*, *S. sprattus*), 3 crustaceans (*C. crangon*, *P. logirostris*, *C. volutator*), 2 molluscs (*S. plana*, *L. balthica*), 3 annelids (*N. hombergii*, *N. diversicolor*, *H. filiformis*), 4 algae (*F. vesiculosus*, *F. spiralis*, *U. lactuca*, *U. intestinalis*) for the Loire estuary and 4 vertebrates (*L. limanda*, *P. flesus*, *P. platessa*, *E. vipera*), 3 molluscs (*C. fornicata*, *S. plana*, *L. balthica*), 1 annelid (*N. diversicolor*), 3 algae (*U. intestinalis*, *C. rupestris*, *Ulothrix spp.*) for the Seine estuary. The REE concentrations were measured in organisms by ICP-MS and trophic relations were estimated by stable isotope analysis (C and N). Intra-species differences have been highlighted with greater concentrations of light REEs than medium and heavy ones for all species of the two estuaries. In addition, a higher REE accumulation was observed in lower trophic level species (algae, annelids, crustaceans and molluscs) compared to fish, belonging to higher trophic levels. These inter-species variations demonstrate a phenomenon of trophic dilution. For both estuaries, total mean REE concentrations values were the highest for *U. intestinalis* (34 and 166 µg/g dw) whereas the lowest was for *T. luscus* (0.1 µg/g dw) in the Loire estuary and for *P. platessa* (0.03 µg/g dw) in the Seine estuary. Inter-phylum disparities were also noticeable. For example, in the Loire estuary, *H. filiformis* showed much higher concentrations than the two

other annelid species and among the vertebrates, *C. ramada* presented twenty times higher concentrations than *T. luscus*. Post Archean Australian Shale normalization allowed to highlight gadolinium positive anomalies in the REE patterns of most studied species, especially visible in fish of the Loire estuary and in algae of the Seine estuary.

4.09.P-Mo-114 Bioavailability Evaluation of a European Monitoring Database

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Over the past two decades, bioavailability models (e.g., biotic ligand models, multiple linear regressions) have been developed for several metals to calculate environmental threshold concentrations around the world. Although substantial progress has been made in the development and validation of these models, their application still depends greatly upon the availability of site-specific water quality data for physicochemical parameters, particularly hardness, pH, and dissolved organic carbon. Most water monitoring databases currently do not contain data for all necessary parameters to perform bioavailability analyses or they fail to report specific details surrounding the geolocation of sampling sites. To address these concerns, the metals research organizations and their partners collated data from European member states and ECHA's Waterbase to develop a comprehensive database of water quality parameters measured in European waterways, currently containing nearly one million time-and-location-matched datapoints spanning 1978 through 2022. The database is publicly available in an online tool where users can query multiple fields, including date, location, and chemical parameters. Within the database, over 200,000 datapoints contain reported values of pH, hardness and organic carbon which makes these data eligible for performing bioavailability calculations using a simplified tool such as bio-met. This presentation summarizes the reported data through the lens of bioavailability by using bio-met, to predict the hazardous concentration for 5% of species (HC5) on the eligible data. Although HC5 values differ for each metal due to different interactions occurring between water chemistry parameters and each metal, similar trends can be observed across the substances. In general, regions of Iceland, Croatia and Austria report highly sensitive waters (i.e., low HC5 values) while the regions of Latvia, Estonia, and Finland report relatively insensitive water chemistry conditions (e.g., high HC5 values). These trends correspond most closely with the reported levels of organic carbon in the water. Although organic matter seems to be the driver of HC5 values at either end of the spectrum, interactions involving pH and hardness are also important factors for determining the sensitivity of a local water and their interactions influence the entire distribution of HC5s for each metal.

4.09.P-Mo-115 Long-Term Effects of Copper and Zinc on Microbial Community Structures in Constructed Wetlands

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This study presents the results of the long-term (2007–2014) monitoring of the microbial community structure in the surface sediments of the H-02 constructed wetland system, which was built on the Savannah River Site in Aiken, SC, USA, to treat the wastewater generated at the Tritium facility. The aim of this study was to monitor the changes in the microbial community structure in the surface sediments of a constructed wetland during the first 7 years of operation. Microbial community structure provides valuable information about the functioning of constructed wetlands and helps understand the biogeochemical cycling of nutrients and contaminants.

Phospholipids fatty acid (PLFA) analysis and qPCR were used to identify major bacterial phyla in the sediments. The physicochemical properties of the sediments were also used to deduce potential effects on the microbial community structure over the years. Sulfate-reducing bacteria (SRB) were the most dominant bacterial groups, and their prevalence was progressively increasing throughout the years most likely on the account of methane producers. Concentrations of trace metals (copper and zinc) were negatively associated with methane producers and oxidizer while positively correlated with SRB. Overall, the H-02 wetland system was

efficient in immobilizing copper and zinc through the anaerobic respiration of sulfate by SRB and minimizing methane emission through the progressive elimination of methane producers by SRB and Geobacter.

4.09.P-Mo-116 Spectroscopic Studies of Interaction Between Hg(II) and AgNPs in the Dark

Peter Olusakin Oladoye, Guangliang Liu and Yong Cai, Florida International University

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. Atmospheric Hg (Hg^0 and Hg(II)) can be deposited into natural water by wash-out or rain-out whereby Hg primarily exists as Hg(II) and transformed to other Hg species. Photochemical redox reaction is a major process that leads to transformation of Hg species from one form to another via oxidation or reduction to generate organo-mercury and/or dissolved gaseous Hg. Suspended particles in water can bind Hg species to produce particulate Hg which is expected to participate in Hg transformation. This study focuses on the spectroscopic studies of the interaction between Hg(II) and silver nanoparticles (AgNPs) (20 nm) in the dark to assess the possibility of AgNPs to chemically transform Hg(II) species in water. From X-ray spectroscopic investigation, it was revealed that redox reaction took place between Hg(II) and AgNPs in suspension in the dark. Characteristic peaks of Hg^0 , Ag^0 , AgO and Hg-Ag were found from the processed XPS data, suggesting the feasibility and/or role of AgNPs in transforming Hg(II) in surface water. Furthermore, it was shown that the percentage composition (atom %) of Hg as Hg^0 in 100 ppt Hg (II) suspension (4.02%) was more than 25 ppb (2.05%) owing to the amalgamation of Hg^0 and Ag^0 at high levels of released Hg^0 in 25 ppb suspension. In addition, scanning electron microscopic study revealed that AgNPs increased in size due to aggregation at high metal ion (Hg^{2+}) concentrations. Therefore, from this preliminary investigation, it can be affirmed that AgNPs should play a role in Hg transformation in aquatic environment. Also, it is known that AgNPs exhibit surface plasmon resonance as a result of inherent ability to interact with light. Thus, it is recommended that photoreduction of Hg(II) to Hg^0 by irradiated AgNPs be studied in order to further explore the role of AgNPs in biogeochemical cycling of Hg in the environment in the presence of natural and/or simulated sunlight.

4.09.P-Mo-117 A Predictive Model Created to Understand How Specific Water Quality Characteristics Govern the Association of Microplastics and Cadmium in Freshwater Systems

Lauren Zink¹, Emily Mertens¹, Jackie Zhou¹, Sarah-Ellen Johnston², Matthew Bogard¹, Steve Wiseman¹ and Gregory Pyle¹, (1)University of Lethbridge, (2)University of Alaska, Fairbanks

Environmental characteristics including water quality and sediment properties govern the speciation and partitioning of metals in aquatic systems, altering their bioavailability and toxicity. Our understanding of the partitioning of metals between water, sediment, and biotic ligand components are relatively well understood; however, alternate ligands are being introduced into aquatic systems through anthropogenic activity. Microplastics are a ligand in which metals interact through adsorption to the plastic surface. Using a combination of laboratory experiments and machine learning, we have developed and tested a model that outlines the environmental characteristics that govern the association of cadmium, a metal, to microplastics. This tool has been formatted to a dichotomous-style key, not requiring highly precise measurements but rather requiring whether an environment falls above or below the given thresholds for each water quality parameter which together determine the amount of cadmium bound to microplastic in the system, increasing the model's usability and accessibility. Our model has shown that alterations in water quality can result in anywhere from 10% to 95% of cadmium being bound to microplastic – which, if ingested, could result in varying toxicity. Further experimentation using fathead minnows assessed the coordination of how the environmental characteristics determined by our model (assessing cadmium-microplastic interactions) works in tandem with the Biotic Ligand Model (assessing cadmium-gill interactions) to provide insight on how cadmium-microplastic ligand interactions relate to cadmium toxicity.

4.09.P-Mo-118 Variation of Mercury-Dissolved Organic Matter Stability Constants: Complexation of Mercury(II) with 2-Aminothiophenol Using Ultraviolet Spectroscopic and Isothermal Titration Calorimetric Techniques

Mayowa Ezekiel Oladipo, Guangliang Liu and Yong Cai, Florida International University

Mercury (Hg) is a global pollutant that has raised a high risk to the ecosystem and human health, especially through the intake of high levels of methylmercury bioaccumulated in the food chain. Dissolved Organic Matter (DOM) has been known for its sequestering ability towards Hg, and quantification of mercury-dissolved organic matter (Hg-DOM) stability constants has been proved to improve the predictive power of transport and fate of Hg in the natural water. However, the reported stability constants with using different analytical methods differed significantly which complicated the modeling of Hg speciation in natural water. Therefore, the need for a comparative study of these methods to understand the factors causing the variation of stability constant of Hg-DOM complexation is reasonable to estimate reliable binding constants and elucidate the Hg-DOM binding mechanisms, which will further clarify the importance of Hg-DOM complexation in aquatic Hg cycling. The present study compared the analytical methods i.e., ultraviolet absorbance titration spectroscopy and isothermal titration calorimetry (ITC) to understand the possible causes responsible for the huge variations of the stability constants of Hg-DOM complexation using DOM model ligand (2-Aminothiophenol) of known structure and reaction sites - thiol (-SH) and amine (-NH₂) involved in Hg binding. For ITC experiments, titrations of 0.075 mM 2-Aminothiophenol in 100 mM 2-(N-morpholino) ethanesulfonic acid (MES) buffer at pH 6 (the sample cell) with 1.5 mM Hg(II) in MES buffer at pH 6 (syringe) was conducted at 25⁰C with a stirring rate of 150 rpm for 20 times sequential 2.5 μL injection at an interval of 300 s. The stability constants from fitting the data with independent model using Nanoanalyzer was 5.20 4.98. Furthermore, the UV titration experiment was carried out by the successive addition of 0.001M Hg(II) from 0 to 3 equivalent in the solution of 2-Aminothiophenol (40μM) and the stability constant was determined using HypSpec program. The result showed 1:1 stoichiometry with stability constant of 2.10 1.25. The wide difference observed is because of the lower range of ligand concentrations used in UV titration in comparison with ITC experiments. It can be deduced that the variation of the stability constant depends on the principle and assumption that governs each analytical method and on different experimental conditions required for each method to be valid.

4.09.T Fate and Effects of Metals: Biogeochemical Perspective

4.09.T-01 Controls on Porewater Pb in the Floodplains and Lateral Lakes of the Lower Coeur d'Alene Basin in the Bunker Hill Superfund Site

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Riverine floodplains downstream of mining and other industrial activities are often long-term repositories of contaminated sediment. Fluctuating water levels in these floodplains are believed to cause spikes in metal contaminant solubility by shifting sediment redox regimes, weakening thermodynamic stability of metal hosts. One notable contaminant is lead (Pb), whose redox-dependent transitions and solubility have been the focus of much recent study. Here, we examine sediment and porewater Pb in the floodplains and lateral lakes of the Coeur d'Alene River Lower Basin, part of the Bunker Hill Mining and Metallurgical Complex Superfund Site (Bunker Hill) in northern Idaho. Bunker Hill is laden by 8 million tons of Pb that were released in contaminated mine tailings and smelter waste now spread out along a 37-mile river network. In 2021, sediment Pb concentrations in the Lower Basin averaged over 3,000 mg kg⁻¹, posing a threat for surrounding communities and ecosystems. For our study, we measured 0-4 cm sediment and porewater chemistry in 30 plots scattered across the Lower Basin floodplains and lateral lakes. Plots were sampled twice in 2021 – June (flooded conditions) and October (dry conditions) – to maximize hydrologic differences. Our objectives were to: 1) determine when and where porewater Pb concentrations are highest, 2) analyze how these increases are

related to water level, co-contaminants, and environmental covariates, and 3) aim to use these results to predict porewater Pb hotspots in the Lower Basin. Average porewater Pb was significantly higher in June (231 $\mu\text{g/L}$) than in October (209 $\mu\text{g/L}$). Seasonally inundated floodplains, which dried out in fall 2021, drove this trend. Lateral lakes and permanently inundated floodplains did not show a significant difference in seasonal porewater Pb. A notable exception was a group of plots adjacent to a natural levee, in a backswamp area that floods annually. Porewater Pb at these plots spiked in October (dry conditions), which we hypothesize was due to oxidation of deposited Pb sulfides. Permanently inundated floodplains and lateral lakes had higher sulfide concentrations, lower dissolved organic carbon, and lower dissolved oxygen year-round. Overall, results suggest highest porewater Pb concentrations are in seasonally inundated floodplains and can occur either in the spring or the fall depending on sediment deposition and porewater chemistry.

4.09.T-02 Selenium Bioaccumulation in a Riverine Aquatic Ecosystem: Nonsupport for the Lentic/Lotic Risk Paradigm

Chris Mebane, A Robin Stewart, Erin M Murray, Terry M Short, Veronika A Kocen and Lauren M Zinsser, U.S. Geological Survey

In the United States, the nationally recommended EPA aquatic life criteria for selenium (Se) are intended to protect 80% of water bodies from risk of Se toxicity to aquatic communities by maintaining dissolved Se concentrations below 1.5 $\mu\text{g/L}$ in lentic waters and 3.1 $\mu\text{g/L}$ in lotic waters. These dissolved criteria concentrations were in turn back calculated from an egg/ovary concentration in fish of 15.1 mg/kg dry weight (dw), a concentration protective of most fish species tested, using abiotic accumulation and biotic trophic transfer factors. The calculations assumed lower accumulation potential in lotic waters. That assumption did not hold for the aquatic communities in a large, oligotrophic river, the Kootenai River in ID and MT. Over 3 seasonal events, we collected and analyzed Se in water, sediment, periphyton (3 types), benthic invertebrate (~37 taxa), and fish (5 focal species). Samples were collected from major geomorphic reaches spanning 150 km from Libby Dam, MT to the ID/BC border: 3 fast water reaches (dam tailwater, narrow canyon, broad-braided) and one backwater reach with barely perceptible flows. Dissolved Se concentrations ranged between 0.6 to 1.2 $\mu\text{g/L}$ with about 80% as selenate, 10% as selenite, and the remainder undetermined. During the fall low-flow conditions, dissolved Se concentrations were similar over the 150 km study area. Se concentrations in periphyton were typically between 0.5 and 1.5 mg/kg dw and were not higher at the lentic backwater site. Tissue concentrations in aquatic invertebrates ranged from about 2 to 54 mg/kg, with the higher values occurring in annelids (oligochaetes and leeches) and some mayflies. Invertebrate tissue concentrations at a site varied seasonally up to 6-fold for the same taxa at the same site, although most differences were within a factor of 2. Se in fish tissues tended to be higher in the fast water canyon and braided reaches, contrary to expectations that the lentic backwater or dam tailwater sites would be highest. Among species, Se egg/ovary concentration ranks were roughly Kokanee salmon < rainbow trout < slimy sculpin < northern pikeminnow < mountain whitefish < reidside shiner. Mountain whitefish and reidside shiner consistently exceeded the EPA egg/ovary criterion concentration at all lotic sites, with occasional exceedences observed in other species, except Kokanee. Fish tissue Se concentrations in all species were consistently lower in the lentic backwater site.

4.09.T-03 Dolomite Amendment, pH Neutralization, and Metal (loid) Immobilization in stormwater Bioretention Beds

Abdullah Al-Amin, Erica R. McKenzie and Robert J. Ryan, Temple University

The importance of pH in stormwater bioretention beds cannot be overstated since it impacts plant health, microbial populations, particle stability, and removal of metal(oids) from stormwater runoff. This study investigated the impact of dolomite amendment on bioretention media to neutralize the acidic pH (~ 2.9) caused by iron amendment and subsequent metal(loid) immobilization in the media. To assess dolomite dissolution and metal(loid) leachability, samples of dolomite-amended soil were collected from two bioretention beds during two sampling rounds (one and two months after installation). In the two bioretention beds, the pH was 4.49 and

5.52 in round one, while in round two, it changed to 5.37 and 5.66, respectively. Leachate Mg and Ca concentrations supported comparatively fast calcium carbonate and relatively slow magnesium carbonate dissolution from dolomite. The two bioretention beds had different impacts on metal(loid) immobilization: in one, higher metal(loid) leachate concentrations were observed but there was a substantial percent reduction in metal(loid) leaching followed the order of $Cr > Fe > As > Cu > Pb > Cd > Zn > Mn$; by contrast, the second bioretention bed exhibited no change except for Cu and As, and effectively immobilized the metal(loids) before the first sampling. Additionally, a column study was conducted to evaluate the effects of hydrologic conditions (low flow, high flow, and intermittent flow) on dolomite dissolution and metal(loid) immobilization. Among the treatments, intermittent inflow had the highest porewater Mg and Ca concentrations, indicating faster dolomite dissolution, while low inflow condition exhibited higher porewater metal(loid) concentrations. Metal(loid) normalized concentrations ($[\text{porewater}]/[\text{influent}]$) at the end of the operational period were generally less than 1, except for Mn, Fe, and As. However, as pH became more neutralized, porewater As concentrations increased with operational time. Overall, the study suggests that pH neutralization and metal(loid) immobilization depend on bioretention media deployment period, soil – water contact time, and hydrological properties such as inflow rate and frequency. In addition to these findings, incorporating factors – such as soil to dolomite ratio, ponding, and antecedent dry periods in bioretention media design – can be effective measures for intercepting contaminants, improving ecosystem health, and enhancing biochemical contaminant breakdown.

4.09.T-04 Microbial Communities: The Unsung Heroes in The Biogeochemical Cycling of Trace Metals in Constructed Wetlands

Zeinah Elhaj Baddar^{1,2}, *Xiaoyu Xu*² and *Breann ShangPing Spencer*², (1)*Savannah River Ecology Lab*, (2)*University of Georgia*

The H-02 constructed wetland (CW) was built in 2007 to treat the process water generated at the Tritium Facility on the Savannah River Site (SRS) in Aiken, SC, USA. High levels of copper (Cu) and zinc (Zn) in the wastewater are attenuated through the passage into two adjacent wetland cells. Microbial assisted sulfur reduction plays an important role in immobilizing Cu and Zn by forming insoluble sulfides under anaerobic conditions. In this study, we focused on the spatiotemporal changes in the microbial communities in the H-02 CW using qPCR and next generation sequencing (NGS). To this end, we compared microbial community structures between the cool months (October 2021 – February 2022) and warm months (May 2022 – September 2022), and microbial community structure changes between influent (IF) and effluent (EF) locations and between the two wetland cells (WC1 and WC2). Preliminary analysis of qPCR data of bacterial groups of interest showed that during the cool months, methane oxidizing bacteria (MOB) were more prominent at the IF for both cells whereas *Desulfotomonas spp.* (DSM) were the most dominant bacteria at the EF. During the warm months, regardless of the location or cell, sulfate (SO_4^{2-}) reducing bacteria (SRB) were the most dominant. We conclude that sulfur (S) reduction is the dominant process that took place in the H-02 CW system regardless of the season. Our previous work showed that SO_4^{2-} concentrations in warm months were significantly lower than cool months, most likely due to SO_4^{2-} depletion by the flourishing SRB. Therefore, the anaerobic respiration of SO_4^{2-} was the main process by which sulfide (S^{2-}) was produced in warm months. Whereas It is likely that S reduction was the main mechanism driving S^{2-} production in cool months, since DSM are incapable of sulfate reduction, but rather use elemental S as their ultimate electron acceptor. Preliminary analysis of NGS at the phyla level revealed that Firmicutes were the most dominant in both cells at both locations and during both seasons, albeit higher in warm months, and we are currently analyzing data at the genus level to better understand the specific processes associated with the biogeochemistry of Cu and Zn in the H-02 CW. Overall, the activities of the microbial communities helped in the metal removal in the H-02 CW majorly through sulfur reduction and the subsequent formation of insoluble metal sulfides.

4.09.T-05 Development and Application of a Unit World Model for Flowing Waters

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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 simplified two-layer lake or impounded river. Although created to consider generalized environmental settings (i.e., the unit-world), 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. Since it only has two model “cells,” TICKET-UWM is not well-suited to describe flowing systems that are closer to plug flow than well-mixed. In this work, 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. It includes multiple water column and sediment compartments. Transport processes modeled in the TICKET-2D are advection, dispersion, settling, resuspension, burial and diffusive exchange. In addition, chemical speciation calculations are performed in all model cells (surface water and sediment) using WHAM VII and a solid/solution/seed approach to model precipitation reactions. TICKET-2D calculates equilibrium speciation in every model cell at every time step in the simulation. The ability that sets it apart from other 2-D riverine fate models is the model’s robust treatment of partitioning to natural organic matter. Only precipitated or solid-bound species (i.e., particulate phase species) are permitted to settle, resuspend or be buried. The newly developed TICKET-2D was used to model copper transport and fate in relatively simple “proof of concept” simulations to assess its capabilities. First, TICKET-2D output was compared to an analytical solution. Second, the model was used to simulate copper dynamics in the Flint River, Michigan, USA. Finally, TICKET-2D was used to assess the behavior of copper from a hypothetical release of roof runoff to an actual stream. These simulations demonstrated the model’s veracity and its capability to reproduce key fate controlling processes in a generalized environment.

4.09.T-06 Ecological Risk Classification of Inorganic Substances

Rebecca L. Dalton, Rachel Bouwhuis, Melissa Ma, John Prindiville, Michael Beking and Mei Lin, Environment and Climate Change Canada

The complexity of biogeochemical processes poses a challenge to understanding and predicting the fate and effect of metals. While mechanistic models can improve this understanding, existing hazard and exposure data can also be leveraged to characterize the potential for ecological risk in a regulatory context. This presentation will provide an overview of the Ecological Risk Classification of Inorganic Substances (version 2) (ERC-i-2), an approach developed under the Chemicals Management Plan, a Government of Canada initiative aimed at reducing the risks posed by chemicals to Canadians and the environment. ERC-i-2 is a risk-based prioritization approach for classifying the potential risk that an inorganic substance may pose to the environment by considering its hazard potential and multiple lines of evidence for exposure. Site-based risk classifications are considered within a rule-based framework to produce a final prioritization score. ERC-i-2 uses extensive empirical and estimated exposure information for metals and inorganics and currently includes over 1000 exposure locations and over 1 million individual data points for 44 elements. ERC-i-2 is intended to be an evergreen approach and given the high number of data points, it is built using a computational framework to allow for future integration of new and updated datasets. This presentation will describe key aspects of ERC-i-2 and its application in identifying inorganic substances that may be of low concern to the environment and those of relatively higher concern within a regulatory context.

4.10.P-We Fate of Plastics in the Environment: Towards Unifying Laboratory Experiments, Field Observations, and Modelling

4.10.P-We-093 A Low-Tech, Mass-Based Community-Scientist-Oriented Method for Routine Microplastics Monitoring in Coastal Systems

Troy Langknecht, Dounia Elkhatib, James Farnan, Robert M. Burgess and Kay T. Ho, U.S. Environmental Protection Agency

Sediments are a major sink for microplastics (MP) and while methods for extracting and identifying MP in sediment have improved in recent years, these methods continue to be time and cost intensive and scientifically rigorous. The cost of specialized identification instrumentation can be prohibitive for state agencies, universities, nonprofit organizations, and participatory science groups (i.e., citizen or community science). Further, the analysis time can restrict sampling sizes and replication, geographical area covered, and number of times each site is visited (i.e., single sampling vs. multi-year). Consequently, routine monitoring is resource intensive, thus limiting the availability of baseline and long-term datasets for MP, which are essential for policy development and understanding threats to humans and the environment. The objective of this study was to create and validate a method for detecting MP in sediment that is cost-effective, suitable for scientists of any level, and less time intensive. A series of experiments were conducted on MP spiked sediment samples to optimize the method, including testing various digestion solutions, lengths of digestion and density separation, and combustion, among others. The finalized method, called Oxidation of Microplastics in Sediment (OMS), consists of a 24-hour density separation with a high density (1.5 g/mL) sodium bromide solution to remove dense particulates followed by two 24-hour digestions with muriatic acid (5%) and bleach (7%) to eliminate inorganic carbon and non-polymers. The samples are then filtered over a pre-weighed polycarbonate filter and re-weighed to determine the remaining sample mass (i.e., operationally defined MP). OMS is being validated on 14 marine sediment samples collected off the coast of Massachusetts, USA. This work provides a fast, low-tech, mass-based method for detecting MP in aquatic sediments. With this method, we attempt to open opportunities for routine monitoring of MP across larger areas and allow for rapid detection of changes in MP concentration in coastal systems.

4.10.P-We-094 Development of Neural Network Models for Distributions of Plastics Patches in the Ocean by Sentinel-2 High-resolution Data

Yutaka Kameda and Emiko Fujita, Chiba Institute of Technology

Microplastics (MPs) are widespread in surface water of a boundless expanse of ocean. However, MP ranges from less than 1 μm to 5 mm and their analytical methods as well as sampling methods have not been developed nor have not been integrated in the world. Moreover, recent reports suggested that large MPs such as macro MPs or meso MPs aggregates in surface layers of the ocean and are floating with various debris as big islands or long lines accumulated by tide. It is also known that their distributions in the ocean change dramatically. Though these results indicate size and distributions of MPs in the ocean are very complex, it is important to reveal time trends of geographical distributions of these plastic patches in order to estimate their ecological risk and to control their concentrations. But it is hard to monitor their distributions by general grab sampling of MP samples from the ocean. In this study, the estimation methods to display seasonal geographical distributions of plastic patches in the ocean are developed by using satellite data and generic models. We used 4 bands spectral data from Sentinel-2 satellites according to a previous report by Biermann et al. We also used two indicators such as Floating Debris Index (FDI) and Normalized Difference Vegetation Index (NDVI) to distinguish plastic pixels to others. One strong point in our study is to establish a generic model which can download satellite data from websites, to easily correct the data by Atmospheric Correction for OLI lite version 20181210.0 (ACOLITE), and to calculate FDI values and NDVI values for each pixel. The model can also classify each pixel from 9 materials such as plastic, ships, water, pumice released from volcanoes, sand, clouds, rocks plant and woods by K-nearest neighbor algorithm in python. But the accuracy of the K-nearest neighbor

algorithm is not high enough, approximately 60%. Further revisions such as replacement of the algorithm to neural network models are needed. However, this novel model will enable all users including non-experts to generate geographical distributions of plastic patches in widespread areas such as the Japan sea, the Mediterranean Sea, north Atlantic Sea and other bays and lakes all over the world.

4.10.P-We-095 Development of Semi-Automatic Analytical Methods for Fine Microplastics Greater Than 1 μm by Raman Imaging Microscopy

Yutaka Kameda and Emiko Fujita, Chiba Institute of Technology

It is highly concerned that ultra fine microplastics (UFMPs), which range from 1 μm to 20 μm , may have adverse effects on human health and wildlife. Occurrences of UFMPs in the ocean are also issues which should be revealed in views of ecological risk assessment on aquatic environment and environmental behaviors in marine environment. However, it is difficult to quantify and identify them in water samples because each particle of UFMPs must often be identified by operators so that lots of time and hard work is needed. Adding to that, Raman spectroscopy is not appropriate for observation of a wide range of areas to quantify and identify all UFMPs in samples, which will involve high extrapolation to estimate UFMPs concentrations and their high limits of detection. Pretreatment processes for UFMPs with little contamination are also needed. This presentation shows semi-automatic analytical methods for UFMPs in tap water including pretreatment methods. The pretreatment process of 100L tap water is constituted of general process of microplastics and a novel process. At first, digestion of hydroperoxide and density separation by Sodium Iodide were conducted to remove degradable organic materials and heavier matrixes. But fluorescent matrixes, which prevent microplastics analysis by Raman spectroscopy, cannot be removed. Therefore, novel analytical methods to remove fluorescent matrixes were developed. Our analysis of the matrixes by Raman spectroscopy revealed that they were butyric acid-like compounds, nonanoic acid-like compounds and methyl octadecenoate-like compounds. Adding to that, they were lipid and specific rates were less than NaI solution. Therefore, fat-soluble solvent, which could not affect microplastics and whose specific rate was less than plastics, were used with centrifugation. Finally, the settlement was passed through an area of 4 mm diameter of a silica membrane. UFMPs on the membrane can be analyzed by DXR3xi Raman Imaging Microscope (Thermo Fisher Scientific). The Raman microscope has Ultra-fast chemical imaging capability so that it has possibility to analyze UFMPs to be observed in more than 50 % area to total for 88 hours. By this analytical method, 0.3 μm of microplastics could be detected. The analytical time can be shorter by smaller filtration area of the silica membrane.

4.10.P-We-096 Spatial Distribution of Microplastics in Overbank Deposits and Sandbar Sediments of the River Loire (France)

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Sediments from rivers constitute sinks for microplastics (MPs) but the understanding of microplastics depositional mechanisms within sediments remains fragmentary. The aim of this study is to identify factors that influence MP contamination and its variability in surface sediments at very small spatial scale: intrinsic MP properties, sediment composition, sediment depositional environment. In this context, 14 surface sediment samples were collected in an 8 square km area of the river Loire in overbank deposits and flooded channels. These samples correspond to different depositional environments with various connectivity levels and sediment compositions which are characterized by grain size analysis and organic matter contents. After treatment of samples (organic matter digestion and densimetric separations), the quantities, types and sizes of MPs in each sample are analysed by μFTIR imaging (Fourier Transform Infrared Spectroscopy coupled to microscopy). Results show MP levels ranging between 867 and 10,635 MPs/kg dry weight sediment averages. An internal variability is observed in sediment samples, MP types and sizes being consistent between replicates whereas MP abundance shows a high variability. Sediment deposition mode is shown to be an influencing factor of internal variability of MP abundance. At the floodplain scale, a substantial spatial variability is illustrated.

Sediment composition (< 63 µm sediments and organic matter), as a proxy of hydro-sedimentary processes, is shown to have an influence on abundance of total MPs, polypropylene and polyester particles, and MPs smaller than 500 µm. This observation is verified by a negative correlation between river width and MP abundance. The geomorphology of sampling sites also has an influence on MP abundance, flooded channels being more contaminated than overbank deposits. Width of river appears to be an important influence factor, as the narrower the river is, the more contaminated it is. However, river width is also correlated to fine and very fine sand contents, which implies that sediment composition is a main influence factor for MPs. MP abundance is highly positively correlated to the frequency of connexion to the river of sampling sites in the case of flooded channels.

4.10.P-We-098 Spatial and Temporal Patterns of Plastic Pollution in the Matagorda Bay System: Domestic or Industrial Source Concern?

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There are concerns on the ecological threat posed by plastic debris (including microplastics, MPs) and other chemical pollutants to Matagorda Bay system resulting in water quality decline, with largely unknown adverse impacts on various species and human health. These uncertainties associated with local emissions and transport of mismanaged plastics into aquatic compartments, highlight an urgent need to provide data on inventories and fluxes of these particles in Matagorda coastal waters. Spatial variability of plastic transport dynamics within the Bay system and temporal variability due to various seasonal activities (i.e., tidal fluxes) are crucial indexes in understanding the effect of plastic pollution in the environment and possible impact on human health. To address these knowledge gaps, a plastic monitoring campaign was carried out in twelve different locations across the Matagorda coastal waters, the second largest estuary on the Texas Gulf Coast. Sampling campaigns were carried out between July 2021 and March 2023 to investigate the plastic occurrence and distribution, polymer types, and factors contributing to observed abundance in each location within the Bay system. A 1 by 5 m transect was laid in quintuplicate for each beach site and plastics were sampled within these transects (surface extraction). Using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), a total of 1189 plastic items have been characterized into different polymer types. Our preliminary data show that greater plastic concentrations (63%) were found near Point Comfort throughout the sampling campaign period which coincidentally is a known hotspot for pre-production plastic pellets emission by the plastic company in this location. Also, there is a larger input from land-based sources through storm runoffs. This study will provide important data to inform transport modelling, source, fate, and transport evaluation, and eventual assessment of risks of plastic pollution within the Matagorda Bay system to better manage this ecosystem's health while also educating stakeholders in the region about this pollution.

4.10.T Fate of Plastics in the Environment: Towards Unifying Laboratory Experiments, Field Observations, and Modelling

4.10.T-01 Processes of Environmental Plastic Weathering and Biodegradation in Natural Systems

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Plastics are universal, versatile materials that are ubiquitous in current society. However, the ever-increasing accumulation of waste in the environment reflects the rate of anthropogenic production, use, and mismanagement of waste and has gained global attention from environmental, human health, and sustainability perspectives. One such component of waste is plastics and the factors affecting fate and transport in the

environment are still poorly understood. Here, we aimed to identify and measure the consequences of direct plastic-microbe interactions (e.g., biofilm growth, biodegradation) that can affect plastic fate and transport by conducting laboratory experiments with both pure and mixed-species environmental enrichment cultures. We established a phylogenetically-diverse culture collection consisting of 10 bacterial and 5 fungal species of polyethylene (PE) degraders. From these, we cultivated *Rhodococcus ruber*, *Pseudomonas putida*, and all 5 fungal species on PE as a sole carbon source and observed CO₂ production as a result of PE carbon remineralization. The fungi produced 10X more CO₂ than the two bacterial species, suggesting that fungi have a substantially enhanced potential for PE degradation. We also performed comparative genomic analyses of 7 bacterial PE degraders and found few shared genes involved in cross-membrane transport of polymer-derived molecules, suggesting diverse microbial mechanisms for accessing PE carbon. The application of multi-omics tools is informing the discovery of new PE degradation mechanisms. Further, given that microbes in nature work together in multi-species consortia, we report the development of environmental enrichment cultures from lakewater and sediment sources to study the cooperative interactions that can contribute to PE biodegradation using omics techniques and mass spectroscopy-based imaging. Altogether, these results will help to develop new metabolic models of PE-degrading microbes and microbial consortia that can be used to predict impact of genetically-engineered improvements, to identify the factors necessary for difficult-to-grow microbes, and to predict outcomes for different PE compositions and environmental conditions.

4.10.T-02 Specific Surface Degradation Rates of Non-Biodegradable Plastics: What We Know and Implications for Future Degradation Studies

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There is no available life cycle analysis (LCA) framework to quantify the end-of-life impact of plastic wastes that are leaked into the environment. To fill this gap, it has been proposed to estimate the fate factor, a term based on the distribution and residence times of plastic waste in specific environmental compartments. This project aims to calculate the residence times by using the specific surface degradation rate (SSDR), which allows for comparison of the degradation of plastics regardless of their composition, shape, degradation mechanism, or environmental compartment. Readily extractable literature data is mostly available for analyzing biodegradable plastics measured using standard methods, while this research expands SSDR estimation to recently published literature on the degradation of non-biodegradable plastics via abiotic pathways (photo/photochemical and mechanical degradation). Appropriate literatures will be searched and collected using a critical review approach, and those with the necessary information (e.g. initial mass, length, and final degradation products) will be included for calculating SSDR. By using existing data in literature, the SSDRs of highly produced non-biodegradable plastics (i.e. polyethylene, polypropylene, and polystyrene) can be estimated through dissolved organic carbon and carbon dioxide production measurements (produced by photodegradation) and through particle concentration and size distribution measurements (produced by photodegradation and mechanical degradation). Initial estimates indicate that polyethylene has a SSDR between 0.2 and 36.3 m/yr, polypropylene has a SSDR between 0.6 and 1.2 m/yr, and polystyrene has a SSDR between 4.31 and 16.3 m/yr. The preliminary calculations for polystyrene disagree with previously reported estimates (0.001 m/yr), indicating the importance of further studying plastics which degrade through abiotic pathways. The conversion of lab scale accelerated weathering to field study will be carefully considered. Future work will assess the quality and availability of current data in calculating SSDR, identify knowledge gaps and limitations in experimental data for plastic degradation, and highlight the best practice of accurately reporting data. In addition to the fate of plastics, this research lays important groundwork for novel LCA that will inform sustainable design of future plastics.

4.10.T-03 Seasonal Variability of Microplastics in Hamilton Harbour: Do Season Affect the Microplastics Distribution in Hamilton Harbour?

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Hamilton Harbor is identified as one of the Areas of Concern in Lake Ontario due to its vicinity to wastewater treatment plants, river streams, and various industries. This has led to degradation in water and sediment quality measures and arrays of pollutants, including microplastics, have been reported in Hamilton Harbour. The detrimental effect of microplastics on living organisms has been proven so far, and they have been detected even in the human bloodstream, showing the significance of microplastic detection and analysis studies. Different hydro-environmental factors can affect the presence of microplastics in the environment, one of which is thermocline formation during hot seasons. Thermocline formation, caused by temperature-induced stratification, has been shown in lakes to affect water quality indexes, including pH, dissolved oxygen, and turbidity, among other factors. This study aims to investigate the distribution of microplastics in Hamilton Harbor. Microplastics are not passive contaminants; their densities actively affect their in-depth distribution. Combined with mixing caused by the thermocline formation, their density, size, and shape will govern the distribution of microplastics in Hamilton Harbor. To conduct the research, bulk water samples were collected from Hamilton Harbor at hot and cold seasons, August, September, and October, with various expected thermocline formations. Also, in-depth turbulent dispersion profiles were collected using a MicroCTD device. Water samples then were filtered on small-pore filters to assure the exact estimation of microfiber which can easily escape from larger nets. Various preparation methods were used, including digestion and filtration. The prepared samples were then analyzed using microscopy techniques and were categorized based on their size, shape, and color. Furthermore, using an advection-diffusion model, the possible distribution of the microplastic contaminant particles is simulated, implementing the in-situ dispersion profiles. Using this method allowed for a more detailed analysis of the samples and provided additional information about the composition of the microplastics found in the water. Combined with the results of numerical simulations, this study assesses the role of microplastic characteristics and stratification in their in-depth distribution, showing the significance of thermocline formation during hot seasons.

4.10.T-04 The Migration of Polycyclic Aromatic Compounds in Plastics

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Plastics have become a necessity of modern-day life with a range of uses from medical supplies, children's toys, and food packaging. As a product that is derived from fossil fuels, it would be expected that plastics contain a multitude of polycyclic aromatic hydrocarbons (PACs). It has been widely accepted that many plastic types are capable of leaching into its surrounding medium, yet very little published data exists for this phenomenon. This aim of this study will identify and quantify the PACs that exhibit the leaching ability of plastics and thus the migrations of PACs from plastic containers into food based mediums. Early data suggests that this effect occurs under certain conditions, although typical conditions have yet to be investigated.

4.10.T-05 Leveraging Physiology & Behavior to Better Understand Exposure, Uptake, & Elimination of Micro- and Nanoplastics (MNP) in Pelagic & Benthic Species 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 Hermesen 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.

4.10.T-06 Relevance & Reliability of Environmental Sampling Data for Use in Quantitative Risk Assessment – Towards Developing Best Practices & Guidance in Sampling & Reporting

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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.

4.10.V Fate of Plastics in the Environment: Towards Unifying Laboratory Experiments, Field Observations, and Modelling

4.10.V-010 Distribution and Ecological Risk Assessment of Microplastics in the Water of St. Lawrence River, Estuary, and Saguenay Fjord in Canada

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Microplastics are plastic particles ranging in size from 1 µm and 5000 µm and are contaminants of environmental concern because of their persistent, bio-accumulative, and toxic properties. However, little is known about the microplastic contamination in St. Lawrence (SL) Estuary and adjacent riverine environments. This data gap impedes risk assessments of plastic pollution in this distinctive and significant ecosystem. The objective of this study is to examine the spatial and vertical distribution of microplastics in the water of the SL Estuary, as well as the SL River and the Saguenay Fjord, which are the primary waterways that flow into the SL Estuary. Furthermore, the data generated will be used to evaluate the ecological risks of microplastics. Surface water samples (2.4-7.2 L/site) were collected in 2022 from 22 sites along the SL River (n=6), SL Estuary (n=9), and Saguenay Fjord (n=7) of the Quebec (Canada) region in June 2022 as a pilot study. Surface and depth (Saguenay Fjord only) water samples will be collected from the same sites in 2023. Preliminary results from 2022 found suspected microplastic particles with a mean abundance and size of 3.1 ± 0.5 (count; n/L; SE) and 180 ± 17 µm, respectively, after blank subtraction (30 field and procedure blanks). Fourier transform infrared spectroscopy will be used to determine the particle type at a later date. Transparent fibers (33%) were the dominant suspected microplastic particles with an average length of 255 ± 34 µm. Although not statistically significant, higher abundances were found in samples from Saguenay Fjord (4.3 ± 1.0 n/L), followed by the SL River (2.8 ± 0.9 n/L) and the SL Estuary (2.4 ± 0.6 n/L). Samples from the SL River showed a decreasing trend of suspected microplastic particles from Montreal (4.5 ± 1.0 ; n=3) to the upstream of Quebec City (not detected; blank subtraction n=4; 3 films and 1 fiber). In the Saguenay Fjord, two samples close to Jonquière (7.5 n/L) and La Baie (7.5 n/L) showed higher abundance than other sites, possibly due to more industrial activities in these upstream areas. The data produced by this study will be input into a polymer-based ecological risk assessment followed by pollution load and hazard index to better elucidate the plastic pollution in this aquatic system. Preliminary results will be shared in this poster presentation.

4.11.P-Th Human Exposure to Organic Chemicals of Current Concern

4.11.P-Th-069 Polyfluoroalkyl Substances (PFAS): Ligands that Bind the Orphan Nuclear Receptor 4A1 (NR4A1, Nur77)

Amanuel Esayas Hailemariam, *Gregory Martin*, *Srijana Upadhyay*, *Garhett Wyatt* and *Stephen Safe*, Texas A&M University

Polyfluoroalkyl substances (PFAS) have been produced since the 1950s and are used extensively in multiple commercial products including food packaging, paints, lubricants and fire retardants. Mixtures of these compounds are now widely found in the environment, wildlife and humans and are considered to be a major class of persistent organic pollutants. Human studies show that higher levels of some PFAS compounds are associated with increased incidence of cancers and other diseases and these can also be observed in laboratory studies and are compound/compound class specific. Some responses modulated by PFAS resembled those observed in cancer cells expressing and overexpressing the orphan nuclear receptor 4A1 (NR4A1, Nur77) and its ligands. Initial studies investigated the binding of perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) to the ligand binding domain (LBD) of NR4A1 using a binding assay which measures the loss of fluorescence of a tryptophan residue in the LBD. The K_D values for binding PFOS and PFOA were 2.3 µM versus 19.4 µM respectively and structure-activity studies indicate that PFOS containing

the octafluoro moiety exhibited the highest binding affinities (lower K_{DS}). In addition, we also observed that PFOS bound NR4A1 using an isothermal titration calorimetric assay and the K_D value was 69 nM indicating relatively high affinity ligand-receptor interactions. We further investigated the effects of 10, 25 and 50 μ M PFOS on the growth of several cancer cell lines and growth promotion was variable and included a 3- to 5-fold increase in growth in the highly responsive MIA PaCa-2 pancreatic and Rh30 rhabdomyosarcoma cells, respectively. Further studies in Rh30 cells showed that the remarkable growth promoting activity was NR4A1-dependent and exceeded the effects of growth factors. Additional studies on both functional and genomic responses demonstrated that PFOS is an NR4A1 agonist in cancer cells and this was consistent with studies suggesting that exposure to this widespread environmental contaminant may enhance carcinogenesis. These effects are structure-dependent and the NR4A1 activity of other PFAS are currently being investigated.

4.11.P-Th-070 Sewer Gas Siphon: A Mitigation Approach for Sewer Gas Vapor Intrusion

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For decades, volatile organic compounds (VOCs) have been migrating from subsurface sources into indoor spaces, affecting residents with acute and chronic health effects. This phenomenon is widely known as vapor intrusion (VI). Vapor intrusion pathways through sub-slab cracks due to volatility of the compounds have been well-established. Mitigation of VI through foundation cracks has several well-defined and accepted approaches, such as sub slab depressurization systems. A growing number of studies show that VOCs can also migrate into buildings through sewer connections and defective pipe fittings. In the present work, multiple residential buildings near a VI site were impacted by a sewer pipeline that was reported to be one of the preferential pathways. This study investigated an approach that uses a gas siphon in the sewer laterals that service the residential units with the intent of reducing migration of vapor-phase contaminants prior entering into the buildings. Pre- and post-mitigation concentrations data were collected to examine the removal efficiency of gas siphon. Comprehensive analytical approaches are incorporated to evaluate the VOC concentrations in sewer system and to investigate variation in risks levels for residents in the building. Following installation of the gas siphon, vapor-phase concentrations of TCE and PCE in sewer system declined drastically which suggests siphons are a viable approach for mitigating sewer gas VI. The results of this study highlight the benefits and challenges of gas siphons and provide a strategic approach in mitigating VI risk associated with sewer pathways.

4.11.P-Th-071 Dietary PFASs Exposure from Home-Grown/Raised and Local Foods for a Midwestern PFAS-Impacted Community

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Dietary exposure to per- and poly-fluoroalkyl substances (PFASs) has been raised as a concern due to elevated PFAS found in water, biosolids, and soil. Elevated concentrations have been identified in livestock and wildlife as well as produce in impacted areas. While elevated PFASs in drinking water clearly contribute to elevated exposures, less is known about the contribution of home-produced and locally-grown foods. Therefore, we conducted a detailed exposure assessment for a Midwestern community where drinking water contamination was discovered in 2018 (>1400 ng/L PFOA+PFOS). During the growing seasons of 2020/21, we collected samples of produce and soil from the gardens of 27 homes as well as chicken eggs (n=2) and venison (n=3). Data were collected for each sample and participants completed a detailed exposure questionnaire. Additionally, 166 local food samples of 10 different food types were collected from farms within a 15-mile radius of the community as well as from background locations from a neighboring county. Samples were combined by food type into 28 composite pairs. All samples were tested for 48 PFASs using LC-MS/MS (animal products) and LC-QTOF-MS (soil and produce) with improved methods designed to minimize bias during sample extraction.

Among the home samples, modestly elevated concentrations of PFOS were found in eggs (3.5 ng/g) and venison (14 ng/g). PFOS was detected infrequently at low levels in produce (Max = 0.20 ng/g) with PFBA at the highest concentrations, which may reflect greater plant uptake of shorter chain PFAS and/or ongoing low levels of PFBA in tap (irrigation) water. Among the farmer's market samples, PFAS concentrations were elevated near the site compared to the background for eggs, spinach, and butternut squash (Sum PFAS ranged up to 6 ng/g). In addition to sharing details of these findings, we will report exposure estimates for home-produced and locally grown foods.

4.11.P-Th-072 PFAS in Me: Which Ones and How Much?

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Bioaccumulation of PFAS in the human body resulting from environmental exposure is a growing public health concern. Recent studies have linked PFAS exposure to adverse health outcomes including childhood health complications, reduction in kidney functions, thyroid disease, hormone suppression, decreased fertility, increased cholesterol levels, and diabetes, among others. Given the prevalence and ubiquitous nature of PFAS in the environment and every-day consumer products (including our drinking water supply), there is a critical need to develop quantitative tools capable of accurately and precisely detecting low-levels of PFAS in biological fluids to understand the impact on the human body. In this study, we combined low volume blood sampling with the SCIEX QTRAP 7500 system for the analysis of trace level of PFAS. The analysis was performed on the author and the results of the analysis are shared to demonstrate what PFAS exposure looks like in a typical American. A finger-prick was used to draw capillary blood. The first drop of blood was wiped away with a PFAS-free gauze and the Mitra device is applied to the subsequent drops of blood. The four Mitra tips contained approximately 30 μ L of blood and were stored at -20°C until extraction. Absorptive Mitra tips were then placed in polypropylene vials with isotopically labelled internal standards and acetonitrile to aid with protein removal. The samples are sonicated and allowed to equilibrate prior to a centrifuge step to condense the precipitated protein for easier removal. The extracts were then combined and solid phase extraction (SPE) was performed. Injection internal standards (ISs, or recovery standards) were added to the SPE extract immediately prior to placing it in a new polypropylene vial for analysis. Data were collected using a SCIEX QTRAP 7500 system using electrospray ionization (ESI) in negative mode. The total amount of perfluorooctanoic acid (PFOA) detected was 0.82 ng/mL, however, only the linear version of PFOA was detected. This value was slightly lower than the median value of 0.9 ng/mL for Americans aged 18-49 according to the United States Environmental Protection Agency. The total amount of perfluorooctanesulfonic acid (PFOS) detected was 1.862 ng/mL, which again is lower than the median value listed by the EPA of 2.6 ng/mL. Finally, perfluorohexanesulfonic acid (PFHxS) was detected at a value of 1.558 ng/mL or 2.7 times higher than the listed median EPA value of 0.6 ng/mL.

4.11.P-Th-074 Assessment of Per- and Polyfluoroalkyl Substances (PFAS) in Tap Waters from Miami-Dade, South Florida

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Per- and poly-fluoroalkyl substances (PFAS) are a class of synthetic chemical compounds widely used in various industrial and commercial products due to their unique properties, including resistance to heat, water, and oil, which make them useful in a wide range of applications, such as non-stick cookware, water-resistant textiles, and firefighting foams. However, their persistence in the environment and the human body has led to concerns about their potential human health effects. Exposure to PFAS through drinking water has been associated with various adverse health outcomes, including developmental delays, reduced immune system function, and an increased risk of certain cancers. One of the most concerning routes of exposure to PFAS is through drinking water contaminated with these chemicals. Drinking water contaminated with PFAS has become a growing public health concern in recent years. There is a need for more data on the prevalence of

these chemicals in different regions and to understand how these substances may affect human health. To address this concern, levels of PFAS are being determined in 60 tap drinking water samples which were collected between March and May 2023 from nineteen zip code areas in Miami Dade County, South Florida. The samples were processed using Solid Phase Extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), a sensitive and reliable method for analyzing PFAS in water samples, based on the recent draft EPA Method 1633 (2nd draft). In this study, we will focus on assessing the levels and distribution of 40 legacy and emerging PFAS across Miami-Dade County. These results will provide valuable insights into the extent of PFAS contamination in drinking water sources in Miami-Dade and add to the growing evidence of the prevalence of PFAS contamination in some county regions near PFAS point sources. Further studies will be conducted in other counties evaluating the potential associations between PFAS exposure through drinking water and certain types of cancer.

4.11.P-Th-075 The Optimization of Arsenolipid Detection Methods in Seafood: Identification and Quantification of Prevalent Arsenolipid Species

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Arsenic (As) in seafood poses a potential health risk due to the popularity of seafood in the diet and the toxic effects of As, which include cancer, neurodegenerative disease, cardiovascular disease, etc. In vitro studies have shown that arsenolipids (AsL), a class of organometallic As species that is found in seafoods, are cytotoxic to human bladder and liver cells and have neurotoxic properties. However, the prevalence and toxicological effects of AsL remain poorly understood in humans. The detection of AsL in seafood is challenging due to the lack of standard reference materials (SRMs) and the complexity of fish tissues, hindering accurate identification, detection, and toxicological analyses. The present study aimed to optimize an established method for identifying AsL in seafoods, specifically focusing on determining the predominant types of AsL in fish and seaweed SRMs and in fish collected from local markets. To evaluate As extraction efficiency, two different extraction methods were applied to three SRMs: Dogfish liver (DOLT-5), hijiki seaweed (CRM 7405-b), and tuna fish (BCR 627). Additionally, 48 seafood samples were collected from local markets for analysis: 22 tuna, 12 salmon, 6 shrimp, 4 clam, and 4 mackerel. A sequential extraction method consisting of hexane, dichloromethane/methanol, water, and 1% nitric acid, followed by liquid chromatography hyphenated to inductively coupled plasma mass spectrometry (HPLC-ICPMS), was used to identify water-soluble As and non-polar AsL species. Total As extraction efficiencies for the SRMs were 92 to 98%. Previously unidentified AsL were detected in the SRMs; arsenofatty acid 362 (AsFA362) was detected in all three SRMs (16 ± 5 $\mu\text{g}/\text{kg}$ in CRM 7405-b; 6 ± 1 $\mu\text{g}/\text{kg}$ in BCR and 5 ± 1 $\mu\text{g}/\text{kg}$ in DOLT-5) while arsenohydrocarbon 444 (AsHC444) was detected in DOLT-5 (0.5 ± 0.007 $\mu\text{g}/\text{kg}$). Unidentified AsL were also detected in DOLT-5 (8 ± 2 $\mu\text{g}/\text{kg}$), CRM 7405-b (580 ± 7 $\mu\text{g}/\text{kg}$), and BCR 627 (14 ± 7 $\mu\text{g}/\text{kg}$). In the fish samples, AsFA362 was detected in 58% of samples (5 to 281 $\mu\text{g}/\text{kg}$) while AsHC444 was detected in 12% of the samples (1 to 30 $\mu\text{g}/\text{kg}$). The sum of unidentified AsL in the tuna samples were 5831 ± 822 $\mu\text{g}/\text{kg}$ (3.18%), 857 ± 21 $\mu\text{g}/\text{kg}$ in salmon (5.32%), 244 ± 3 $\mu\text{g}/\text{kg}$ in shrimp (17.22%), 882 ± 9 $\mu\text{g}/\text{kg}$ in clam (19.3%), and 346 ± 8 $\mu\text{g}/\text{kg}$ in mackerel (18.5%). The molecular structures of the unknown AsL will be determined in future work by ESI-MS, and in vivo toxicological studies will be performed to understand their effect on human health.

4.11.P-Th-076 Updated and Novel Methods for Investigating Organophosphate Esters in Particulate Matter

Adelaide E Clark, Tess Bonanno, James Bateman, Parker Keller and Anne Gathof, Providence College

According to the World Health Organization (WHO), "one of the biggest environmental threats to human health" is air pollution. Since organic chemicals can efficiently sorb to particulate matter (PM), the health effects associated with these chemicals must also be considered a hazard. One such "high production-volume" chemical class are the organophosphate esters (OPEs), which are a "reemerging" pollutant with a wide-range of

uses in consumer products, including as a flame retardant (FR) and plasticizer. Despite known and suspected health effects of OPEs, there is no international regulation in place for OPEs and studies of these chemicals in PM in the US are limited. Previously, methods have been developed for the extraction of OPEs from PM. However, these methods are either time-consuming (e.g. using Soxhlet extraction) or hazardous to human and environmental health (e.g. using dichloromethane as an extraction solvent). An updated high-throughput pressurized liquid extraction method has been developed for the extraction of OPEs from PM using non-chlorinated solvents. Additionally, a method has been developed using pressurized liquid extraction to investigate OPEs from surface grime samples. Results of reproducibility studies, analysis of SRMs, method detection limits, and other confirmation studies will be presented.

4.11.P-Th-077 Investigating the Presence of Organophosphate Esters in Particulate Matter from Wildfires

Adelaide E Clark, Parker Keller, James Bateman, Tess Bonanno and Anne Gathof, Providence College

The World Health Organization (WHO) considers air pollution “one of the biggest environmental threats to human health”. Wildfires cause changes to atmospheric particulate matter (PM), which change the health outcomes associated with PM. Since organic chemicals can efficiently sorb to particulate matter (PM), the health effects associated with these chemicals must also be considered a hazard. Organophosphate esters (OPEs), which are a “reemerging” pollutant with a wide-range of uses in consumer products, including as a flame retardant (FR) and plasticizer, are one such “high production volume” chemical. Despite known and suspected health effects of OPEs, there is no international regulation in place for OPEs and studies of these chemicals in PM in the US are limited. The Pacific Northwest is becoming more and more inundated with wildfire smoke from increased dry conditions due to climate change. As wildfire occurrence increases, so does the likelihood of structures containing consumer products being caught up in their destruction. Filter-based samples of total suspended particulate (TSP) and PM_{2.5} have been collected before, during, and after smoke events from wildfires. Samples were extracted to determine the concentration of 37 OPEs in a rural area as a function of size fraction, bulk PM_{2.5} concentrations due to wildfire smoke, and PAH concentrations. Comparison of partitioning between size fractions will allow for a better understanding of health-related exposures during wildfires, since PM_{2.5} is the respirable fraction of PM. Additionally, since PAHs are well-documented byproducts of incomplete combustion, but not commonly used a marker of rural air pollution, their presence further enforces that air masses sampled are from wildfires. Limited data on indoor PM levels will also be presented.

4.11.P-Th-079 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.

4.11.P-Th-081 The Guts of PFAS Fish Consumption Advisories

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Last year we co-chaired a session at the SETAC North America 43rd Annual Meeting titled, “To eat, or not to eat? The Guts of PFAS Related Fish Advisories”. The session highlighted the complexity of PFAS fish consumption advisories (FCAs). This discussion extended beyond SETAC and initiated a larger collaborative effort to develop a publication highlighting the session findings. This publication will highlight successful parameters involved in deriving FCAs and underscores the necessity for continued interdisciplinary collaborative efforts by regulators, academic researchers, and industry professionals to develop robust and meaningful FCAs for the protection of human health. The forthcoming review paper addresses existing challenges, knowledge gaps, and needs related to issuing FCAs for PFAS. We explore epidemiology, analytical challenges, confounding factors, study designs, risk communication and policies used in developing PFAS related fish advisories. Information is presented for three focus areas: 1) methods and sources leading to the variability in numerically derived FCAs; 2) sampling and analytical methods used to supply data for generating FCAs and associated their associated challenges; and 3) key risk management, risk communication, and policy challenges for PFAS-related FCAs. Conclusions focus on data gaps, recommendations for research, and considerations for future management and regulatory strategies to protect public health.

4.11.P-Th-082 Development of a Highly Sensitive Analytical Method to Detect 1,4-Dioxane and Co-occurring Contaminants in Drinking Water and Blood Samples

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1,4-Dioxane is a synthetic industrial chemical that has been widely used in commercial products. The United States Environmental Protection Agency (EPA) classified 1,4-dioxane as a probable human carcinogen based on evidence from experimental animal studies. Approximately 7% of drinking water samples tested across the US exceed the federal cancer risk guideline level of 0.35 µg/L, including most locations at Nassau and Suffolk counties on Long Island, New York, as suggested by data from EPA’s Third Unregulated Contaminant Monitoring Rule (UCMR-3). Despite this significant environmental contamination, no federally enforceable drinking water standard has been established in large part due to the lack of understanding of 1,4-dioxane exposure level and its health impacts to humans. While biological monitoring provides a more direct means of assessing exposure and has the advantage of aggregating across all sources and routes, low concentrations in blood present measurement challenges. We present an analytical protocol to detect 1,4-dioxane and co-occurring contaminants (1,1-dichloroethane; 1,1,1-trichloroethane; trichloroethylene) in blood and water samples, providing sensitive detection of low levels in the ng/L range. This method extracts samples by headspace solid-phase microextraction with a carboxen/polydimethylsiloxane fiber and uses gas chromatography-high resolution mass spectrometry under selected ion monitoring for targeted analysis of 1,4-dioxane and co-occurring contaminants. This novel analytical method will be applied to paired drinking water and blood samples collected from healthy adults residing in areas near known Superfund sites on Long Island, NY with known groundwater 1,4-dioxane contamination. Our research will enable the evaluation between

environmental and biological concentrations of 1,4-dioxane (and its co-occurring contaminants), addressing important scientific questions in addition to urgent community questions about 1,4-dioxane exposure and human health.

4.11.P-Th-083 Trends of New Flame Retardant, PFAS, and Plasticizer Notifications in Canada

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The New Substances Notification Regulations (Chemicals and Polymers) of the Canadian Environmental Protection Act, 1999 (CEPA) are jointly administered by Health Canada and Environment and Climate Change Canada, through the New Substances (NS) program. These regulations ensure that new substances (i.e., not on the Domestic Substances List) are not imported into or manufactured in Canada before undergoing ecological and human health risk assessment. The NS program has received over 21,000 notifications for new substances since 1994. Due to their potential to cause risk to human health and the environment, certain substances have been identified as needing a more thorough examination under the Chemicals Management Plan (CMP). These include flame retardants, per- and polyfluoroalkyl substances (PFAS) and phthalates. To mitigate risks, the Government of Canada has taken action on several of these substances. Although many of these substances have been assessed and managed, they may be replaced by similar substances notified to the NS Program. The NS program's database was searched to analyze trends in notifications of new flame retardants, PFAS, or plasticizers (including phthalates). An overall decrease in notifications for all three substance types was observed: **(A)** Flame retardant notifications were submitted to the NS program every year between 1994 and 2021. NSN submissions for brominated and organophosphate flame retardants have declined. **(B)** Long-chain PFAS have not been notified to the NS program since 2004. PFAS continue to be notified with either short-chain (chain length ≤ 3 carbons) or medium-chain (chain length 4-7 carbons). Most PFAS were notified as a refrigerant/blowing agent, water and oil repellent, or solvent. **(C)** Most plasticizers notified to Canada were non-phthalates. Traditional phthalates represent a small proportion of new plasticizers notified to the NS program. Despite the decrease in notification of new flame retardants and long-chain PFAS, the number of notifications for other structural types of flame retardants and short-chain or medium-chain PFAS has remained constant over the years. When a potential risk to human health or the environment was identified for a particular substance, the NS program imposed risk management measures (for example, Ministerial Conditions to limit the use). Continuing attention should center on whether these groups may show risk in the future.

4.11.P-Th-084 Evaluation of Environmental Impact of Vehicular Emission on Soil and Vegetables from Farmlands along the Major Highways in Enugu State"

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Air, soil and vegetation in the three selected highway, Enugu-Abakiliki, Enugu-Onitsha and Eke-Iwollo 5 meters and 500 meters from the road in Enugu State were investigated for the presence of toxic air gaseous constituents, Polycyclic aromatic hydrocarbon (PAHs) and heavy metals in the soils and vegetables. Two of the highway roads (Enugu-Abakiliki and Enugu-Onitsha) are associated with vehicular emission due to high level traffic density while Eke-Iwollo road is associated with low traffic density site. The polycyclic aromatic hydrocarbon (PAHs) in the soil and food cultivars were determined using Gas Chromatography-Mass Spectrophotometer while heavy metals were analysed by Atomic Absorption Spectrophotometer. Air-vehicular emission of (NO₂, SO₂, PM_{2.5}, PM₁₀ and total carbon) were measured morning, afternoon and evening with Aeroqual/Crowcon Gasman monitors and GPS Garmin GPS. The results of the investigation reveals that high levels of the toxic gaseous substances (NO₂, SO₂, PM_{2.5}, PM₁₀) in Enugu-Abakiliki and Enugu-Onitsha 5 meters from the highway is due to emission of pollutants by vehicles into the atmosphere as a result of traffic density emanating from high fleet. Low, medium, high molecular weight PAHs and the mean concentration of PAHs in soil were highly present and above WHO 0.3mg/kg permissible level in both Enugu-Abakiliki and Enugu-Onitsha 5 meters from the road, but below detection level in other study sites, except for flouranthene 5 meters

and 500 meters of Eke-Iwollo. Vegetables (bitter leaf, pumpkin leaf and cassava leaf (were equally proportionately contaminated with these low molecular weight PAHs and in Dibenzyl (a – h) anthracene of high molecular weight PAHs of Enugu-Onitsha and Enugu-Abakiliki 5 meters from the road. Heavy metal contaminants of soils and vegetable mirror exactly the same trend associated with recalcitrant PAHs. The results reveal that vehicular emission due to high traffic density is responsible for the preponderance of PAHs and heavy metals in the soils and vegetables of Enugu state highway road. Given the appreciable accumulation of these vehicular emission toxicants in vegetables, it is suggested that the food remain a major endogenous source of PAHs and heavy metals, among the people of the area. The impacts of pollution (PAHs, heavy metals) associated with vehicular emission on air, soil and vegetables from roadside farmland are presently alarming due to high level of traffic density.

4.11.P-Th-085 Screening House Dust for PFAS: Revealing the Extent and Diversity of Contamination

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Per- and polyfluoroalkyl substances (PFAS) are a group of compounds widely used in industrial and consumer products due to their unique water- and oil-repellent properties. Recent studies have highlighted the presence of PFAS in various environmental matrices, including house dust, raising concerns about potential exposure and associated health risks. House dust is a complex matrix composed of various particles, fibers, and organic matter and acts as a reservoir for environmental contaminants, including PFAS. These compounds can enter house dust through various sources, including treated textiles, carpet and upholstery treatments, cleaning products, and consumer goods treated with PFAS. Understanding the amount and types of PFAS compounds present in house dust is crucial for identifying the sources for these compounds as well as for assessing human exposure and managing associated risks. In this study, a series of dust samples from several residential homes were screened for PFAS using high resolution mass spectrometry. Dust samples were collected using a small vacuum and sieved to only contain particles less than 150 µm. The samples were then diluted in 50:50 methanol and water containing 0.03% NH₄OH. Solid phase extraction was then performed using Strata-PFAS and eluted with methanol and 1% NH₄OH. An aliquot of the sample was then injected into a SCIEX X500R QTOF system for analysis. A panel of 50 PFAS compounds were screened for using a targeted approach, additionally, samples were screened using the NIST List of Possible PFAS. Several PFAS compounds were identified and quantified when possible, and, when native standards were not available, a semi-quantitative approach was taken.

4.11.T Human Exposure to Organic Chemicals of Current Concern

4.11.T-01 Exposure of Young Children to Semi-Volatile Organic Compounds (SVOCs) in the Sleeping Micro-Environment

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/Young children spend up to 15 hours/day in sleeping micro-environments (SMEs). The SME consists of items in the bed like mattresses, beddings, toys, and air in the immediate contact where children are likely exposed to semi-volatile organic compounds (SVOCs). Non-dietary exposure to elevated levels of some SVOCs including di(2-ethylhexyl)phthalate (DEHP) and tris(2-butoxyethyl)phosphate (TBOEP) may cause adverse effects such as the development of childhood asthma. We aimed to measure SVOC levels in SMEs and identify influential factors on their emission rates through in-lab and in-home testing. Target SVOCs were phthalates (PAEs, n=8), organophosphate esters (OPEs, n=28), benzophenones (BPs, n=3) and salicylates (SALs, n=5). In-lab testing assessed the impact of temperature (37.5 °C), weight (7.5 kg) and a combination of both on SVOC emissions from brand-new mattresses (n=8) using passive silicon rubber samplers deployed for 3 days on mattresses.

SVOC content of bedding materials (e.g., mattress foams, covers) were determined by direct extraction. Results showed mattress foams and covers as sources of PAEs (e.g., diisononylphthalate [DiNP], and DEHP), OPEs (e.g., tris(1,3-dichloro-2-propyl)phosphate [TDCiPP], TBOEP, and tris(chloropropyl)phosphate [TCPP]), BPs (e.g., BP-8 and BP-3) and SALs (e.g., phenyl salicylates, and 2-ethylhexyl salicylate). The relationship between emission rates and both weight and temperature varied by compounds. For example, the TCPP emission rate increased with temperature, while TBOEP emission rate increased with weight. In-home testing measured SVOC levels in children's SMEs (1 to 4 years) in the Greater Toronto Area using passive silicon rubber samplers. Participants' parents (n=16) installed three samplers in their children's SMEs: 1) on the mattress but under the bedsheet covered to air (to capture mattress emissions), 2) on top of the mattress and open to the air, and 3) beside the bed (to monitor ambient bedroom air). Results from mattress samplers indicated detection frequencies of 0-90% for PAEs, 0-80% for OPEs, 50-90% for BPs and 50-80% for SAL. Among the targeted compounds, the dominant chemicals were phthalates (DnBP, DEHP and DiNP) and organophosphate esters (TDCiPP, TBOEP and TCPP). Our findings from the lab and from homes confirm that mattress foams and covers contribute to SVOC levels in SMEs resulting in early-life exposure. Moreover, a child's body temperature and weight could impact SVOC emission rates in SMEs.

4.11.T-02 Gas Chromatography - High Resolution Mass Spectrometry Analysis of Human Placenta for Environmental Chemicals of Concern

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Exposure to organic chemicals during fetal development has been associated with adverse birth outcomes, such as low birthweight and prematurity, and some outcomes are fetal-sex dependent. Most studies have relied upon measurements of maternal serum collected during pregnancy; however, biomonitoring of the placenta may offer new insights into fetal in-utero exposures to environmental chemical mixtures. To address this data gap, we quantified 124 semi-volatile organic chemicals in placenta samples collected from a prospective cohort study in Durham, NC, USA. Nearly 200 placentas have been collected to date, and participant recruitment is ongoing. Placenta samples have been dissected to separately analyze maternal and fetal sections of the placenta to assess differential chemical transport and accumulation. To support this study a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction method was developed to reduce sample preparation time and solvent usage. Extracts were analyzed for pesticides, organophosphate esters, phthalates, phenols, polybrominated diphenyl ethers and polycyclic aromatic hydrocarbons using gas chromatography- high resolution mass spectrometry (GC-HRMS). The developed method can simultaneously be used for quantitative measurement of environmental chemicals and further analyzed via suspect screening approaches to identify other chemicals of concern. In a pilot study with 20 placental tissues, we observed fetal-sex and maternal/fetal chemical concentration differences in placenta tissue. Detection frequencies ranged from 0 – 100% among all target compound classes with a total of 37 analytes detected in greater than 50% of samples. Phthalates and organophosphate esters were among the most frequently detected compounds with concentrations as high as 190 ng/g wet weight. Preliminary results suggest that a few chemicals, and notably nonylphenol, were present at higher concentrations in placentas from male fetuses compared to female. Analysis of chemical features obtained from the suspect screening highlighted a stark fetal-sex difference in chemical profile. These preliminary results, along with results from a subsequent batch of 50 placentas will be presented.

4.11.T-03 The Nexus between Wristband-Associated Flame Retardants and Polycyclic Aromatic Hydrocarbons, Their Urinary Metabolites and Human Health

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Silicone wristbands can successfully capture over thousand industrial and consumer product additives, and they provide integrated and longer-term exposure to SVOCs. A few recent studies documented correlations between

the levels of FR and PAH on wristbands with those in human matrices, particularly their urinary metabolites. One of the key questions that still remains unanswered is if wristbands can be used to provide information on the link between the personal exposome and health effects. To shed some light on the nexus between levels of two groups of SVOCs -organophosphate esters flame retardants (OPEs) and polycyclic aromatic hydrocarbons (PAHs)- in wristbands and health effects, we measured the concentrations of the parent compounds in silicone wristbands, their corresponding metabolites in urine, and selected health biomarkers such as salivary cortisol, dehydroepiandrosterone (DHEA), and C-reactive protein (CRP) and serum immune response markers like immunoglobulins (i.e. IgA, IgG, IgM, IgE, IL-6) in serum and saliva. Thirty-one participants were recruited in December 2018 at University of Turin, Italy. OPE metabolites were detected in all the urine samples with total concentrations in the range of 1.12 – 32.5 ng/mL. Diphenyl phosphate (DPHP), with a median concentration of 3.03 ng/mL, was the most abundant urinary OPE metabolite, which is not surprising since DPHP is a metabolite of several OPEs. Most of the targeted mono-hydroxylated PAHs were detected in $\geq 85\%$ of the urine samples, with 2-hydroxyfluorene (2-OH-FLOR), and 3-hydroxyfluorene (3-OH-FLOR), reported together as 2/3-OH-FLOR, being the most abundant metabolites. In a tertile regression, we detected significant association and large effect size between wristbands levels of several chemicals and their main metabolites as well as between urinary metabolites and biomarkers. Among the measured biomarkers, IgM displayed the largest number of significant effects from the concentrations of chemicals in wristbands while DHEA seemed to be the least affected by the presence of chemicals on wristbands, suggesting that it's not a sensitive biomarker to assess the impact of exogenous chemicals in humans. Among metabolites, 2/3-OH-PHEN showed the highest number of correlations with PAHs on the wristbands and BCEP, BCIPP, and DPHP with a variety of OPEs in wristbands, indicating that they are good urinary metabolite to assess PAHs and OPEs exposures.

4.11.T-04 Investigating PFAS Levels in Paired Silicone Wristband and Blood Serum Samples

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Exposure to per- and polyfluoroalkyl substances (PFAS) occurs via contaminated drinking water and food; however, individuals are also exposed to PFAS in the indoor environment. This study sought to evaluate PFAS exposure from the ambient indoor environment by utilizing silicone wristbands as personal passive samplers. Paired wristband and human serum samples (n=87) were collected from participants residing in a midwestern community that had PFAS impacted drinking water. Each participant wore a wristband for 7 days to assess personal exposure to PFAS. Individuals in this study ranged from 7 to 84 years and the majority were white (84%) and female (66%). Serum samples were analyzed for 35 PFAS via liquid chromatography tandem mass spectrometry (LC-MS/MS) and the most frequently detected analytes were PFHxS, n-PFOS, br-PFOS, PFHpS and PFOA. Wristbands were analyzed for 43 total PFAS using both LC-MS/MS and gas chromatography high resolution mass spectrometry (GC-HRMS). The most frequently detected analytes on the wristbands were 6:2 diPAP (LC-MS/MS) as well as 6:2 FTOH and MeFOSE (GC-HRMS). Analyses revealed a sex-dependent difference in exposure to 6:2 diPAP and age-dependent differences in exposure to 6:2 FTOH and MeFOSE. The current data indicate that PFAS are commonly detected on wristbands, and that exposure to PFAS precursors (e.g., diPAPs) is greater than exposure to legacy PFAS (e.g., PFOA/PFOS) in the indoor environment. To our knowledge, no other studies have been published which quantify volatile PFAS precursors on silicone wristbands via GC-HRMS. Therefore, this is a novel method which helps fill an important data gap in our knowledge of PFAS exposure and can provide information which can be useful for understanding PFAS exposures from indoor environment sources (e.g., paint, furniture, carpeting, etc.).

4.11.T-05 Rapid Detection and Targeted Analysis of Fluorinated Compounds in Smart and Fitness Watch Bands

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Per- and polyfluoroalkyl substances (PFAS) are added to consumer goods to promote stain, oil, and water repellency and to improve durability. The persistence of PFAS in the environment and their links to adverse to human health risks have spurred investigations of PFAS presence in various consumer goods and products where they find use. The presence of PFAS in smart and fitness watch bands, many of which are advertised as containing fluoroelastomers, have not been previously described. These products are worn daily against the consumer's skin for long periods of time, representing a direct exposure pathway for PFAS. Additionally, at the end of their lifespan, smart and fitness watch bands end up in landfills, where they can contribute to PFAS concentrations in landfill leachates, ultimately leading to indirect human exposure. This work presents a combination of a rapid screening method, *ex-vacuo* particle-induced gamma-ray emission (PIGE) spectroscopy, with traditional targeted analysis methods to measure the occurrences of PFAS in watch bands. Watch bands from a variety of brands, compositions, and price points were first screened for total fluorine concentrations. Samples were then subjected to liquid chromatography tandem mass spectrometry for targeted PFAS analysis of 21 analytes. This study represents the first investigation of PFAS concentrations and human exposure estimates for smart and fitness watch bands.

4.11.T-06 Insights from Recent Investigations on Neutral PFAS, their Presence in Consumer Products and in the Environment, and their Potential Exposure Assessments

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Neutral per- and polyfluoroalkyl substances (PFAS) refer to groups of fluorotelomer (FT)- or electrochemical fluorination (ECF)-based PFAS that are volatile since they occur in uncharged states at environmental conditions. These compounds are primarily used as building blocks to some ionic PFAS and in the production of side-chain fluorinated polymers (SCFPs). Investigations of the neutral PFAS presence in consumer products and in the environment, and preliminary exposure assessments these compounds will be presented. The main analytical technique employed is gas chromatography coupled with mass spectrometry (GC-MS) with liquid injection or with sorbent tube-based thermal desorption (TD). Two sources of potential artifacts were identified: formation of fragment ions from mass-labelled neutral PFAS corresponding to neutral PFAS fragment ions under positive chemical ionization mode and formation of FTOHs from the thermal degradation of FT-based phosphate diester occurred at 280 °C in the GC inlet. Artifacts such as these indicate the need to test for and control for neutral PFAS arising from processes that occur during sample analyses. The FTOHs were quantified in firefighter turnout gear, facemasks, and indoor household paints. Neutral PFAS in consumer products likely originate from SCFPs or impurities during the production of consumer products. Consumer products likely end up in the landfill post-use and data from landfills in southeastern U.S., analyzed by TD-GC-MS, indicate two orders of magnitude higher levels of FTOHs compared to levels reported in ambient air collected downgradient of landfills. The ECF-based neutral PFAS were below limit of quantitation (<LOQ). Levels of neutral PFAS were below or near LOQ (ug/L) from field-collected light non-aqueous phase liquids collected in the U.S. Inhalation was estimated to be the most dominant exposure route for neutral PFAS found in facemasks and indoor household paints. Using ConsExpo and a reference dose of 5 µg/kg-day, increased exposure to neutral PFAS is likely for adults who wear facemasks with levels of 6:2 FTOH ≥ 1000 µg/m², during high physical activity, and for an extended period, or for individuals who paint using paints with levels of 6:2 FTOH ≥ 34 µg/g during the first 132 min of painting. Screening of dermal absorption of neutral PFAS from gas phase revealed negligible uptake for FTOHs commonly found in consumer products, but provided positive result for shorter chain ECF-based neutral PFAS.

4.12.P-Mo Identifying and Linking Environmental Exposure to Biological Effects

4.12.P-Mo-120 A Pull-Down Assay Coupled to Non-Target Analysis as a Tool for the Identification of Thyroid Hormone Disrupting Chemicals from Complex Environmental Mixtures

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Surface waters are contaminated with a wide range of micropollutants of anthropogenic or natural origin occurring in highly complex mixtures. Those may cause deleterious effects in aquatic biota through diverse pathways. Endocrine disruption, e.g., effects on thyroid regulation, has been investigated in complex environmental samples, however, the causative chemicals remain largely unknown. The identification of effect drivers has been addressed by high-performance liquid chromatography with high-resolution mass spectrometry (HPLC-HRMS) for target analysis and non-target screening. These are usually coupled with bioassays and sequential fractionation schemes to reduce sample complexity. The main limitations of this approach are the potential loss of bioactivity after fractionation and the low specificity of the fractions. To overcome these issues, a novel approach for the identification of active compounds in complex mixtures based on protein-ligand interactions was developed. We have optimized a method to facilitate the separation and identification of compounds with the potential to bind to human transthyretin (hTTR), a transporter protein of thyroid hormones (T3 and T4). This method uses an engineered in-house recombinant hTTR protein serving as a highly specific bait for its ligands potentially occurring in environmental mixtures. The hTTR protein is purified via His-tag and its affinity to nickel cations, which is also utilized in the following pull-down assay. Eluted ligands are subjected to TTR inhibition bioassay and non-target screening workflow based on HPLC-HRMS data for differential analysis of different pull-down assay eluates. We have applied the optimized hTTR pull-down assay method in a case study focused on treated wastewater from a small stream in the Czech Republic, Europe. We have identified several known and a set of novel ligands of hTTR. These compounds were confirmed and quantified in the targeted analysis of the original samples. The pull-down eluates of treated wastewater retained the effect in TTR binding assay, and the effects and potencies of individually identified ligands were determined in bioassays. The contribution of identified ligands to the overall effect was calculated based on mass balance. The presented method is a promising tool for the effective identification of bioactive compounds from complex mixtures. The project has received funding from Czech Science Foundation (GACR) under grant agreement GX20-04676X.

4.12.P-Mo-121 Assessing Dried Blood Spot and Foam Tip Devices for PFAS Detection and Quantitation

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals of great concern due to their bioaccumulative properties and links to adverse health effects. PFAS are found worldwide in the water, air, fish, soil, and blood of over 98% of people. In toxicological studies, blood samples from animals are commonly used as indicators of environmental contamination. However, blood collection in the field is a challenging process as current blood-based sampling methods require rapid sample refrigeration or risk sample degradation. Therefore, it is desirable to assess the utility of devices that can perform blood sampling under ambient conditions. This project evaluated two different ambient sampling devices and compared their results to traditional whole blood studies. The devices tested included the HemaXis 903 whole blood microfluidic chip which spots onto a Whatman 903 Card and Mitra® foam sampling tips. Dried blood spots have previously been used for PFAS biomonitoring as an effective method to monitor toxicants, however, there is no study analyzing foam tip-based devices or the HemaXis 903 microfluidic chip for PFAS biomonitoring. This project aims to benchmark the detection limit for each method in comparison to whole blood sampling to determine whether ambient sampling

methods are a viable alternative for biomonitoring studies. To determine extraction efficiency, defibrinated sheep blood was spiked with heavy-labelled PFAS standards and “in-the-field” sampling methods were mimicked. Whole blood was kept on ice for six hours and then frozen at -20 °C prior to analysis, while the HemaXis 903 chips and Mitra® foam tips were kept at ambient conditions during and after sampling. The samples were then extracted, and extraction efficiency was determined by quantifying 24 PFAS using a non-targeted LC-IMS-MS platform to assess their losses and limit of detections using the different methods. Comparisons were then made to whole blood sampling for each method to evaluate advantages and disadvantages. If promising results are illustrated for the ambient sampling devices, they may be able to aid in further detailing the environmental contamination of PFAS in remote locations. In addition, these methods could also be readily applied to mail-in samples for humans where sample degradation is a risk.

4.12.P-Mo-122 Anthropogenic Compounds Found in Placental Tissue

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Whereas traditional targeted analysis methods have characterized few well-known chemical stressors, non-targeted analysis (NTA) methods now enable broad characterization of many understudied or previously unknown compounds. High-resolution mass spectrometry (HRMS) platforms, chemical databases, and sophisticated software tools now allow for the rapid and confident identification of hundreds to thousands of chemicals in any given environmental or biological sample. These methodological improvements, serving as a foundation for exposomics research, have rapidly led to an improved understanding of the complex exposures encountered by individuals and populations.⁴ Yet, due to the nascent nature of the field, there remain gaps in characterizing the exposome, as well as relating the exposome to measurements of biological function to better understand the impacts of chemical exposures on health. What remains to be established are approaches to integrate such measures, resulting in the increased understanding of dynamics between systems-level exposure and biology. In this study we apply a nontargeted approach to evaluate placental tissues to identify novel chemical-biological interactions. This study tests the hypothesis that understudied groups of xenobiotic chemicals are present in placenta.

4.12.T Identifying and Linking Environmental Exposure to Biological Effects

4.12.T-01 Using High Resolution LC-MS and GC-MS and Nontargeted Analysis to Identify Potential Mammary Gland Carcinogens in California Drinking Water

Gabrielle Black and Thomas Michael Young, University of California, Davis

Kitchen tap water contaminants were investigated for their potential correlation with elevated breast cancer occurrence rates. One hundred and twenty household taps and fifteen bottled water samples were sampled twice in 2020 (winter and summer). Eight regions of fifteen households each were sampled of which four of regions have previously been identified by the California Public Health Institute as regions with disproportionately high breast cancer rates. Additionally, within these eight regions, four water source types were represented: large surface water (San Francisco Bay Area), small surface water (rural Northern California), groundwater (Central Valley), and a mix of ground and large surface water (Southern California) and two disinfection techniques. A suite of targeted, suspect, and nontargeted analysis of GC- and LC-QTOF-MS data was performed to investigate contaminants in water samples correlated to estrogen reporter-gene bioassays and/or breast cancer hot spot zones. Targeted results identified several regions with low ng/L concentrations of perfluorooctanoic and hexanoic sulfate and perfluorooctanoic and hexanoic acids. Suspect screening discovered pesticides like atrazine, simazine, and their degradates, plasticizers like bis(2-ethylhexyl)phthalate, and phosphate flame retardants like tris(chloropropyl)phosphate. Nontarget analysis utilized a SIRIUS:CSI FingerID-MSFINDER dual platform scoring and validation system, QSAR toxicity prediction and retention time indexing to focus

identification efforts on compounds most likely to be related to breast cancer. Identifications include transformation products of vulcanization accelerators, plastics, household solvents, and polychlorinated biphenyls.

4.12.T-02 Non-Targeted Analysis (NTA) and In-Vitro High-Throughput Screening Approaches for the Evaluation of Polymer Additives and Transformation Products Sources in Drinking Water

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Identifying agents responsible for adverse health outcomes in environmental samples has always been a challenge. Any sample taken from the environment usually contains thousands of different components and even the most modern techniques cannot single handily identify them all. Combining different analysis workflows from high-resolution mass spectrometry (HRMS) to in-vitro cell analysis, metabolomics, real-time cell analysis and extracellular flux analysis we demonstrate the use of effect-directed analysis (EDA) to identify different toxic products derived from the transformation of polymer leachates in drinking water. Analysis of drinking water (n=20) shows median concentrations of extractable organic chlorine (EOCl) of 33.4 µg/L and extractable organic bromine (EOBr) of 11.2 µg/L, with highest concentrations observed at locations using different point-of-use (POU) devices such as aerators. Further analysis of polymer gaskets and O-rings leachates shows a large contribution of leaching additives from such POU devices. NTA analysis showed the presence of 57 different additives in leachate samples. While siloxanes were the major leaching product of silicone materials, additives that have gained recent attention due to potential toxic effects such as 1,3-diphenylguanidine (DPG) and 6PDD were also found to be leaching from polymer materials in POU devices. The presence of HOCl and NH₂Cl (water disinfectants) can also significantly contribute to the leaching of polymer additives as well as promote the formation of DBPs. A chlorinated by-product of DPG (1-(2,4-dichlorophenyl)-3-phenylguanidine) was detected in 100% of drinking water samples; median concentration of 1.7 ng/L. Using different bioassay approaches and NGS RNA-sequencing, we show that some of the chlorinated by-products of DPG exert significant toxicity. DPG and its chlorinated by-products also can alter cell bioenergetic processes, affecting cellular respiration rates and ATP production, moreover, DPG and two of its chlorination products also have an impact on mitochondrial proton leak, which is an indicator of mitochondria damage. Evidence of genotoxic effects in the form of DNA double strand breaks (DSBs) was suggested by RNA-sequencing results and further validated by an increased expression of genes associated with DNA damage response. Immunofluorescence analysis of phosphorylated histone H2AX, another DSB biomarker, also confirmed the potential genotoxic effects observed for the chlorinated products.

4.12.T-03 Identification of Toxic Contaminants in California Sediments using Nontargeted analysis and *Hyalella azteca* Toxicity Tests

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Since 2008, the Stream Pollution Trends (SPoT) Program has monitored up to 100 sites for sediment contamination and toxicity, providing crucial information for impaired waterbody listings across California. While their efforts have been extremely important for sediment quality assessments, the broad scope of this campaign limits the number of targeted contaminants that can be monitored. To provide greater protection of ecological health and beneficial uses, it is essential we combine targeted approaches with nontargeted analysis to identify unknown and emerging toxic chemicals within California streams. In this study, both liquid and gas chromatography with high-resolution mass spectrometry (LC-HRMS and GC-HRMS) were used to screen for tens of thousands of suspect compounds in 30 sediment samples. Nontarget features correlated with *Hyalella azteca* (HA) growth and mortality were investigated further through targeted mass spectrometry and standard purchasing for confirmation. Out of the 5548 total features detected, 403 were correlated with HA mortality and growth. Pesticides including bifenthrin, permethrin, clomazone, and fipronil significantly correlated with HA

mortality. These compounds were found in several sediment samples from across California and were confirmed with authentic standards. Ongoing work is focused on identifying compounds linked to toxicity but not yet confirmed through standards. These findings suggest that multi-mode nontargeted analyses combined with standard toxicity testing provide more complete toxicity profiles within complex environmental mixtures.

4.12.T-04 Using High-Resolution Gas Chromatography-Mass Spectrometry to Discover Byproducts of Potential Health Concern in Community Water Supplies Impacted by Polycyclic Aromatic Hydrocarbons

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Polycyclic aromatic hydrocarbons (PAHs) can be introduced into community water supplies from diverse sources, including wildfires within the source watershed. In a study of household drinking water quality in eight California communities, two communities relying on surface water supplies from watersheds with recent wildfire impacts exhibited elevated levels of a wide range of PAHs. Although PAH levels did not exceed relevant regulatory standards, we sought to identify less commonly studied PAH species, including potential disinfection byproducts, and to understand their impacts on measured bioactivity of the samples. Fifteen households in each community provided water samples on two dates. Samples were passed through multi-layer solid phase extraction cartridges and eluted with a series of solvents to produce samples for analysis using multiple instrumental techniques. Of relevance to this part of the work, a portion of each extract was directed to gas chromatography with high-resolution mass spectrometry (GC-HRMS) and a separate portion was analyzed with the chemically activated luciferase estrogen receptor in vitro bioassay (CALUX-ER α). Data from all samples was aligned (separately by season) using the open source application MS-DIAL (v. 4.90) and identified by spectral retention index matching against the NIST17 database. Additional identification for individual samples was also performed using Agilent Unknowns Analysis (v. 10.2). Target analysis of selected PAH compounds indicated that concentrations ranged from < 0.21-16.9 ng/L for pyrene and 4.3-234 ng/L for fluoranthene, for example. A variety of PAH compounds widely detected using suspect screening were potentially created by disinfection processes, including 9,10-anthracenedione at levels as high as 371 ng/L. Strong correlation ($\rho=0.98$ in winter and $\rho=0.84$ in summer samples) between anthracene and 9,10-anthracenedione, a known disinfection byproduct formed from anthracene, led to a search for other potential PAH byproducts in the samples. Using literature sources and correlation analysis led to discovery of chlorinated and oxygenated PAH derivatives. Although the health impacts of these findings are not clear, a statistically significant ($p<0.05$) negative correlation was observed between the abundance of numerous PAH derivatives and the ER α agonism observed, consistent with previous findings that aryl hydrocarbon receptor agonists act as ER α receptor antagonists.

4.12.T-05 Exploring Novel Passive Sampling Devices (SPATTs) for Non-Target PFAS Assessment and Data Visualization

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Persistent organic pollutants (POPs) are an ensemble of man-made chemical compounds that are resistant to degradation and known to cause adverse outcomes in human health, wildlife, and the environment. Specifically, per- and polyfluoroalkyl substances (PFAS) contamination is an emerging environmental concern in the Cape Fear River Basin in North Carolina due to local fluorochemical manufacturing and recent outflows which affect drinking water quality for nearby residents. Here we evaluate the efficacy of low-cost passive sampling devices (Solid Phase Adsorption Toxin Tracking, or SPATTs) for spatial PFAS profiling using ion mobility spectrometry-mass spectrometry (IMS-MS). In addition, we also highlight key software advancements developed for rapid assessment of data extraction, evaluation, and visualization using Skyline and MetaboAnalyst. These tools are freely available for public use and enhance both the throughput and confidence of molecular annotations in non-targeted data acquisition. Preliminary results demonstrated that while legacy

PFAS were observed in relative equal abundance at all sampling sites (PFOS, PFHxS, PFBS, and PFOA), emerging replacement compounds were localized predominantly downstream of a known fluorochemical manufacturer (PFO4DA, GenX, Hydro-EVE, Nafion Byproducts, and NVHOS). These results are congruent with our initial hypothesis that emerging PFAS observed in the Cape Fear River are likely attributable to local fluorochemical manufacturing processes and corresponding outfalls. While these results are not quantitative, they effectively highlight the potential utilization of SPATTs as low-cost passive sampling devices for the presence of known and novel PFAS.

4.12.T-06 Toxicity of Automobile Spray Paints Could Transcend to Occupational Health Hazards in Unexposed Second Generation Offspring

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Paint particles from spraying gun consist of myriads of hazardous volatile organic compounds, some inspirable carcinogenic heavy metals, preservatives and pesticides. Automobile sprayers and other exposed population are potentially endangered by these toxic chemicals. One of the major problems in interpreting toxicological studies is in estimating the degree of exposure. The traditional approach has been the use of lymphocytes and red blood cells as biomarkers of mutagenic effects where they are mainly used as sentinel cells types to provide early warning signals for adverse health outcome. However, it is important to get more data on the concentration-dependent toxic impact with respect to the health implication to an exposed individual by paying further attention to the effects on the liver marker enzyme, renal indices, oxidative stress markers and reproductive hormones in exposed first and unexposed second generation organism. The experiment was carried out with a total of healthy 30 males and females Wistar albino rats which were randomly divided into 5 groups of 6 rats per group with at least a male in each group. They were exposed to different concentrations of the volatile organic compounds (VOCs) of the paint in "Whole Cell Inhalation Chamber" while allowed free access to food pellets and water *ad libitum*, and maintained at a temperature of $25\pm 2^{\circ}\text{C}$ and relative humidity of 45-55% under 12-h light: 12-h dark cycle. The experiment lasted for 8 weeks with 15 minutes short inhalation time limit daily. Thereafter the toxic effects on the liver marker enzyme, renal indices and reproductive hormones of the exposed first generation and the unexposed second generation albino rats were determined. The results showed varied degrees of significant differences among the exposed group and when compared with the control. Histological studies of the lungs, liver and kidney showed various degrees of changes, while the hormonal level and other biochemical markers were also altered relative to the control. Highly significant changes in the results of chronic exposure suggest possible organs dysfunction both in the exposed first and unexposed second generation off-springs. Hence humans could be susceptible to health disorders when exposed to VOCs from automobile paint. There is therefore the need for total adherence to safety rules, and that regulatory agencies should increase workers sensitization on the possible health problems from exposure to automobile paint.

4.11.V Human Exposure to Organic Chemicals of Current Concern

4.11.V-025 Assessing PFAS Exposure Pathways in a Midwestern Community: Source Apportionment and Relative Contributions from Drinking Water and Diet in a Former Paper Mill Area

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Human exposure to perfluoroalkyl and polyfluoroalkyl substances (PFAS) is multifaceted, spanning consumption of contaminated food and drink, contact with house dust and indoor air, and dermal exposure. Studies have demonstrated that drinking water can be a crucial pathway for PFAS exposure when concentrations are elevated, whereas diet is considered to be the most significant exposure route for the general population. However, certain communities may also face significant exposure via locally sourced and homegrown food. Hence, we conducted an exhaustive biomonitoring study of a Midwestern community that

experienced historic (pre-2018) PFAS drinking water contamination, which measured at 1600 ppt. With 129 participants from 92 households, the study surveyed dietary habits, collected serum and drinking water samples, and recorded data on past drinking water concentrations. Compared to the general population, we observed higher levels of PFOS, PFOA, PFHxS, PFHpS and NMeFOSAA in serum samples. We utilized source apportionment methods to identify predominant PFAS mixtures in the serum samples using note the three methods and conclusion. We then calculated the molar sum for each mixture and determined relative contributions of drinking water and diet to serum PFAS concentrations. Our multivariable linear regression models used log-transformed serum concentrations with drinking water concentrations data along with data on water consumption, diet, and demographic characteristics from the questionnaire. Age, gender, race, body mass index, and sampling date were considered as covariates. We will share these findings along with implications for exposure reduction interventions.

4.13.P-Th Identifying PFAS Sources Near and Far

4.13.P-Th-086 PFAS in Largemouth Bass Across Rhode Island: Concentrations of Pollutants and the Social Profile of Visitors

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Largemouth Bass (*Micropterus salmoides*) are high trophic-level piscivores commonly found in freshwater ponds and lakes within Rhode Island (RI), USA. The increased detection of Per- and polyfluoroalkyl substances (PFAS) in freshwater environments has raised questions over PFAS concentrations in the RI Largemouth Bass population. This study utilized archived Largemouth Bass dorsal muscle tissue collected between 2005 and 2015 from 11 freshwater bodies across RI. Archived tissues were extracted and analyzed for 24 PFAS compounds using LC-MS/MS. Six compounds were consistently detected including long-chain perfluoro carboxylic acids (PFCAs) and Perfluorooctanesulfonic acid (PFOS). \sum PFAS ranged from 1-323 ng/g (\bar{x} =41 ng/g) wet weight in muscle samples. Archived fish were previously analyzed for stable isotopes ($\delta^{15}\text{N}$) and mercury (Hg). No correlation was determined between \sum PFAS and Hg, but fish exhibited increasing PFOS concentrations with increasing $\delta^{15}\text{N}$ values. Largemouth Bass are a popular wild game fish consumed by some humans, leading to the potential for increased PFAS dietary exposure. Linking PFAS concentrations to waterbody visitors' demographic data can help identify populations that are more likely to consume contaminated fish. The demographics of visitors to each waterbody were estimated to better understand the populations visiting each site. Commercial cellphone location data was used to infer the demographics of the visitors to these waterbodies by their home census block groups. These data can help refine future studies of fish consumption advisories and identify populations with potentially higher PFAS dietary exposure from freshwater fish.

4.13.P-Th-087 Occurrence of Per- and Polyfluoroalkyls Substances (PFAS) in Groundwater from Miami-Dade, South Florida

Maria Guerra de Navarro and Natalia Quinete, Florida International University

Per- and polyfluoroalkyl substances (PFAS) have been in continuous use since their initial production over 80 years ago, becoming part of consumer products, non-stick pans, and specific applications like aqueous firefighting foams (AFFF). The exceptional stability of C-F bonds triggers their multiple uses in different applications and prevents their biodegradation. Additionally, their high-water solubility facilitates their movement through different environments; nowadays, they are considered ubiquitous. They are not removed from water through traditional treatment, and the health risk associated with exposure to PFAS has brought the need to understand better its fate and transport in the environment. Once these compounds are released, they enter the water cycle and follow different transport mechanisms toward the groundwater. The sources of PFAS in the environment are related to human activities. Miami has been known to be the third city with the highest

concentrations of PFAS in its groundwater among 44 locations assessed. South Florida is an area that comprises military bases, firefighter training facilities, airports, wastewater treatment plants, and an overload of septic tank discharges, which could be part of the potential point sources of PFAS in this region. The main source of tap water in South Florida is primarily groundwater. Biscayne Aquifer is one of the most productive aquifers in the world. Its infiltration capacity is fast, facilitated by conduits and sinkholes, making it prone to contamination. This study assessed the occurrence and composition of legacy and emergent PFAS in monitoring wells in the Miami-Dade area, South Florida. For this purpose, 15 groundwater and 7 point-of-exit samples were taken in 6 locations in Miami-Dade County during the dry season in April 2022 (N=21). The methodology involved the preconcentration of 250 mL of groundwater by solid phase extraction process (SPE) using a weak anion exchange (WAX) cartridge, followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using isotopically labeled internal standards. The method used was validated in terms of linearity, detection limit, precision, and accuracy. Perfluorooctanoic sulfonate (PFOS) was detected in all the collected samples at concentrations above the EPA's proposed Maximum Contaminant Levels (MCLs) of 4 ppt. PFOS, PFOA, and PFBA were the most frequent and abundant compounds, and Miami-Springs wells exhibited the highest sum of PFAS concentration.

4.13.P-Th-088 Target Analysis of Per- and Polyfluoroalkyl Substances, (PFAS) in Surface Water from Biscayne Bay Canals

Courtney Heath and Natalia Soares Quinete, Florida International University

It is well established that per- and poly- fluoroalkyl substances, (PFAS) are present in the environment as “forever chemicals” due to their stable, persistent, and accumulative properties. While some PFAS, such as perfluorooctanesulfonic acid, (PFOS) and perfluorooctanoic acid, (PFOA), were phased out in the U.S., new alternatives are being produced and these shorter-chain PFAS can be as persistent and toxic as the previously banned compounds. Therefore, there is a need to monitor levels of PFAS in the environment to understand their effects on humans and aquatic organisms. Anthropogenic input is leading to raised levels of contaminants, such as PFAS, in the Biscayne Bay. These contaminants and excess nutrients are leading to fish kills and seagrass die off, which is a major concern in the local community. There is still a knowledge gap in regard to the fate and transport of these compounds in South Florida. While it is known that anthropogenic sources are discharging along Biscayne Canal(C-6), Little River(C-7), and Miami River (C-8), levels along the canals, specific point sources, have not been investigated in detail. In this study, we have initially collected surface water samples from 45 different sites, (N=51) from C-6, C-7, and C-8 in April and May of 2023. We have run solid phase extraction (SPE) on 500 mL surface water samples using weak anion exchange (WAX) cartridges and we analyzed the samples by liquid chromatography tandem mass spectrometry, (LC-TQ-MS). A sensitive and robust method was developed and validated for the detection and quantitation of 40 legacy and emerging PFAS, following the EPA Method 1633.

4.13.P-Th-089 Non-Targeted and Targeted Analysis of PFAS in Household Media from the American Healthy Homes Survey II

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Household drinking water is a major source of PFAS exposure for communities with contaminated water supplies. Although mitigation of PFAS in drinking water is an effective remedy for this source of exposure, additional sources (e.g., textiles and consumer products) can also contribute to exposure. House dust can aggregate chemicals such as PFAS, serving as both an additional source of exposure and a medium which can be analyzed for PFAS presence. There is also the possibility of inter-medium transfer from water to dust (e.g., from cleaning) dependent on physicochemical properties such as volatility and logP. We analyzed the PFAS present in samples from the 2018 American Healthy Homes Survey (AHHS) II. AHHS II was initially intended

to monitor changes in the presence of lead-based paint and associated lead hazards from hundreds of homes across the United States. While not considered nationally representative, results provide a large survey of PFAS presence and abundance. We investigated AHHS II collected tap water using both non-targeted and targeted analysis. We applied liquid chromatography-high resolution mass spectrometry (LC-HRMS) with data-dependent acquisition of tandem MS spectra for non-targeted analyte identification. This presentation focuses on the non-targeted results and their interpretation in the context of the multimedia targeted results. Alongside heavily-studied sulfonic (PFASs) and carboxylic acids (PFCAs), we have identified several classes of PFAS in drinking water from U.S. homes, including short chain sulfonates and fluorotelomers with various head groups, as well as other novel perfluorinated species. We will discuss spatial patterns in the observed PFAS as well as co-occurrence patterns. This study is part of a broader effort to understand household exposure to PFAS via alternate routes and classify PFAS based on their exposure route patterns using additional datasets. *The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.*

4.13.P-Th-090 Investigating the Presence of Per- and Polyfluoroalkyl Substances (PFAS) in Water Samples Collected Around the Island of Okinawa, Japan

Camden G. Camacho, Kaylie Anne Costa and John A Bowden, University of Florida

Per- and polyfluoroalkyl substances (PFAS) are a resilient class of anthropogenic contaminants of growing concern. While the majority of PFAS work to-date has focused on PFOA and PFOS, there are over 10,000 individual PFAS used for industrial applications, consumer goods, and food packaging materials. Interestingly, one of the largest known contributors to PFAS pollution are aviation facilities, specifically military bases. Due to its large-scale U.S. military presence, Okinawa Island in Japan is suspected to have high concentrations of PFAS in both drinking water and surface water. However, despite what is known about the correlation between the detection of high PFAS levels near military installations, there have been extremely limited peer-reviewed studies characterizing the distribution of PFAS (presence and concentration) on and around the island. The purpose of this work was to employ our rapid PFAS analysis to detect and quantify 31 individual PFAS in both surface and drinking water samples taken throughout the island of Okinawa. In total, 50 coastal sites were selected for collecting 250 mL of surface water with sites selected based on their proximity to suspected PFAS contamination sources, as well as sampling an even distribution around the island. The chosen sampling sites for collecting 500 mL of drinking water were 20 public water fountains which often were found in/near parks and recreational areas. Samples were spiked with isotopically-labeled PFAS standards and subsequently extracted via weak anion exchange solid phase extraction. Samples were then analyzed employing a Thermo Scientific Vanquish ultra-high performance liquid chromatograph coupled to a TSQ Quantis triple quadrupole mass spectrometer, in negative mode using selected reaction monitoring. This work hopes to inform an area where PFAS are understudied, in relation to the amount of suspected exposure due to the highly concentrated military presence. The data can be applied to provoke action towards increasing awareness and regulations, management of PFAS waste, and/or highlight PFAS hotspots around the island. Island communities, specifically Okinawa where commercial fishing and ecotourism are essential to the economy, are particularly vulnerable ecosystems impacted by high levels of PFAS contamination. A comprehensive PFAS monitoring effort, as described in this work, is the first step in realizing the issue.

4.13.P-Th-091 Dispersion and Stratification of Per-and Polyfluoroalkyl Substances (PFAS) in Surface and Deep-water Profiles: A Case Study of the Biscayne Bay Area.

Olutobi Daniel Ogunbiyi Sr and Natalia Soares Quinete, Florida International University

Per-and polyfluoroalkyl substances (PFAS) are group of synthetic chemical compounds known for their persistence, bioaccumulation, and toxic characteristics in all environmental compartments. As industrial and domestic applications of PFAS increases, their discharge into water bodies becomes of human and ecological concerns. Our research focuses on providing better understanding on the occurrence, vertical distribution, and dispersion of PFAS compounds in surface and bottom water from inshore and coastal area of Biscayne Bay,

Miami, Florida. We screened a total of 30 PFAS compounds from inshore (N= 38) and offshore (N=48) water samples using a semi-automated solid phase extraction (SPE) followed by instrumental analysis using liquid chromatography-mass spectrometry techniques (LC-MS/MS). Our findings show a general surface-enrichment and depth-depletion pattern from inshore to coastal area. Average Σ PFAS loadings inshore (surface vs bottom; 29.52 ± 15.26 ng/L vs 21.45 ± 7.85 ng/L) is significantly greater than offshore (surface vs bottom; 5.18 ± 2.68 ng/L vs 2.42 ± 2.11 ng/L). Σ PFOS had the highest concentration both inshore (120.78 ng/L) and offshore (13.23 ng/L). The most frequently detected (D.F = > 91%) PFAS congeners are PFOS, PFOA, PFHpA, PFHxA, PFBA, PFBS and PFHxS in surface water samples. PFOS/PFOA > 1 suggests that point sources are the major contribution to PFAS burden in the Biscayne Bay. An innovative Inverse distance weighted interpolation (IDW) special modelling approach was implemented to predict the potential contribution of oceanic current on the dispersion of Σ PFAS loadings in surface and bottom profiles from canals (inshore) to offshore areas. This will provide insights into transport mechanisms of PFAS compounds from source emissions, and risk assessments of potential impacts on human and aquatic life in the Bay.

4.13.P-Th-094 Co-Occurrence of Per- and Polyfluoroalkyl Substances (PFAS) with Known Industrial Contaminants in the State of Kentucky

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Per- and polyfluoroalkyl substances (PFAS) are a group of persistent anthropogenic chemicals that have been widely used in industrial and commercial processes since their production began in the 1940s. Given their thermal and environmental stability coupled with their widespread use, PFAS are ubiquitous in the environment with some having the tendency to bioaccumulate throughout the food chain. Focusing on Kentucky as a case study, we conducted an exhaustive literature and state and federal government databases review for PFAS industries, users, discharge and landfill permits to identify unknown polluters and establish a correlation between well-studied contaminants (e.g., heavy metals, trichloroethylene (TCE), and polychlorinated biphenyl's (PCBs)) and PFAS. Kentucky, as most states, is faced with high environmental concentrations of PFAS, sparse environmental assessment data, slow development of analytical methods (with associated high costs) to detect and quantify the growing list of PFAS species, and a growing pressure from the community to understand their health-effects and implications, coupled with limited state and local resources to respond to all of these needs. There is an urgent need for simple and cost-effective methods to prioritize sample locations for PFAS around the state that result in more efficiently use available resources to sample, detect, and quantify. Here we present a geospatial analysis of PFAS detection in geographic areas in Kentucky and predicted possible PFAS "hot spots" based on their co-existence with known industrial contaminants to serve as aid in prioritizing sampling locations in the state.

4.13.P-Th-095 Deriving Whole Fish to Fillet Conversion Equations for Per- and Polyfluoroalkyl Substances (PFAS)

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Since 2010, the New York State Department of Environmental Conservation (NYSDEC) has sampled 81 unique water bodies and 53 unique fish species across New York State for per- and polyfluoroalkyl substances (PFAS). Perfluorooctanesulfonic acid (PFOS; one of up to 40 PFAS tested) is most frequently detected in fish and thus of primary interest to federal and state managers. Given that monitoring programs often sample differently, conversion equations between fillet and whole body PFAS will be useful for future comparisons among studies, and to enable evaluation of concentrations for both human consumption risk (fillet) and ecological assessments (whole fish) from a single analysis per fish. Here we present conversion equations for several species including Largemouth Bass (*Micropterus salmoides*), Yellow Perch (*Perca flavescens*), and Brown Trout (*Salmo trutta*), derived from fillet and whole fish analyses on the same individuals. We test whether the ratio of whole fish to fillet concentrations is variable within a species at a site, among species, and

among locations. A significant positive linear regression was found for each species for PFOS (Largemouth Bass, $R^2 = 0.98$; Yellow Perch, $R^2 = 0.84$; Brown Trout, $R^2 = 0.89$). Beaverdam Lake, a PFAS point source recipient, also showed a linear relationship among all tested species ($R^2 = 0.97$). Other legacy PFAS had either much lower R^2 values, such as perfluorohexanoic acid (PFHxA) ($R^2 = 0.26$ in all tested species), or did not exhibit a linear relationship by species, such as perfluorononanoic acid (PFNA). These results demonstrate highly reliable interconversion between fillet and whole fish concentrations for PFOS, the dominant PFAS in fish, but suggest that conversion may be less robust for other PFAS. Further analysis will be presented for all PFAS with substantial prevalence in the New York State dataset.

4.13.P-Th-096 Assessment of Mercury and Per- and Polyfluoroalkyl Substances (PFAS) in New York's Seneca Lake Sport Fishes

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Seneca Lake is one of the New York State (NYS) Finger Lakes and serves as a freshwater water source and major fishery for many communities in the Finger Lakes watershed. Very little is known about how diet influences contaminants in Seneca Lake sportfish, especially for per- and polyfluoroalkyl substances (PFAS). The Seneca Lake Trout Derby is an annual fishing event attracting >600 anglers, many of whom consume their catches. Samples were donated by anglers to conduct analyses of diet (stomach content and carbon and nitrogen stable isotope analyses) and contaminants (mercury and PFAS). Four species (Lake Trout, *Salvelinus namaycush*; Landlocked Salmon, *Salmo salar*; Brown Trout, *Salmo trutta*; and Rainbow Trout, *Oncorhynchus mykiss*) were collected in May 2022. We hypothesized that mercury and PFAS would be detected in all four species. We found similar diets and trophic positions among the Landlocked Salmon, Brown Trout, and Rainbow Trout, but found Lake Trout consumed more benthic species such as sculpins and had a significantly higher trophic position. Mercury and perfluorooctanesulfonic acid (PFOS), were also significantly higher in Lake Trout. Relative to other Finger Lakes, Seneca Lake Trout had elevated PFAS concentrations, and were within the ranges found for the much larger Laurentian Great Lakes. However, Seneca Lake Trout had elevated concentrations of perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluorooctanoic acid (PFOA) relative to Lake Ontario, suggesting a unique source in the watershed. This is the first report of PFAS concentrations in several important sportfish species of Seneca Lake. Future work is recommended to evaluate PFAS point sources to the Seneca Lake fishery.

4.13.P-Th-097 Quantifying Hidden Fluorine in Aqueous Film-Forming Foams via 19F NMR

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Twenty-four aqueous film-forming foams (AFFFs) of military and commercial formulations from four manufacturers with manufacturing dates spanning a 36-year period were analyzed by ¹⁹F NMR to determine total fluorine concentration and characterize synthetic PFAS production as electrochemical fluorination (ECF) and fluorotelomer (FT) based processes. Current analytical methods used to quantify PFAS in AFFFs such as liquid chromatography-mass spectrometry (LC-MS), are often complicated, time-consuming, and expensive. Additionally, LC-MS is best fit for determining individual concentrations of select target PFAS rather than rapid characterization (ECF vs. FT) of PFAS synthesis processes and for total fluorine. As the concern over PFAS contamination grows due to health issues such as increased cholesterol levels, decreases in infant birth weight, and an increased risk of certain cancers, rapid and inexpensive analytical methods are needed to characterize and quantify total fluorine in complex AFFF mixtures. The amount of total fluorine in each AFFF determined by ¹⁹F NMR compared PFAS concentrations by liquid chromatography-quadrupole time-of-flight (LC-QTOF) for the PFAS listed in EPA Method 8327 and the Department of Defense's Method DoD AFFF01. An AFFF obtained from a military storage tank of an unknown origin that had been extensively characterized

by LC-QTOF was also analyzed by ^{19}F NMR. All NMR data were collected on a Bruker 800 MHz Avance IIIHD NMR spectrometer equipped with a 5 mm TCI cryogenic probe, with the ^1H coil tuned to ^{19}F . Increased sensitivity of the cryoprobe and decreased experiment time led to high quality data with acquisition times under 30 minutes. This method is quick, simple, and routine for the determination of total fluorine, which is a key advantage when compared to LC-QTOF. Visual and multivariate cluster analyses of ^{19}F NMR spectra differentiate between ECF- and FT-based AFFFs by complexity in CF_3 and CF_2 regions of the spectra. Total fluorine ranged from 56 to 845 mM with no difference in total fluorine between MilSpec and non-MilSpec AFFFs. The AFFF of unknown origin gave higher complexity that was consistent with an ECF origin. Fluorotelomer-based AFFFs gave poor mass balance when the sum of individual PFAS was compared to total fluorine by NMR on a mM basis. More in-depth analyses will be performed on the AFFFs that gave poor mass balance to identify additional PFAS that are not observed by LC-QTOF.

4.13.P-Th-098 Discharge of Per- and Polyfluoroalkyl Substances to the Environment: Temporal Implications for Water Quality Assessment and Management

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Sustainable water resource management is challenged by emerging water quality threats, particularly in regions experiencing climate change. Per- and polyfluoroalkyl substances (PFASs) have been used for decades in both consumer and military products but have recently been under increased scrutiny due to their risks to public health and the environment. Wastewater treatment plants (WWTPs) represent a significant source of PFAS to aquatic ecosystems because many PFASs that are found in consumer products, such as laundry and cookware, are highly resilient to many of the removal processes in WWTPs. Water reuse within Texas is quite common with many downstream communities utilizing reclaimed wastewater for drinking water, agriculture, and industrial uses. To understand the potential impacts of 24 priority PFASs on surface water quality, we examined PFAS in effluents from 2 Texas cities of similar size over a 2 year period. Data generated were then used to calculate environmental introduction concentrations and subsequently perform environmental hazard assessments (PEHAs). Among all samples, we detected at least one PFAS with PFBA, PFPeA, and PFHxA being detected at the highest levels. Of the 24 PFAS studied, only one was not detected in any samples while 12 were detected in all samples with total PFAS concentrations ranging from 58 to 270 ng/L. Total PFAS levels differed between the first and second year of study (ΣPFAS : 126 vs. 85 ng/L) while seasonal differences were less pronounced. Using the recent guidance put forth by the USEPA in response to environmental PFAS contamination, we performed PEHAs and found that while PFOS concentrations exceeded the new MCL (4 ppt) in over 50% of samples, PFOA exceeded the same value in nearly all samples tested. Hazard indexes calculated for other compounds were found to be below 1; however, compounds we detected most frequently in this study do not currently have regulatory criteria or guideline values for environmental concentrations. Given individual and mixture toxicity concerns for PFAS, the prevalence of these studied compounds in combination with PFOS and PFOA indicate future exposure studies are needed for downstream uses in these effluent-dominated and dependent systems.

4.13.P-Th-099 Domestic Wastewater as a Nonpoint Source of PFAS Contamination in the US

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Per- and poly-fluoroalkyl substances (PFAS) source identification studies often focus on point sources such as large or industrial primary contamination sources. However, PFAS are present in numerous common household products including cosmetics, cleaning products, and toilet papers, among others. Prior studies have detected PFAS in domestic wastewater, further indicating that it is a diffuse source of PFAS to groundwater. This poster estimates the PFAS loading from US septic systems based on i) measurements of PFAS in household products as reported in the literature and ii) estimated average annual down the drain product load per household. The results are compared to measurements of PFAS in domestic wastewater in septic systems and in wastewater

treatment plants. The results indicate that domestic wastewater is a quantifiable source of PFAS that should be further studied to better understand its contribution to PFAS in the environment, particularly in communities that rely heavily on septic systems.

4.13.T Identifying PFAS Sources Near and Far

4.13.T-01 Atmospheric Releases of PFAS From Wastewater Treatment Plants and Implications for Worker Exposure

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During wastewater treatment, particularly during aeration, per- and polyfluoroalkyl substances (PFAS) can be released to air as a result of volatilization and aerosolization. The overarching aim of this research was to evaluate the release of PFAS to the air above aeration basins in wastewater treatment plants by determining PFAS concentration in the aerosol and gas phases. Specific goals were to assess the importance of wastewater treatment plants as sources of atmospheric PFAS and to estimate occupational exposures for treatment plant operators. A mass balance approach was used to assess PFAS partitioning and mass flows. Both neutral and polar PFAS were measured in the aqueous phase, gas phase, and aerosol phase at three wastewater treatment plants. A high-volume air sampler equipped with a glass-fiber filter and an XAD resin/polyurethane foam cartridge was used to obtain aerosol and gas-phase PFAS, respectively. A total of 52 PFAS were measured including neutral PFAS by gas chromatography tandem mass spectrometry (GC-MS/MS) and polar PFAS compounds via liquid chromatography tandem mass spectrometry (LC-MS/MS). Among the targeted polar analytes, perfluoroalkyl carboxylic acids were dominant in the aerosol phase while 6:2 fluorotelomer alcohol (6:2 FTOH) was dominant neutral PFAS in the gas phase. Neutral PFAS accounted for 15 to 47% of quantified PFAS in the air above aeration basins, with summed concentrations of polar analytes ranging from 1210 to 8120 pg/m³, and summed concentrations of neutral PFAS ranging from 397 to 7340 pg/m³. For the three facilities sampled, the estimated atmospheric emissions for quantified PFAS ranged from 268 to 5300 mg/yr, suggesting that wastewater treatment plants are not an important source of atmospheric PFAS emissions. However, worker exposure may be important. During an 8-hr work day, a wastewater treatment plant operator could inhale between 7.8 and 28 ng of PFAS targeted in this study, including up to 4.4 ng PFOA.

4.13.T-02 Sources Of Per- and Poly- Fluoro Alkyl Substances to The Influent and Effluent of Municipal Wastewater Treatment Plants

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Municipal wastewater treatment plants (WWTP) receive PFAS from multiple sources. Several studies have demonstrated that measurable PFAS levels are higher in the effluent than the influent to such plants, presumably because during the treatment process, precursors that are not measured are converted to PFAS. Because of this, determining the sources of PFAS to WWTPs is difficult. In this study, a dataset on PFAS in paired influents and effluents of 24 WWTPs was obtained from the California GeoTracker database and analyzed using positive matrix factorization (PMF) to investigate PFAS sources. The data was compiled such that analytes measured in the influent and effluent of paired samples were considered to be different analytes. PMF analysis generated three fingerprints that described PFAS sources. The first was characterized by high contributions from PFOA, PFPA, and PFHA in both influent and effluent, suggesting that it represents PFAS that are already in a stable form upon entering the WWTP and therefore undergo little alteration during treatment. The second fingerprint was characterized by a high contribution of PFOS, PFHxS, and 6.2FTS in the influent, and increased amounts of PFOA, PFPA, PFHA, and other perfluorocarboxylic acids (PFCAs) in the

effluent. This pattern suggests that this source includes unmeasured precursors that are converted to PFCAs during treatment. The third fingerprint was characterized by very low contributions of all measured PFAS in the influent, but high levels of PFOA, PFPA, and PFHA in the effluent, suggesting that it represents PFAS from precursors that are converted to PFCAs during treatment. The three identified fingerprints were roughly equally abundant in the samples, indicating the diversity of PFAS sources in WWTPs. Notably, the WWTPs with the highest overall PFAS levels were dominated by the first and second fingerprints. These findings shed light on the complex nature of PFAS inputs into WWTPs and highlight the need for comprehensive monitoring and better control measures to minimize PFAS contamination in wastewater systems.

4.13.T-03 Investigation of PFAS in Domestic Water Supplies in an Oil and Gas Producing Region of Northern West Virginia

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Sources of per- and polyfluoroalkyl substances (PFAS) to groundwater supplies remain poorly understood, despite groundwater serving as a major source of drinking water globally. Additionally, limited scientific attention has focused on possible environmental impacts from the use of PFAS in the oil and gas industry, though several US patents detail the use of PFAS as surfactants, corrosion inhibitors, and additives used in drilling and fuel storage. We measured PFAS in samples from 45 wells used as household drinking water sources and 8 surface water sources in northern West Virginia, an area heavily impacted by both conventional and unconventional oil and gas extraction. All surface water and 67% of the wells sampled contained quantifiable levels of at least one targeted PFAS, and four wells (8%) had concentrations above the proposed US EPA maximum contaminant level for PFOA. Semi-quantitative non-targeted analysis results showed concentrations of emergent PFAS that are potentially higher than those measured for targeted PFAS. Comparison of our results with previous studies on PFAS in regional precipitation and extrapolation of trends from the historic PFOA releases from the Chemours Washington Works Plant (southwest of our study area) are unable to fully account for the concentrations we measured. We further investigated relationships between potential PFAS sources and groundwater concentrations using a multivariate latent variable hierarchical Bayesian model combined with data on the geology of the area and potential point sources such as wastewater treatment plants and oil and gas wells. Model results reveal i) increased contamination in upland recharge zones relative to areas with older groundwater, ii) negative correlation between PFAS size and concentration, indicating the role of sorption to soil organic matter for longer chain compounds, and iii) possible influence from nearby point-sources, including oil and gas wells.

4.13.T-04 Preliminary Analysis of PFAS in an Agricultural Ecosystem following Irrigation with Treated Effluent Wastewater

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As freshwater scarcity increases, municipalities have sought to re-purpose treated effluent wastewater as a drinking water source, an irrigation source for public grounds, or for agricultural practices to cultivate crops. Although many studies have centered on biosolids as a source of PFAS contamination in the environment, less is known about PFAS residuals in the treated effluent. Studies have shown that PFAS have been observed in effluent wastewater at detectable concentrations, and therefore there is a need to better understand the fate and transport of PFAS in the environment following the application of treated effluent (Arviniti et al., 2015; Houtz et al., 2018). The objective of this work was to evaluate the distribution of PFAS in a semi-rural, municipal wastewater re-use irrigation scenario including a) the accumulation of PFAS in hay grazer grass (*Sorghum bicolor (L.) moench*) b) the soil accumulation of PFAS, and c) the potential for infiltration of PFAS through the top layers of soil during the season as an indicator for potential impacts to groundwater. Treated effluent water concentrations were analyzed for PFCAs (PFBA, PFPeA, PFHxA, PFHpA, PFOA, and PFDA), PFSAs (PFBS, PFHxS, PFHpS, PFOS), and two precursor species (FOSA and 6:2 FTS). Effluent water concentrations

of PFAS ranged between 10-100 ng/L, which are lower than concentrations typically associated with concentrated biosolids. However, soil accumulation of PFAS did occur over the growing season, with PFHxA and PFHpA showing statistically significant increases ($p=0.025$ and $p=0.085$, respectively). Short-chain PFAS concentrations were similar in both shallow and deep soils, indicating a possibility for infiltration. In the cultivated grass, PFBA and PFHxA concentrations increased significantly ($p=0.007$ and $p=0.072$, respectively) over the season, which suggests the potential for accumulation, specifically for short-chain PFAS. Although PFAS was present in the effluent water at low concentrations, the increase in PFAS in both the soils and crops during the growing season indicates the application of this water could pose a risk of environmental exposure that has previously been overlooked. Mass loading of PFAS to the system resulting from high irrigation rates was suspected to be a key contributor to this seasonal accumulation. Overall, the results of this preliminary study indicate that effluent wastewater reuse may be an important factor when estimating PFAS exposure to an ecosystem.

4.13.T-05 Trifluoroacetic Acid and Other Ultrashorts: Understanding Sources, Measurement and Occurrence

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Per- and Polyfluoroalkyl Substances (PFAS) such as trifluoroacetic acid (TFA) with chain lengths of 3 or fewer are referred to as ultra-short PFAS and there is a significant data gap on their sources, occurrence and fate. These acids have multiple known sources into the environment with TFA arising from atmospheric degradation of hydrofluorocarbons (HFCs and HCFCs), the oxidation and chain shortening of fluorotelomer PFAS and more. PFPrA is also likely formed from the degradation of fluorotelomer PFAS. PFMS is used widely in organic synthesis and in lithium ion batteries. The limited information on TFA available in the literature suggests that background concentrations are steadily increasing. In addition, there is a significant gap in the applicability of forensic tools such as the total oxidizable precursor assay (TOP) due to omissions of ultrashort PFAS from the target lists. Methods used for short and long-chain PFAS do not typically work for ultra-short PFAS necessitating new/modified measurement approaches. In this study, we discuss new measurement tools for ultrashort PFAS, and their application to study their use in forensics using the TOP assay, and their prevalence as a source of PFAS in wastewater treatment plants. We developed and validated an isotope dilution/surrogate standard quantitation UPLC-MS/MS method for the measurement of 5 ultrashort PFAS TFA, perfluoropropionic acid (PFPrA), perfluoromethanesulfonic acid (PFMS), perfluoroethanesulfonic acid (PFEtS) and perfluoropropanesulfonic acid (PFPrS) in aqueous samples. Reporting limits for the method ranged from 1-20 ng/L. The analytical approach was designed to be fully compatible with the preparation protocols from EPA 1633 draft enabling measurement of the ultrashorts from the final preparation step of EPA 1633. We apply this new method to expand the list of TOP targets and partially close the mass balance on fluorotelomer PFAS oxidation. We also apply this new tool to assess the source strength of ultrashort PFAS in wastewater treatment plant effluents. In a reagent water spike/recovery experiment ($n=5$) recovery values of 76-106% and RSD values of 0.8-2.4 was achieved. Preliminary results also indicated suitability in surface water and robustness testing in wastewater and leachate samples is ongoing. This study will shed light on an understudied source of PFAS.

4.13.T-06 From Watersheds to Dinner Plates: Evaluating PFAS Exposure through Fish Consumption in Michigan

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In response to the growing concern of per- and polyfluoroalkyl substances (PFAS), the Michigan Ecology Center partnered with other organizations and community anglers to collect and analyze filet, gut (stomach plus intestines), liver, and eggs in multiple fish species from popular fishing areas for PFAS. The direct oxidizable

precursor (dTOP) assay was shown to be useful to investigate the presence of precursors that can't be detected using targeted analysis. The study composited over 100 different fish from six different species to measure PFAS in gut, eggs, filet, and liver using a targeted suite of 66 PFASs. A subset of samples was also analyzed using the dTOP assay (n=6) and total extractable organic fluorine (EOF, n=22). The results from the targeted analysis were evaluated for source apportionment by use of positive matrix factorization (PMF). Spearman correlation showed a positive relationship ($P < 0.0005$) between the Σ PFAS and EOF, indicating a significant overlap of targeted and EOF compounds. The median PFOS level in fish filet, gut, egg, and liver was 4.5, 16, 23, and 38 ng/g wet weight (w.w.), respectively. The dTOP assay showed the highest percentage increase in targeted PFAS in the Catfish fillet (>500%) while the smallest percentage increase was observed in the Catfish liver (32%) compared to the original levels. Catfish fillet showed an increase of 1.3 nMole F g⁻¹ dry weight (d.w.), whereas Catfish liver showed an increase of 8.1 nMole F g⁻¹ d.w. In this work, the levels of PFOA, PFOS, PFNA, PFHxS, and PFBS in all liver, egg, and intestine samples exceeded the limited consumption guidelines, while 9 of the 11 filet samples exceeded the guidelines and none exceeded the "do not eat" advisory. Furthermore, the PMF analysis showed that three PFAS sources were identified, with a factor dominated by PFOS explaining 73% of the data. This study identifies potential sources of PFAS contamination in fish tissues and emphasizes the need for continued monitoring and investigation of the associated health risks. Results suggest that consuming PFAS-contaminated fish represents a potential health risk for humans and the high levels in fish organs indicate greater risks to the ecosystem, underscoring the importance of ongoing monitoring of PFAS in aquatic organisms, particularly in areas with known contamination.

4.14.P-Tu Measurement Challenges and How to Tackle Them: Per and Polyfluoroalkyl Substances (PFAS) and Other Contaminants of Emerging Concern (CEC)

4.14.P-Tu-145 Development of an Analytical Method for Simultaneous Determination of PFAS in Japanese Drinking Water by Liquid Chromatography/Tandem Mass Spectrometry

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This study seeks to develop a liquid chromatography/tandem mass spectrometry analytical method for the simultaneous determination of 80 Per and Polyfluoroalkyl Substances (PFAS) in drinking water to support future regulations in Japan. Twenty-one perfluoroalkyl acids (PFAAs), such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), telomer sulfonates (FTS), perfluorooctanesulfonamidoacetic acids (FOSAAs), perfluorooctanesulfonamides (FOSAs), perfluorooctanesulfonamidoethanols (FOSEs), telomer acids (FTAs), and other PFAS were selected as analytical targets. Although some PFAS have linear and branched isomers, only the linear isomer of each PFAS was assigned because it is the main component of standard mixtures used in the study. A standard solution of each PFAS was measured by LC/MS/MS (LCMS-8050, Shimadzu Corporation) via the electrospray ionization (ESI) to acquire the mass spectrum of each PFAS. After identifying the quantifier and qualifier ions of each PFAA, the mobile-phase gradient conditions for LC were optimized using an octadecyl silica (ODS) column (InertSustain AQ-C18, 2.1 x 150 mm, pore size 3 μ m, GL Sciences Inc.), and a mobile-phase gradient comprising 10 mM aqueous ammonium acetate solution and acetonitrile. The pretreatment method for the PFAS analytes was developed based on existing standard analytical procedures such as those of the ISO and U.S. EPA. A 500 mL water sample was concentrated and purified by solid-phase extraction (SPE) to 0.5 mL (1000x concentration), after adding 13C-PFAS extraction standards (ES) as surrogates. The prepared samples were analyzed by LC/MS/MS. Losses sustained during pretreatment were corrected by the 13C-PFAS-ES. The validity of analytical method was evaluated based on the results of the recovery test of each chemical added to drinking water. Further, we have conducted environmental monitoring of these PFAS in Japanese drinking water and river water. We have collected 300 drinking water and river water samples in 25 prefectures in Japan during 2020 to 2022. Then, these samples were analyzed by the method developed in this study. As a result of the environmental monitoring, we have

detected 29 and 40 PFAS in the drinking water and river water samples, respectively. The results obtained from this study can be applied to risk assessments of PFAS in Japan.

4.14.P-Tu-146 Which Side are You On? The Interesting Partitioning Behavior of Ionic Per- and Polyfluoroalkyl Substances in Octanol/Water Systems.

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The octanol/water partitioning coefficient (K_{ow}) is an important chemical property used to predict a compound's distribution in the environment, uptake into biota, and biological response. Similarly, K_{ow} can provide insights into a compound's behavior during chemical analysis. For example, compounds with a high $\log K_{ow}$ (> 4.0) tend to sorb to container walls during the sample collection and storage of aqueous matrices, which can lead to a significant loss of chemical from the solution. The partitioning behavior of many non-ionic compounds can be determined accurately and quickly from model predictions. However, for per- and polyfluoroalkyl substances (PFAS) model predictions are highly variable (e.g., PFOA ranges from $\log K_{ow}$ 3.11-7.75 according to CompTox Chemicals Dashboard v.2.2.1) which is likely due to their ionization state, surfactant character, and that quantitative structure-activity relationship models were not trained with highly fluorinated compounds. Additionally, there is little experimental data available regarding the partitioning behavior of PFAS in their ionic forms—which are the dominant fraction in the environment and biota. To elucidate ionic PFAS partitioning, slow stir experiments (OECD Slow Stir Method 123) were performed with perfluoroalkyl carboxylates (C6, C8, C10), perfluoroalkyl sulfonates (C6, C8, C10), perfluoroalkyl sulfonamides (C4, C6, C8; likely non-ionic), and polyfluoroalkyl telomer sulfonates ($n = C6$ and $C8$). In addition, the effects of solute concentration, pH, ionic strength, and salt composition were explored. Not surprisingly, there is a linear increase in $\log K_{ow}$ with perfluoroalkyl chain length. However, one interesting finding is a concentration dependence on perfluoroalkyl sulfonate partitioning that is not observed for perfluoroalkyl carboxylates or sulfonamides. The presentation will outline the experimentally derived K_{ow} values for those selected PFAS and how additional parameters affect partitioning. This abstract does not necessarily reflect U.S. E.P.A. policy.

4.14.P-Tu-147 Verification of Free Chemical Concentration in 96-well Plate-Based High Throughput Testing of Per- and Polyfluoroalkyl Substances (PFAS)

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High throughput testing in a 96 well plate format is an efficient and cost-effective way to screen many chemicals, in concentration response, to determine toxicity, biological activity, and associated points of departure. However, many chemicals interact with well surfaces and other materials in an exposure system, leading to decreased chemical exposure concentrations relative to nominal concentrations. Additionally, stock chemical may be lost during storage due to degradation, volatility, and binding to storage vessels. Failure to account for the bound (unavailable) versus free chemical fractions (available) and potential chemical loss can lead to inaccurate potency estimates. Although, there are models that can predict the free fraction within a well plate for some compound classes, such models have not been developed and validated for per- and polyfluoroalkyl substances (PFAS). As part of development of ecologically relevant high throughput assays, this study quantified recoveries of 22 PFAS after a 24-hour exposure in 96 well plates containing *Pimephales promelas*, *Daphnia magna*, *Chironomus dilutus* or *Raphidocelis subcapitata*. The PFAS tested included carboxylic acids with different chain lengths, as well as alcohols and sulfonates, all of which were measured by liquid chromatography–mass spectrometry (LC-MS). Nominal concentrations ranged from $0.03\mu\text{M}$ to $100\mu\text{M}$. Free chemical concentrations detected in the aqueous phase after 24 h of exposure ranged from 50-90% for most chemicals. However, six out of the 22 PFAS had less than 20% recovery in all exposures. Notably, almost all chemicals with low recoveries had chain lengths of 8 carbons or more. This suggests that alkyl chain length is an important parameter to consider in developing partitioning models for these types of multi-well plate-based exposures. The present results also emphasize the importance of exposure

verification for deriving accurate points of departure for use in risk-based screening or prioritization. *The contents of this abstract neither constitute, nor necessarily reflect US EPA policy.*

4.14.P-Tu-148 Canadian Building Materials are Significant Sources of Per- and Polyfluoroalkyl Substances (PFASs) to the Environment

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We hypothesized that building materials and products, used to reduce deterioration due to weathering, are likely sources of per- and polyfluoroalkyl substances (PFASs) to the environment. To test this hypothesis, three categories of outdoor products sold on the Canadian market, namely outdoor textiles, paints and sealants, were characterized for total organofluorine (TOF) using ¹⁹F nuclear magnetic resonance (¹⁹F NMR) and individual PFAS by liquid chromatography-high resolution mass spectrometry (LC-HRMS) and gas chromatography-mass spectrometry (GC-MS). ¹⁹F NMR revealed large variations in TOF concentrations and compositions between these products. Specifically, the TOF content ranged from 0.3-74.0, 0.1-42, and 0.6-2500 µmol/g in outdoor textiles, paints and sealants, respectively. The TOF in textiles was composed of three types of side-chain fluorinated polymers (SCFPs), including those derived from 6:2 fluorotelomers (FTs), 8:2 FTs, and perfluorobutane sulfonyl fluoride (PBSF). The TOF in paints consisted of two kinds of nonpolymers, including those with an aromatic-CF₃ group and derived from 6:2 FTs, while those in sealants had both SCFPs and nonpolymers: SCFPs derived from 6:2 FTs, nonpolymers with an aromatic-CF₃ group and those derived from 6:2 FTs and PBSF. LC-HRMS results for paints and sealants showed the dominance of three 6:2 FTs, including two 6:2 FT phosphate esters (PAPs) and one 6:2 FT pyrophosphate, which constituted 23–61% of the TOF, indicating the presence of additional unidentified 6:2 FTs. GC-MS analysis is ongoing to identify 6:2 FTs in these products. Weathering experiments involving exposure to UV radiation in a chamber showed that two textiles with PBSF-derived SCFPs, one paint with nonpolymeric 6:2 FTs, and one sealant containing 6:2 FT SCFPs all released short-chain perfluoroalkyl carboxylates or sulfonates (PFCAs/PFSAs). These results support the hypothesis that outdoor textiles, paints and sealants are not only significant pools of polymeric and nonpolymeric PFAS, but also could be significant sources of short-chain mobile PFCAs/PFSAs to the environment.

4.14.P-Tu-149 Enrichment of PFAS in the Surface Microlayer of Water Bodies Does Not Cause Significant Bias in Bulk Water Samples

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Per- and polyfluoroalkyl substances (PFAS) exhibit measurable enrichment in the surface microlayer (SML) of surface water bodies. This leads to a concern that entrainment of a PFAS-enriched SML during sample collection could cause overestimates (bias) of PFAS concentrations in bulk surface water, particularly for samples collected using a partially submerged sample bottle. To evaluate the potential for PFAS enrichment in the SML to bias measures of bulk water PFAS concentration, we sampled PFAS in SML and bulk water at 11 water bodies with varying concentration and water quality characteristics. SML samples were collected using glass plates, and bulk water samples were collected using peristaltic pumps, submerged bottles, and partially submerged bottles. Samples were extracted and analyzed for PFAS using Isotope Dilution and LC-MS/MS compliant with Table B-15 of DoD QSM 5.3. The results described here are based on SML and bulk water samples for up to 7 water bodies per PFAS compound (depending on analytical detection frequency). Analyses of the additional 4 water bodies are ongoing. Fourteen PFAS, primarily perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs), were detected with sufficient frequency to calculate enrichment factors (EFs) and evaluate potential bias of bulk water measurements. Results indicated that EFs were generally greater

in PFAS with greater hydrophobicity, as C18 retention time (RT), although water body and PFAS family may also affect the EF. For example, of the 8 PFAS with RTs ≤ 10.3 min, EFs ranged from 0.96 to 5.0. For the 6 PFAS with RT ≥ 10.4 min, EFs ranged 0.80 to 42. However, 8:2 FTS and PFOSA, with RTs of 12.8 and 14.0 min, had EFs ranging 3.5 to 42 and 1.6 to 13, respectively. In addition, for most PFAS, the greatest EFs were from the same water body, while the least EFs were from one of three water bodies, suggesting a water chemistry or site-specific component also influences the EF. No consistent or significant differences were observed among results for the bulk water sampling methods for the 14 PFAS, indicating that inclusion of the SML did not contribute significant bias to the measured PFAS concentrations in the bulk surface water, and the use of a peristaltic pump, submerged, or partially submerged bottle for bulk water sample collection is unlikely to bias PFAS assessments for surface water bodies for these PFAS in the range of conditions and concentrations surveyed in this study.

4.14.P-Tu-150 Enabling State Adoption of Non-Targeted Analysis (NTA) to Address Pressing Public Health Needs: Maryland, Minnesota, and California Leading the Way

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Environmental health concerns over per- and polyfluoroalkyl substances (PFAS) are spurring states to adopt non-targeted analysis methods (NTA). The need for NTA is underscored by the approximate 100-fold difference between the number of chemicals making up the class relative to the few (<50) compounds that are routinely included in targeted analysis methods. The use of NTA can provide a more comprehensive assessment of PFAS environmental occurrence to inform public health protective strategies based on source attribution, exposure potential, hazard and risk assessment, and management strategies. However, there are significant barriers to state adoption of NTA including instrument cost, analytical expertise, and complex data processing workflows and tools. To overcome or mitigate these barriers, 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. To assess barriers more comprehensively and evaluate developed tools, EPA/ORD and regional offices (Regions 3, 5, and 9) partnered with three states (MD, MN, and CA) leading efforts to deploy NTA to address location-specific scenarios of environmental health concern. This presentation will provide an overview of lessons learned through this collaboration, state motivations to adopt NTA, study plans, barriers to implementation, and strategies for overcoming those barriers. Together, this collaboration will provide a roadmap and best practices for EPA engagement as other states and regions develop an interest in pursuing NTA. *Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US EPA.*

4.14.P-Tu-151 Development of A Solid-Phase Extraction Method to Distinguish Inorganic and Organic Fluorine and to Separate Ultrashort from Longer Chain PFAS

Yukun Jin, Heather D. Whitehead, Alyssa Wicks and Graham F. Peaslee, University of Notre Dame
Per- and polyfluoroalkyl substances (PFAS) are a large group of anthropogenic chemicals that have been widely used in industrial and commercial applications. They are well known for their extreme persistence, bioaccumulation, and toxicity. Of particular concern is their mobility in water that allows them to pollute drinking water supplies. Traditional analytical methods for PFAS measurements, such as liquid chromatography with tandem mass spectrometry (LC-MS/MS) and combustion ion chromatography (CIC), tend to be lengthy and cost prohibitive. Additionally, these techniques must also overcome unique issues. Inorganic fluoride often exists in real-world water samples, and thus it may need to be distinguished, separated, and removed from organic fluorine (*i.e.*, PFAS) in sample preparation prior to analysis for select analytical techniques. Recent

studies have found ultrashort chain PFAS to be largely present in environmental waters, though, measurements of ultrashort chain PFAS in environmental waters using LC-MS/MS are still limited and challenging. As an alternative method, and to quickly screen for the presence of PFAS, a low-cost and rapid solid-phase extraction (SPE) method using a graphitized activated carbon felt has been developed to preconcentrate and capture PFAS from drinking water samples *in situ*. These SPE felts are analyzed by particle-induced gamma-ray emission (PIGE) spectroscopy to rapidly screen for the level of total adsorbable organic fluorine (AOF). Results from the development of a rapid and cost-effective methanol rinse method to distinguish inorganic fluoride and organic fluorine in water samples will be presented. In addition, a nitric acid washing method to separate ultrashort chain PFAS (*i.e.*, C2 and C3) from longer chain PFAS will also be described. These developed methods were applied to screen for the presence of PFAS in northern Indiana drinking water samples which were collected from public water sources. The total AOF results measured on PIGE were then compared with targeted PFAS analysis by LC-MS/MS. Preliminary results from this work demonstrate the differences in the amounts of PFAS measured by PIGE and LC-MS/MS, and further examine the presence of ultrashort chain PFAS in these drinking water samples.

4.14.P-Tu-152 Assessment of PFAS in Fluorinated Polymers Applied to Firefighting Gear

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Per- and poly fluoroalkyl substances (PFAS) are extensively used in functional textiles as water and stain repellents. The unique requirements for personal protection in firefighting turnout gear result in application of specialty textiles and coatings, which may include PFAS-based side-chain polymers. Treated fabrics in firefighting gear may generate PFAS- containing fragments under stresses including heat, sunlight, wear, and washing. These fluorinated polymers may transform to environmentally-persistent perfluoroalkyl acids (PFAAs), which could be released to the environment and may cause exposure to firefighters using these treated garments. Characterization of PFAS in textile-associated fluorinated side-chain polymers is challenging in comparison to non-polymeric PFAS, due to polydispersity in molecular weights and chemical complexity. The objective of this study was to analyze PFAS side-chain polymers in firefighting turnout textiles. We have studied 8 new and 6 pre-worn samples of fabrics used in firefighter turnout gear using targeted PFAS quantitation by LC-MS/MS, total oxidizable precursors (TOP) assay analysis, non-targeted analysis using high resolution mass spectrometry (HRMS), as well as accelerated solvent extraction (ASE) and solvent-based textile dissolution techniques to optimize estimation of total PFAS concentration and identity in fabrics. TOP analysis of textiles from firefighter turnout gear showed that 7 of the new (unworn) textiles contained a dominant 6:2 fluorotelomer (FT)-based PFAS fluorinated backbone in the external fabric's coating, while the underlying moisture barrier was distinct, with a characteristic C4-based electrochemical fluorination (ECF) signature. This implies that at least two distinct PFAS-containing polymers were used in these fabrics, likely to achieve different performance goals. Results from analysis of pre-worn turnout gear by TOP assay indicated an increase in perfluorobutanoic acids 30-150x relative to the same new, unworn fabrics, which could be due to release of C4-based ECF polymer breakdown products from the moisture barrier into the outer layer of firefighter uniforms. This result has implications for firefighter PFAS exposure during wear and use. Further characterization of firefighter turnout gear using accelerated solvent extraction (ASE) and total fabric solvent dissolution coupled with HRMS are ongoing to further characterize the PFAS polymer used in these textiles.

4.14.P-Tu-153 Development of A Sensitive Method for Determination of Per and Polyfluoroalkyl Substances (PFAS) in Biosolids Leachates

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Per and polyfluoroalkyl substances (PFAS) are recalcitrant environmental contaminants of global concern. They are extensively used in a variety of household and industrial products and are found ubiquitously in the

environment and in humans. PFAS are extremely persistent and not removed by traditional wastewater treatment processes. In wastewater treatment plants (WWTPs), biodegradation is limited (some PFAS precursors might degrade to mobile perfluoroalkyl acids), and PFAS can be adsorbed on suspended solids during activated sludge process resulting in accumulation in biosolids. Land application of biosolids is a common agricultural practice and a sustainable method of disposing treated sewage sludge. However, limited studies have investigated the presence and quantity of PFAS in biosolids and their potential impact on agricultural lands. The uptake of PFAS from soil to crops could constitute a significant exposure pathway to humans and animals through the food chain. Therefore, it is critical risk factors and there is a need to evaluate the quality of biosolids produced at WWTPs and their impact in the environment for PFAS content. In this study, biosolids were collected in 500 mL polypropylene jars from the South District and Central District wastewater treatment plants in Miami Dade County and leachate experiments were conducted for a period of 30 days. A methodology based on the semi-automated solid phase extraction (SPE) was developed and validated followed by analysis of PFAS using a high-performance liquid chromatography-tandem mass spectrometry (HPLC-LCMS/MS). The analytical method focused on the determination of 40 legacy and emerging PFAS using isotopic labeled standards, following the EPA method 1633. The preliminary results generated in this study demonstrate a sensitive method to assess PFAS levels and composition in biosolids leachate, which will further inform recommendations for appropriate biosolids testing and land application practices in Florida, aiming to minimize the potential risks associated with PFAS contamination.

4.14.P-Tu-154 Techniques to Determine Total PFAS and Fluorine Mass Balance in Biological Samples: A Review

Nawagamuwage Lilani Dilani Perera, Wesley P Scott, Rachel Smolinski, Leenia Mukhopadhyay and Carrie McDonough, Carnegie Mellon University

Per/polyfluoroalkyl substances (PFASs) are a large group of synthetic compounds that have been used in a wide range of industrial applications and commercial products. PFASs are called “forever chemicals” as they are extremely stable, leading to their widespread contamination of environmental and biological matrices. It is crucial to identify and characterize the chemicals that comprise total PFASs in these samples, as they may cause serious negative health effects in exposed humans and wildlife. Although there has been significant progress in PFAS analysis during the past decade, a significant fraction of total organofluorine measured in biological matrices remains unidentified, and in some cases overlooked. Here we will review traditional and recent analytical approaches in screening PFASs along with their advantages and limitations. We will outline progress to date on closing the organofluorine mass balance gap in biological samples using expanded chromatography techniques as well as high-resolution mass spectrometry (HRMS) techniques, ion mobility spectrometry (IMS), and other advanced techniques. We also summarize progress being made in comprehensively measuring total organofluorine to accurately constrain the unidentified fraction. We suggest that combining advanced analytical techniques and the development of automated data processing methods will pave the way to closing the PFAS mass balance gap.

4.14.P-Tu-155 Characterization of Dissolved and Colloidal PFAS in Textile Manufacturing Wastewater Impacting North Carolina Drinking Water Sources

Patrick Faught, Marzieh Shojaei, Abigail Joyce and Lee Ferguson, Duke University

Per-and-polyfluoroalkyl substances (PFAS) are used to create water-repellant and stain-resistant fabrics in the manufacturing of textiles. The recent discovery of unexplained high levels of PFAS in public drinking water supplies downstream from textile industry discharges in North Carolina initiated an investigation to find the source of these PFAS contaminants. Targeted analysis of raw industrial wastewaters in an upstream municipality found short-chain (C₄-C₇) perfluorocarboxylic acids (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 the wastewater. We thus 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 specific raw textile wastewaters after TOP assay, revealing the primary source of PFAS to the WWTP. Parallel TOP assays performed on wastewater samples with and without suspended solids suggested that a significant fraction of PFAS precursors were associated with particulates in the textile wastewater, posing a risk for contamination of sludge and resulting biosolids. The flux of textile-associated PFAS precursors into the WWTP was monitored over a two-year sampling period. 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 experiments prior to TOP assay treatment 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. Further characterization of these colloidal PFAS using asymmetric field-flow fractionation (AF4), density gradient ultracentrifugation, nuclear magnetic resonance (^{19}F -NMR) and dynamic light scattering has been performed. 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.

4.14.P-Tu-156 Temporal Variability of PFAS Precursors in Wastewater Treatment Processes

Jean Van Buren and Phillip Potter, U.S. Environmental Protection Agency

Assessing the fate of PFAS in the environment requires improved analytical methodologies to quantify a greater variety of PFAS and application of these methods to investigate their occurrence in complex sample matrices. PFAS precursors are a compound class of emerging concern that are capable of undergoing reaction under environmentally-relevant conditions to transform into per-fluorinated alkyl acids (PFAAs). Most PFAS precursors are not included on the compound lists of common targeted analysis methods and require either amended targeted compound lists, non-targeted analysis via high resolution mass spectrometry, or the Total Oxidizable Precursor (TOP) assay (which does not yield full characterization of the individual precursors). This research includes both method development for PFAS precursor analysis and application of various analytical methods to wastewater samples to assess precursor content.

4.14.P-Tu-157 Super Critical Water Oxidation Coupled with Colorimetric Fluoride Detection for Total Organic Fluorine Analysis in Environmental Samples

Hannah Katherine Teed, Lee Ferguson and Marc Deshusses, Duke University

Per- and polyfluorinated alkyl substances (PFASs) are a collection of widely used compounds, with applications ranging from commercial to industrial. Due to extensive use, persistence, and mobility in the environment, PFASs accumulate in all environmental matrices and pose human health risks when ingested. Due to these health risks, rapid and sensitive analytical methods with the ability to measure total PFAS concentrations are necessary to identify and remediate areas with elevated contamination. However, measuring total PFAS in the environment is complicated by the molecular diversity of PFAS compounds, limited availability of standardized reference materials, and the time-consuming nature and selectivity of sample preparation. We have developed a total organic fluorine (TOF) analysis method by coupling supercritical water oxidation (SCWO) with a colorimetric fluoride (F^-) sensor. Under supercritical conditions with abundant oxidant, temperatures above 600°C, and pressures \sim 3800 psi, SCWO rapidly mineralizes PFAS into inorganic species including CO_2 , F^- , and water. For analysis, F^- levels in the post-SCWO effluent are measured using the optical absorbance of an aluminum (III) phthalocyanine chloride (AlPc-Cl) dye probe. After mixing the AlPc-Cl dye with the post-SCWO effluent, we observe a correlative absorbance shift from 610 nm to 637 nm with a diode array UV/VIS absorbance detector. Background levels of F^- are corrected using a dual loop injection system, which measures and subtracts background F^- levels from evolved F^- measurements. With optimized reactor and optical sensor conditions, we have demonstrated that the SCWO-TOF configuration can achieve rapid and sensitive

measurements for TOF in aqueous film-forming foam contaminated waters and other PFAS-containing samples at concentrations below 5 parts-per-billion (ppb), representing the most sensitive currently available method for TOF measurement. We will discuss the application of SCWO-TOF to analysis of total PFAS in textile wastewaters as well as in the context of drinking water sources around North Carolina. Novel approaches for removal of inorganic fluoride from samples prior to analysis will also be discussed.

4.14.T Measurement Challenges and How to Tackle Them: Per and Polyfluoroalkyl Substances (PFAS) and Other Contaminants of Emerging Concern (CEC)

4.14.T-01 Using Cheminformatics Approaches to Develop a Structure Searchable Database of Analytical Methods

Antony J Williams, Greg Janesch, Sakuntala Sivasupramaniam and Erik Carr, U.S. Environmental Protection Agency

Analytical methods can vary in nature from detailed regulatory methods to more summary in nature. Regulatory method documents can include details of analytes which can be studied, supported matrices, reagents, methodological details, statistical performance, interlaboratory validation and other details. Summary methods provide a general overview of reagents, instrumentation and commonly a short list of analytes. Regulatory bodies including the US Environmental Protection Agency (US-EPA), US Geological Survey (USGS), US Department of Agriculture (USDA) and others provide detailed analytical methods and collections of summary methods from the agrochemical industry, such as the US-EPA Environmental Chemistry Methods (<https://www.epa.gov/pesticide-analytical-methods/environmental-chemistry-methods-ecm>). Instrument vendors also provide access to many hundreds of application notes which can be considered as summary methods. We have developed a cheminformatically enabled database of methods whereby chemicals have been extracted from the methods, with the identifiers (names and/or chemical abstracts registry numbers) mapped to chemical structures. The resulting database of almost 3000 methods can be searched by chemical name, CASRN, structure and similarity of chemical structure. The resulting database has been integrated into a web-based application and includes integration to public domain mass spectral data and filtering of the methods based on analyte, chemical class, method source and other related metadata. Tens of the methods are associated with PFAS and are from regulatory agencies, vendors and the peer-reviewed literature. *This abstract does not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

4.14.T-02 Improved Automated Sample Preparation for Persistent Organic Pollutants using Parallel Gas Assisted Accelerated Solvent Extraction and Automated Solvent Concentration

Chris Shevlin, Rahmat Ullah, Yan Liu, Fabrizio Galbiati and Mingfang Wang, Thermo Fisher Scientific

Accelerated solvent extraction is a high-temperature and high-pressure extraction technique that is widely used in environmental, chemical and food analysis. New advancements have been made in accelerated solvent extraction which allows for a more efficient and controlled extraction to allow for better extraction over traditional methods and devices. Gas assisted extraction is a new method employed for extracting analytes of interest from a solid or semi-solid matrix, that uses a mixture of a liquid solvent at a constant flow rate. Gas assisted extraction can be more effective as compared to static extraction methods. In addition, when done in parallel, this new technique yields many advantages. Furthermore, the solvent evaporation process is fully incorporated into the automated process. In this presentation, we discuss a new parallel gas assisted extraction for pursuing extractions and by quantitation for PFAS and persistence organic pollutants (POPs). We present here performance data of the new method for analyte extraction and evaporation in the same platform from soil samples for different POP. Unlike traditional methods such as liquid-solvent extraction followed by nitrogen stream evaporation, the fully automated solvent extraction and evaporation system saves time, solvent, and labor, while ensuring high reproducibility and productivity for analytical testing.

4.14.T-03 Exploring the use of DLLME Cleanup Approach in the Detection of EPA 1633 PFAS Target List in Mulch, Clam Tissue and Sediment Samples

Hlengilizwe Nyoni and Odette Mina, Pennsylvania State University

In this work, we report advances in the use of a miniaturized dispersive liquid-liquid microextraction (DLLME) cleanup method for Native PFAS standard (mix) PFAC-MXG, MXJ, MXI, MXF and MXH; Extraction Internal Standards, (EIS) – MPFAC-HIF-ES and Non-extraction Internal Standards (NIS) – MPFAC-HIF-IS prior to LC-HRAM analysis. The Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) method was employed to optimize the DLLME solvent pairs and clean up experimental parameters. The most effective cleanup and concentration process involved the use of perfluorotert-butanol as the extraction solvent, combined with acetonitrile as the dispersive solvent in specific volume ratios (120:800 μL). A comparison was made between the DLLME cleanup method, and the traditional wax solid-phase extraction (SPE) method described in the 2nd Draft Method EPA 1633. The results demonstrated that the DLLME approach achieved recoveries within the desired range of 50% to 150%. Notably, the DLLME method significantly improved the recovery of M2PFTeDA from 58% with the SPE method to 80%. Most of the EIS compounds showed recoveries ranging from 58% to 149%, with an average recovery of 97%. However, five isotopically labeled EIS compounds had low recoveries (58% to 66%), while four other EIS compounds displayed recoveries between 77% and 82%. Two compounds, M2-8:2FTS and M5PFPeA, exhibited recoveries above 150% (152% and 169%, respectively). Among the native compounds, PFDoA exhibited the lowest limit of detection (LOD) at 0.34 ng/g, whereas 7:3FTCA had the highest LOD at 97.55 ng/g, with the remaining EIS compounds falling within this range. The limit of quantification (LOQ) for PFDoA and 7:3FTCA was determined to be 1.02 ng/g and 295.60 ng/g, respectively. The SPE procedure required 20 minutes of extraction time per sample using 25 mL of methanol in total, while the DLLME approach only needed 2 minutes with 120 μL of perfluorotert-butanol and 800 μL of acetonitrile per sample for the cleanup step. Overall, these findings highlight the miniaturized DLLME method as a rapid and cost-effective alternative to the traditional SPE cleanup procedure for analyzing PFAS in mulch, clam tissue, and sediment extracts.

4.14.T-04 Per- and Polyfluoroalkyl Substances (PFAS) Interferences in Food Matrices

Brian Ng, Christine Fisher (O'Donnell), Susan Genualdi, Wendy Young, Elsie Peprah and Lowri deJager, U.S. Food and Drug Administration

Due to the extensive use and stability of per- and polyfluoroalkyl substances (PFAS), these compounds have been detected in the environment and certain PFAS can bioaccumulate in the food chain. It is important to accurately quantify PFAS compounds in foods to better understand potential dietary exposure. Targeted methods using triple quadrupole mass spectrometers are commonly used for accurate quantitation of known PFAS compounds. However, the unit mass resolution of these methods is insufficient to differentiate PFAS compounds from potential interferences within 1 Da of the target precursor and transition masses, which can lead to false positives and/or over-estimation of PFAS concentration. This is particularly challenging for complex matrices, such as foods, and for short-chain PFAS compounds with only one transition. In this work, we will first describe key observations in the targeted data that indicate a matrix interferent may be present (e.g., retention time shifts, higher than expected concentrations). We will then describe how we have used high-resolution mass spectrometry (i.e., Orbitrap and time-of-flight) to highlight matrix interferent masses for PFAS compounds in food matrices; these include perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), and 4:2 fluorotelomer sulfonic acid (4:2 FTS) in chocolate, deer meat, and corn snaplage, respectively. Finally, we will discuss approaches that can be applied to confirm false positives using the triple quadrupole instrument. For example, we have successfully used the tandem mass spectrometry spectrum of the interferent obtained on the high-resolution mass spectrometer to determine alternative transition(s) to add to the target list. This work helps raise awareness of these challenges in PFAS analysis and provides practical solutions that can be implemented to help labs avoid these potential pitfalls.

4.14.T-05 Evaluating the Effects of Storage Temperature and Holding Time on Per- and Polyfluoroalkyl Substances in Fish Plasma

Zachary Hopkins and Andrea Tokranov, U.S. Geological Survey

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. For effective and accurate environmental monitoring, appropriate protocols are required for the storage of samples prior to PFAS analysis to assure sample integrity. To date, storage holding time studies for environmental matrices have only been validated for groundwater, soil, biosolids, and sediment as part of the Environmental Protection Agency (EPA) draft method 1633 single laboratory validation study. Another study investigated holding times for bottled water, surface water, and wastewater treatment plant effluent. No studies thus far have investigated holding times for biological matrices. This work aims to fill a knowledge gap on appropriate storage conditions and holding times for plasma using fish as a representative matrix. This study will evaluate the effect of storage conditions at different temperatures (4°C, -20°C, and -80°C) and holding times (0, 7, 28, and 90 days) on quantifying PFAS in fish plasma and plasma extracts. The study will assess PFAS quantified by EPA draft method 1633. The research will also assess the effect of storage conditions on PFAS precursors (compounds that transform into persistent perfluoroalkyl acids) by collecting plasma from fish at an aqueous film-forming foam-impacted site. We hypothesize that storage of plasma samples at -80°C is required for the stability of PFAS in biological matrices, especially for the long-term stability of perfluoroalkyl acid precursor compounds. The results of this storage study will help inform researchers on the optimal holding time and temperature when analyzing for PFAS in plasma to ensure data is accurate and scientifically defensible.

4.14.T-06 Analyzing PFAS via Online Solid-Phase Extraction Coupled with LC-MS/MS: Application to EPA Method 1633 Compound List

Lilit Ispiryani¹ and Helmer Korb², (1) Axel Semrau, (2) iChrom Solutions,

Per- and polyfluoroalkyl substances (PFAS) have emerged as a significant environmental concern due to their widespread presence, persistence, and potential adverse effects on human health. Accurate and efficient analysis of PFAS in environmental, biological and food matrices is crucial for understanding their distribution and assessing potential risks. In this study, we explore the application of online solid-phase extraction (SPE) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) as a robust and efficient approach for analyzing PFAS compounds listed in EPA Method 1633 for environmental samples. There are several EPA and ASTM methods available for the analysis of PFAS in nondrinking water, soil, and tissue. While ASTM methods such as 8421 and 7979, along with EPA Method 8327, commonly employ co-solvation techniques for stabilizing PFAS in the sample prior to preparation, EPA Method 1633 utilizes manual SPE with the entire sample. These methodological differences result in varying sample preparation times, detection limits, and quality control (QC) criteria. By utilizing co-solvation and fully automated online SPE sample preparation methods, we aim to streamline the sample preparation process while maintaining the analytical integrity of the EPA Method 1633 compound list. The reduced time and solvent consumption associated with online SPE techniques offer practical benefits in terms of efficiency and cost-effectiveness and analytical accuracy. Furthermore, online SPE minimizes manual handling, reducing the risk of sample contamination, while LC-MS/MS provides high sensitivity and selectivity for the targeted PFAS compounds. We examine the applicability of online SPE LC-MS/MS for PFAS analysis to meet the specific QC criteria and detection limits required by EPA Method 1633. This work contributes to the advancement of PFAS analysis by improving the efficiency, reliability, and cost-effectiveness of the analytical process, ultimately supporting environmental monitoring, regulatory compliance, and risk assessment efforts.

4.15.A.T Mercury Bioaccumulation, Exposure, and Effects on Wildlife: Understanding How Ecosystem Pressures Drive Mercury Cycling

4.15.A.T-01 Drivers of Mercury Contamination, Methylmercury Formation and Mercury Sources Within Lake Sediments across the Contiguous United States

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Atmospheric deposition is the predominant source of mercury (Hg) to food webs across the United States and is dictated by both international and domestic emissions. This study's objectives are to identify ecosystem drivers of sensitivity to Hg inputs and Hg sources to provide the framework to predict how changes in Hg emissions will impact aquatic ecosystems. This will be accomplished through spatial analyses of existing data, including over 1000 sites measured for speciated Hg concentrations in sediment, and through recent Hg-isotope analyses of a subset of over 400 sites from the USEPA 2012 National Lakes Assessment (NLA). Identifying the spatial drivers for ecosystem sensitivity to Hg inputs and responsiveness throughout the U.S. will rely upon many existing federal datasets sediment chemistry (NLA metadata), atmospheric Hg measurements (e.g., National Atmospheric Deposition Program), emission inventories (National Emissions Inventory), and landscape and physical lake characteristics (LakeCat and others). Stemming from a random forest model, top predictors of Hg concentrations in sediments were catchment characteristics and the GEOS-Chem modeled Hg deposition. Relatively less predictive ability was found for lake trophic status, productivity, or proximity to urban areas. When evaluating Hg isotope heterogeneity across the U.S., we observed isotope clustering within ecoregions to form regional isoscapes. Largely the isotope heterogeneity was driven by differences in precipitation, erosion, and water residency rather than trophic condition or proximity to urban areas. While this work seeks to understand drivers of Hg contamination and Hg sources, it also marks our only known effort to constrain the range of Hg isotope values that may be measured in lake sediment. In that sense, this study serves as a platform for cross evaluation for studies that are comparatively less spatially robust or for ecosystems that lack the wealth of metadata presented here.

4.15.A.T-02 Impacts of Forest Defoliation from Spruce Budworm on Consumer Allochthony and Mercury Bioaccumulation and Biomagnification in Stream Food Webs

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Forested streams are closely linked to their surrounding catchments, and catchment disturbances such as forestry alter stream water quality and food webs, including changes in carbon inputs and quality. Such changes in stream conditions can potentially alter consumers' reliance on autochthonous (in-stream) or allochthonous (terrestrial) sources and mercury bioaccumulation. A recent outbreak of the spruce budworm that feeds on spruce and fir trees has provided the unique opportunity to examine stream food web responses across watersheds experiencing a range of defoliation in the Gaspé Peninsula, Québec, in eastern Canada. This study examined 12 second or third order streams (watersheds 6.33 to 9.85 km²) across a gradient of cumulative forest defoliation (2.5 to 10.4%) to assess whether food web structure and methylmercury (MeHg) cycling were affected. In 2019 and 2020, food sources, several macroinvertebrate taxa representing different functional feeding groups, and fish (were collected and analyzed for stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and MeHg or total Hg (fish only). MixSIAR models and hierarchical partitioning analyses showed that greater watershed defoliation increased allochthony in predatory consumers, and this relationship was strongest for brook trout. Mercury levels in carnivorous invertebrates and brook trout were driven mainly by dissolved organic carbon (DOC), but not consumer allochthony or watershed defoliation. Additionally, rates of trophic magnification (slopes of log

Hg vs $\delta^{15}\text{N}$; 0.27-0.38) varied among streams but were not related to defoliation severity or DOC. These results suggest that forest disturbances from spruce budworm affects carbon use but not mercury cycling in headwater streams.

4.15.A.T-03 The Impact of Impoundment: The Influence of Impoundments on Fish Mercury Concentrations Along an Arid-Land River

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Impoundment is among the most common hydrologic alterations and has profound impacts on aquatic ecosystems, including effects on mercury (Hg) cycling. However, landscape-scale differences in Hg bioaccumulation between reservoirs and other habitats are not well characterized, nor are the processes driving these differences. We examined total Hg (THg) concentrations of Smallmouth Bass (*Micropterus dolomieu*) collected from reservoir, tailrace, and free-flowing segments of the Snake River, USA, a semi-arid river with 22 impoundments along its course that vary in hydrologic and biogeochemical conditions. Preliminary findings indicate that across three size-classes (putative 1-year-old, first reproductive, and the minimum legally harvestable size fish), THg concentrations in reservoirs and tailraces averaged 76% higher than in free-flowing segments. Among reservoirs, THg concentrations were highest in reservoirs with inconsistent stratification patterns – 47% higher than annually stratified and 144% higher than unstratified reservoirs. Fish THg concentrations in tailraces immediately downstream of stratified reservoirs were higher than those below unstratified (38%–130%) or inconsistently stratified (32%–79%) reservoirs. In the two smaller size classes, THg concentrations declined rapidly below stratified reservoirs whereas concentrations in the largest size-class declined more gradually and remained elevated at least 100 km downstream of stratified reservoirs. Stratification regimes influenced the exceedance of fish and human health benchmarks, with 52%–80% of fish from stratifying reservoirs and downstream tailraces exceeding a human consumption benchmark, compared to 6%–17% where stratification didn't occur. Similar differences were observed among habitats and stratification regimes when comparing concentrations in one-year old and early reproductive fish to fish development and reproductive benchmarks, suggesting Hg may pose a risk to some of the imperiled fish species present in the river. These data, together with the abundance of impoundments in the Western United States, suggest that reservoirs may play an important role in determining Hg exposure risks, while also providing a unique and powerful tool for mitigating such risk through careful water management.

4.15.A.T-04 Environmental Drivers of Mercury Bioaccumulation Across Deep and Shallow Water Basins of Lake Champlain

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Lake Champlain is an important freshwater ecosystem bordered by Vermont, New York, and Quebec, and supports a large population of recreational and subsistence anglers. Concentrations of mercury (Hg) in game fish in Lake Champlain frequently exceed the EPA criterion calculated to protect human health. Fish Hg surveys have been conducted on Lake Champlain since the early 1980's, with the most recent in 2022, and have assembled a valuable record of Hg concentrations in five commonly caught fish species. In the past decade, regional atmospheric inputs of Hg have decreased based on data from the Mercury Deposition Network site in Underhill, VT. However, between 2011 and 2017, fish Hg levels increased substantially (50% in yellow perch) across four of the five species monitored, but Hg concentrations in 2022 have returned to levels found in 2011. The increase in Hg concentrations in 2017 was largely driven by fish in the three deep, main basins of the lake, whereas fish Hg in the four shallower basins did not change significantly. Like other large lakes, methylmercury concentrations in the dissolved fraction water column of Lake Champlain water are low ($< 0.025 \text{ ng L}^{-1}$), but bioaccumulation of MeHg from the water to the seston and the base of the aquatic food web is highly efficient. Concentrations of Hg in the food web are therefore highly susceptible to changes in bioaccumulation at the base of the food web. To understand the environmental drivers of methylmercury bioaccumulation at the base of the

food web, we assess the bioaccumulation factors between the water and seston across depth profiles, and methylmercury uptake into zooplankton in the deep and shallow basins of the lake.

4.15.A.T-05 Development of a National-Scale Model to Predict Environmental Mercury Risk Using Dragonfly Larvae as Biosentinels

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Mercury (Hg) contamination is a risk to environmental health, but predicting Hg exposure at the landscape scale is difficult due to high variability in abiotic and biotic factors that influence Hg methylation and bioaccumulation. The Dragonfly Mercury Project (DMP) is a citizen-science program that monitors Hg at a continental scale in U.S. National Parks and other public lands using dragonfly larvae as biosentinels, providing significant spatiotemporal coverage of Hg accumulation data across a range of environmental conditions. Using Hg concentrations collected from more than 20,000 dragonflies (>1,200 site-years) across the U.S., we are developing a landscape-scale model to predict Hg bioaccumulation (i.e., exposure risk) using a Bayesian hierarchical modeling approach. The model variables include landscape features (e.g., landcover, wetland extent, watershed soil carbon), water chemistry (e.g., DOC, pH, sulfate, nitrate), and varying effects of habitat and ecoregion that account for context-dependent responses to Hg exposure. The goals for this modeling effort include predicting Hg risk in unsampled water bodies, forecasting risk by manipulating model inputs, iteratively updating the model with future collection events, and the integration of this model into a dashboard tool. We will present on the ancillary data acquisition and harmonization, constructing and training the model, and preliminary model results.

4.15.A.T-06 Winter Mercury Patterns in Lake Ecosystems and Future Environmental Health Risks

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The health risks and benefits of eating fish are expected to change through the future, as climate change alters fish methylmercury (MeHg) levels. However, our understanding of the influence of climate on MeHg cycling, bioaccumulation, and trophic transfer, is limited. Lake ecosystem processes, including those known to influence MeHg patterns, may be particularly sensitive to climate warming during winter. Thus, one critical knowledge gap is understanding how MeHg patterns and processes in winter compare to other seasons. At least one early study indicated that MeHg concentrations in the water column decline in winter, purportedly due to cold temperatures hindering microbial MeHg production. However, recent winter limnology studies have shown evidence of ecosystem processes during winter that may support MeHg production (benthic oxygen depletion under ice cover), and bioaccumulation (primary productivity, zooplankton growth). We conducted a field study to examine MeHg patterns in two eutrophic bays in Lake Champlain during winter compared to other seasons and relate those patterns to key ecosystem factors known to influence MeHg concentrations in the food chain. We found significant seasonal differences in MeHg concentrations in the water column, but the pattern varied between bays. Specifically, either dissolved or particulate-bound MeHg concentrations were highest in winter compared to other seasons, depending on the bay. Among the ecosystem factors measured, the concentration of total suspended solids emerged as strongly, negatively related to particulate methylmercury concentrations, consistent with algal bloom dilution. Our findings contrast with the conventional wisdom that MeHg supply in the water column is lowest during winter and indicate that dilution processes influence lake MeHg patterns year round. Finally, our results can be used to compare the impact of future climate scenarios on fish bioenergetic rates and MeHg bioaccumulation. These findings help advance our ability to predict future risks of climate-driven changes in MeHg patterns.

4.15.B.T Mercury Bioaccumulation, Exposure, and Effects on Wildlife: Understanding How Ecosystem Pressures Drive Mercury Cycling

4.15.B.T-01 Exploring Nearshore to Offshore Mercury Bioaccumulation Patterns in Lake Huron

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In the Great Lakes, mercury (Hg) continues to threaten wildlife and human health due to its high propensity to bioaccumulate. Lake Huron is one of the least studied lakes for spatial Hg cycling, but statewide and federal assessments of fish tissue Hg have demonstrated relatively constant Hg concentrations in lake trout and increasing concentrations in other game fish within nearshore embayments (e.g., Saginaw Bay). In this study, we investigate how nearshore and offshore ecosystem gradients impact methylmercury (MeHg) dynamics by leveraging Hg concentrations in sediments, surface waters, and seston. We hypothesized that seston would be comparatively more temporally and spatially explicit than longer-lived, migratory fish species and allow us to assess biological hotspots within Lake Huron. Secondly, we were interested in determining if legacy contamination increases Hg bioaccumulation in Saginaw Bay and if differences between nearshore and offshore transects in Lake Huron explain the varying concentrations observed in fish. We found sediment concentrations throughout Lake Huron to be comparable to observations from sediments collected in Lake Superior in 2021, except for elevated Hg concentrations in Saginaw Bay sediments. Hg isotopic data indicates mixed sources contribute to sediments, but industrial Hg predominantly impacts nearshore zones and the southern portion of Lake Huron. Interestingly, the highest sediment hotspot measured in Saginaw Bay was not mirrored in the pelagic food web, indicating a disconnect between sediments and bioaccumulated Hg. There is also a noticeable, but inconsistent, difference between nearshore and offshore Hg bioaccumulation in seston. Spatially, seston MeHg concentrations were lowest in Saginaw Bay ($7.0 \pm 7.2 \text{ ng g}^{-1}$), with higher trends in the main basin ($15.3 \pm 12.7 \text{ ng g}^{-1}$), northern channel ($15.2 \pm 12.5 \text{ ng g}^{-1}$), and Georgian Bay ($13.7 \pm 11.6 \text{ ng g}^{-1}$). The benthic food web had consistent spatial MeHg concentrations with Saginaw Bay ($12.6 \pm 2.5 \text{ ng g}^{-1}$), Georgian Bay ($11.1 \pm 2.4 \text{ ng g}^{-1}$), main lake ($11.3 \pm 1.3 \text{ ng g}^{-1}$), and the northern channel (10.7 ng g^{-1}). Our findings better elucidate bioaccumulation pathways and help identify regions susceptible to elevated Hg bioaccumulation within Lake Huron, though further work is needed to assess driving factors. Ultimately, this information is critical for resource managers in determining long-term remediation strategies for Hg in the food web of Lake Huron and the other Great Lakes.

4.15.B.T-02 Shifts in the Pacific Salmon Community Alter Continental-scale Subsidy Biotransport

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The movement of large amounts of nutrients and organic matter by migrating animals (i.e., biotransport) has ecological benefits for recipient ecosystems that may be offset by co-transported contaminants. Pacific salmon spawning migrations are archetypal of this process, with the annual migration of ~120 million Pacific salmon to spawning grounds in North American watersheds connecting marine with freshwater food webs as a pathway of both nutrient and contaminant transfer. Although Pacific salmon abundance and biomass are higher now than they have been in the last half-century, it is unknown how the underlying shifts in the Pacific salmon community have affected nutrient and contaminant delivery to continental environments. This project combined species-specific subsidy concentrations with 40 years of annual escapement estimates to North America to explore how changes to Pacific salmon community structure affected subsidy biotransport at continental and regional spatial scales. We show that increases in four persistent, bioaccumulative, and toxic contaminants (mercury, PCBs, DDTs, and PBDEs) between 1976-2015 were more than offset by greater increases in

beneficial nitrogen, phosphorus, and fatty acids, and were largely driven by increases in the pink salmon population. Species-specific effects on nutrient versus contaminant biotransport reflected differences in organismal life histories, trophic positions, and proportional contributions to escapement biomass. Our results also show that ratios of biotransported nutrients to contaminants have increased over time. This presentation will explore the ecological implications of the interactions between global environmental change and salmon-mediated transboundary subsidy biotransport in recipient ecosystems.

4.15.B.T-03 Methylmercury Effects on Birds: A Review, Meta-Analysis, and Development of Toxicity Reference Values for Injury Assessment

Josh Ackerman, Sarah Peterson, Mark Herzog and Julie Yee, U.S. Geological Survey

Birds are used as bioindicators of environmental mercury (Hg) contamination and standardized toxicity reference values are needed for ecological risk assessments. We conducted a comprehensive review, summarized data from 168 studies, performed a Bayesian hierarchical meta-analysis, and developed toxicity reference values for effects of mercury on birds using a benchmark dose analysis framework. Lethal and sublethal effects of methylmercury were categorized into nine biologically relevant endpoint categories and three age classes. Effective mercury concentrations where there was a 10% reduction (EC10%) in biochemical function (0.77 and 0.57 $\mu\text{g/g}$ ww adult blood-equivalent Hg concentrations), histology (0.49 and 0.61), and output of juvenile offspring (0.55) were substantially lower than those for behavior (6.23 and 3.11) and survival (2.97 and 5.24) of juveniles and adults, respectively. Within the egg age class, survival was the most sensitive endpoint (EC10%=2.02 $\mu\text{g/g}$ ww adult blood-equivalent Hg concentrations or 1.17 $\mu\text{g/g}$ fww egg-equivalent Hg concentrations). Body morphology was not particularly sensitive to mercury. We developed toxicity reference values using combined survival and reproduction endpoints for juveniles, because juveniles were more sensitive to mercury toxicity than eggs or adults. Adult blood-equivalent mercury concentrations ($\mu\text{g/g}$ ww) and egg-equivalent mercury concentrations ($\mu\text{g/g}$ 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. Maternal dietary mercury ($\mu\text{g/g}$ dw) caused low injury to juveniles at 0.16, moderate injury at 0.6, high injury at 1.1, and severe injury at 2.4. Mercury injected into eggs ($\mu\text{g/g}$ ww) caused low injury at 0.02, moderate injury at 0.11, high injury at 0.25, and severe injury at 0.61. We found few substantial differences in mercury toxicity among avian taxonomic orders. Our results can be used to quantify injury to birds caused by mercury pollution.

4.15.B.T-04 Methyl Mercury Contamination and Diet of Nestling Red-winged Blackbirds

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Red-winged Blackbirds (*Agelaius phoeniceus*) are found throughout North America, often nesting in cattails surrounding ponds and wetlands. Diet studies have revealed that adults feed their nestlings both emergent aquatic insects, like odonates, and terrestrial insects, like lepidopteran larvae. Because emergent aquatic insects can have high concentrations of methyl mercury (MeHg) in their tissues, it has been hypothesized that nestlings whose diets consist of a high proportion of odonates would be contaminated with levels of MeHg that are hazardous to their health. There have been no studies of the relationship between MeHg contamination of nestling Red-winged Blackbirds and their diet. The objective of our study was to measure the concentration of MeHg in the blood of nestling Red-winged Blackbirds and to estimate the proportion of emergent aquatic insects and terrestrial insects in their diet using stable isotopes of nitrogen and carbon and a Bayesian inference stable isotope mixing model. We conducted our study at an atmospherically-contaminated site, the Eagle Mountain Fish Hatchery, Fort Worth, Texas, USA. Previous studies at the Hatchery demonstrated that emergent aquatic insects have high concentrations of MeHg, while terrestrial insects on the pond shorelines

have low concentrations of MeHg. Red-winged Blackbirds nested in cattails in 20 ponds from April 9 to July 30, 2017. We collected 424 blood samples from 243 nestlings from 88 nests (1-2 samples from 1-4 nestlings per nest). Methyl mercury was detected in nestling blood, suggesting that Red-winged Blackbird nestlings were fed emergent aquatic insects. However, concentrations of MeHg in nestling blood were low (mean = 0.020 ug/g ww) and below established risk thresholds. A stable isotope mixing model suggested that terrestrial insects, and not aquatic insects, were dominant in the diets of nestling Red-winged Blackbirds. For a subset of nestlings, we corroborated the results of the stable isotope mixing model using molecular (DNA barcoding) and morphological (undigested hard parts) analysis of nestling feces. Our results suggest that nestling Red-winged Blackbirds may be at limited risk from MeHg contamination when terrestrial organisms dominate their diet.

4.15.B.T-05 Comparing In Vivo Methylmercury Detoxification in Two Species of Duck from Great Salt Lake, Utah

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Great Salt Lake (GSL) biota, particularly waterfowl, have been shown to have elevated mercury (Hg) and selenium (Se) concentrations. While three duck species from GSL have Hg consumption advisories that are designed to be protective of human health, investigations related to wildlife risk assessment and internal detoxification mechanisms within GSL species have been limited. Here, we present Hg concentration and stable isotope measurements of brain, breast muscle, liver, and kidney samples from two duck species with Hg consumption advisories – Northern Shoveler (*Spatula clypeata*) and Cinnamon Teal (*Spatula cyanoptera*). Total Hg (THg) burdens in brain, breast muscle, and kidney were similar in Northern Shoveler and Cinnamon Teal, but Northern Shoveler liver THg burdens were approximately double that of Cinnamon Teal. The percentage of THg existing as methylmercury (MeHg) was lowest in the liver and kidney samples in both species, indicating likely *in vivo* demethylation within these organs as has been documented in other studies of birds. However, %MeHg in Northern Shoveler livers was notably lower than that in Cinnamon Teal livers. Hg stable isotope measurements were made to further examine Hg detoxification pathways in each species by comparing isotopic enrichment factors. The calculated product-reactant isotope enrichment factor for the demethylation of MeHg in Northern Shovelers was consistent with previous studies documenting demethylation of MeHg to a Hg-tetraselenoate complex, suggesting a Se-dependent demethylation reaction. However, this was not observed in Cinnamon Teal tissues, which showed less isotopic variability as a function of %MeHg. As %MeHg values are also higher in Cinnamon Teal tissues, particularly in the liver, our findings suggest notable differences in MeHg detoxification between two species of GSL duck, and highlight the need for species-specific (rather than solely THg) measurements across multiple tissue samples when assessing Hg toxicity risk to wildlife.

4.15.B.T-06 Temporal Trends of Mercury Contamination in Seabirds from Northwest Greenland

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Due to widespread anthropogenic emissions and a global atmospheric cycle, mercury contaminates all aquatic ecosystems, including in the Arctic, at concentrations above pre-industrial baselines. Many seabirds nest in large colonies in the Arctic and are at elevated risk of mercury contamination due to their planktivorous and piscivorous diets and long lifespan. We investigated temporal trends of mercury contamination in five species of seabirds from northwest Greenland. Blood samples were collected regularly since 2010 from adult Atlantic puffins (*Fratercula arctica*), black guillemots (*Cepphus grylle*), black-legged kittiwakes (*Rissa tridactyla*), dovekies (*Alle alle*) and thick-billed murres (*Uria lomvia*). Samples were analyzed for total mercury using direct mercury analysis. All species had average blood mercury concentrations between 212 and 769 ng/g-wet weight, concentrations associated with a lower risk for mercury toxicity. Individual black guillemots and thick-billed murres had blood mercury concentrations >1,000 ng/g wet weight, concentrations associated with moderate risk for mercury toxicity. Preliminary analyses suggest an overall increase in mercury concentrations

in Atlantic puffins, dovekies and thick-billed murrelets over the study period. Comparable temporal studies in the Arctic have shown wide variation in mercury contamination trends. The results of the present study contribute to the understanding of regional mercury trends in the Arctic and efforts to assess the impact of the Minamata Convention.

4.15.P-Th Mercury Bioaccumulation, Exposure, and Effects on Wildlife: Understanding How Ecosystem Pressures Drive Mercury Cycling

4.15.P-Th-101 Temporal Variation in Mercury Concentrations in Emergent Aquatic and Terrestrial Invertebrates of the Great Salt Lake Food Web

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The Great Salt Lake (GSL) in Utah is an interesting system in which to study mercury (Hg) cycling owing to its unique characteristics as a hypersaline terminal lake. The anoxic sediments in lake provide suitable biogeochemical conditions for the conversion of inorganic Hg into the more toxic and bioavailable form of methylmercury (MeHg). The GSL has a relatively simple food web dominated by aquatic arthropods—brine shrimp (*Artemia franciscana*) and brine flies (*Ephydra* spp), both of which have been documented to accumulate Hg. However, it is unclear how much Hg emergent brine flies transfer from the GSL into the adjacent terrestrial food web. This is of significant ecological importance as brine flies make up over 75% of the diet of the millions of breeding and over-wintering waterfowl and shorebirds in this region. To understand temporal variation in Hg transferred from the GSL into the adjacent terrestrial food web, we monitored Hg in brine flies and an abundant terrestrial fly predator, the western spotted orb weaver spider (*Neoscona oaxacensis*). Brine flies and orb weavers were collected using sweep nets around Antelope Island State Park, located within the GSL, each summer from 2019-2023. Brine flies collected in 2019 had an average Hg concentration of 0.61 ± 0.18 ppm (dry weight; n=70 composite samples). There was a significant effect of month, in which flies collected in August had higher Hg than those collected in June or July ($F_{2,68} = 71.15$, $p < 0.001$). Analysis of flies from 2021-2023 is ongoing. Hg concentrations in spiders collected from 2019-2021 averaged 1.02 ± 0.41 ppm (dry weight; n=566). Hg in spiders differed significantly among years with concentrations being the highest in 2019 ($F_{2,565} = 35.96$, $p < 0.001$). Hg analysis of spiders from 2022 and 2023 is ongoing. Overall, preliminary analyses suggest that brine flies do transfer a substantial amount of Hg from the lake into the terrestrial food web and that predators consuming them, such as orb weaver spiders, show biomagnification of Hg as a result. The water level of the GSL has dropped precipitously over the past few years from long term drought; therefore, we plan to compare the annual lake level to changes in Hg in flies and spiders. As an Important Bird Area for migrating waterfowl and shorebirds, as well as the breeding grounds for many songbirds, understanding how Hg cycles in the invertebrate base of the GSL food web is of considerable interest.

4.15.P-Th-102 Mercury and Selenium Concentrations in Lanugo of California Sea Lion (*Zalophus californianus*) Pups of the Southern Gulf of California

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Mercury (Hg) exposure can result in adverse health effects and may influence population dynamics. Selenium (Se)-Hg interactions increase the likelihood of adverse effects. Piscivorous species, such as California sea lions (*Zalophus californianus*), experience Hg bioaccumulation and biomagnification. Speculation exists that some marine mammals display a greater tolerance to the adverse effects of Hg in comparison to non-piscivorous terrestrial mammals, likely in relation to the relatively high total Se concentrations ([TSe]) in prey. At Los Islotes (Gulf of California, Mexico), the total Hg concentrations ([THg]) and [TSe] in the tissues of Z.

californianus have not been studied for the past two decades; the potential influence of sex and body condition index (BCI) on these elements, and comparisons of [THg] with toxicological thresholds of concern (TTC) are unexplored. Thus, using lanugo (hair grown *in utero*, represents dam diet and fetus exposure) samples obtained in 2021, we determined the [THg], [TSe], and TSe:THg molar ratio. The [THg] ranged from 1.25-23.24 ppm, [TSe] 4.41-10.50 ppm, TSe:THg molar ratio 0.65-21.39, and BCI 0.61-1.28. Female pups (average = 7.65 ± 1.33 ppm) had significantly higher [TSe] than males (6.28 ± 1.23 ppm; $p = 0.004$). No significant differences ($p > 0.05$) were observed between sexes for [THg] (median, females = 5.85 ppm; males = 4.37 ppm), TSe:THg molar ratio (median, females = 3.11; males = 3.35), or BCI (median, females = 0.98; males = 1.01). No significant correlations ($p > 0.05$) were observed between BCI and [THg], [TSe], or TSe:THg molar ratio. Using pinniped TTC, the majority of pups (80%) were classified as low risk ([THg] < 10 ppm); 72% of females and 88% of males fell into this group. These results indicate that both Hg and Se accumulate in the lanugo of *Z. californianus*. Studying baseline conditions of *Z. californianus* experiencing population increase may allow better interpretation of the influence of toxicants on declining populations in the Gulf of California, and may be applicable to other species, including Steller sea lions and harbor seals, in other geographical regions.

4.15.P-Th-103 Effects of Nutrients on Mercury Bioaccumulation at the Base of the Coastal Food Web

Anika Agrawal, Rob Mason and Jessica Brandt, University of Connecticut

Coastal systems are at risk of rapid environmental change and are sensitive to stressors such as metal pollution. Specifically, mercury (Hg) and methylmercury (MeHg) concentrations have increased in coastal areas and seafood resources. This has led to concerns about MeHg bioaccumulation as seafood is the primary route of MeHg exposure to human consumers in the United States, and exposure to elevated MeHg concentrations has been associated with a suite of adverse health impacts. Our work is focused in Long Island Sound where Hg concentrations are increasing in bivalves. Nutrient availability can mediate Hg bioaccumulation and studies have shown that selenium (Se) may act as a nutrient mediator of Hg biogeochemistry as well. However, Se's role as a nutrient at the base of the food web has been largely unstudied, despite basal food web interactions greatly influencing bioaccumulation and biomagnification in coastal food webs. To address these gaps in understanding, we are investigating the influence of macro- and micronutrients on the spatial and temporal dynamics of Hg bioaccumulation to phytoplankton and trophic transfer to oysters. This project involves a two-year field study with monthly field collections at four sites distributed along the longitudinal gradient in the Connecticut portion of the LIS. In 2022, dissolved Hg concentrations were 0.0300 ± 0.078 ng/L with no significant differences among sites or sampling events. Adult oyster Hg concentrations were 0.0254 ± 0.0051 $\mu\text{g Hg g}^{-1}$ wet weight and were well below the U.S. EPA/FDA's recommendation level for Best Choice for seafood ($0.15 \mu\text{g Hg g}^{-1}$). The highest concentrations in oysters were seen at our eastern most site. Mean \log_{10} Hg bioconcentration factors, representing concentration enrichment by the food web, were 4.92 ± 0.05 , which are comparable to those found in the literature, and varied by both site and sampling event. Mean dissolved Se concentrations were 0.045 ± 0.016 $\mu\text{g/L}$, with higher concentrations seen at sites with greater freshwater influence from rivers. The influence of nutrient and other abiotic variables on spatiotemporal trends in Se, Hg concentrations, and enrichment factors will be presented and the trends assessed. This presentation will share results to date from the 2022 and 2023 field seasons. We anticipate that the results of this project will inform how nutrient concentrations affect Hg cycling, leading to a better understanding of nutrient/Hg dynamics in coastal food webs.

4.15.P-Th-104 Spatial and Ontogenetic Variation in Mercury, Methylmercury and Selenium Accumulation Dynamics in Bull Sharks (*Carcharhinus leucas*)

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(3)Sam Houston State University, (4)Mote Marine Laboratory, (5)US Department of Commerce, (6)Florida State University, (7)California State University, (8)University of Miami

Mercury is a pervasive global pollutant that can degrade organism health and reduce populations. Large marine predators frequently exhibit high levels of mercury, posing potential health risks to them and human consumers, but mercury concentrations vary spatially, and its negative health effects might be mitigated by selenium. We investigated the factors influencing mercury, methylmercury, and selenium accumulation in bull sharks ($n = 230$, *Carcharhinus leucas*) sampled from estuarine and marine waters of the Gulf of Mexico and western North Atlantic. Across low-salinity nurseries, models including shark body length, nursery location, and trophic interactions inferred from stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) predicted over 40% of the total mercury and 30% of the methylmercury variation among juveniles sampled in nurseries. Juveniles in nurseries of southwest Florida had significantly higher total mercury and methylmercury concentrations than those of Texas. Selenium concentrations were influenced by nursery area, sex and an interaction of nursery and $\delta^{13}\text{C}$. When combining data from nursery-bound sharks ($N=148$) with larger juveniles and adults collected in marine habitats ($N=82$) we found that concentrations of all three trace elements significantly increased as bull sharks grew, and selenium accumulated at a significantly faster rate. As a result, selenium concentrations in 91% of all individuals exceeded those of mercury, potentially mitigating harmful mercury effects. Within some nurseries, however, selenium deficits occurred in up to 19% ($n = 14$ of 74) of juvenile sharks. Risk of mercury toxicity in bull sharks varies based on where the sharks are born and reside for the first few years of life. Despite having higher mercury concentrations overall, mercury toxicity risk is lower in large bull sharks because of the mitigating effects of selenium that accumulates as they increase in size.

4.15.P-Th-105 The Bugs are Alright: Wildfires Increase Particulate Mercury Transport but Not Bioaccumulation in Northwestern U.S. Headwater Streams

Austin Baldwin, Collin Eagles-Smith, James J Willacker, Branden Johnson and Sarah E Janssen, U.S. Geological Survey

The increasing frequency and severity of wildfires in the western US are among the most visible implications of climate change. Soils, which represent one of the largest reservoirs of mercury (Hg) globally, are often destabilized and mobilized after wildfires, potentially increasing Hg concentrations and loads to streams. However, wildfires can also reduce soil Hg concentrations through re-emission to the atmosphere. With these competing mechanisms, the effects of wildfire on Hg cycling in streams and bioaccumulation into aquatic organisms is poorly understood. To address this, we sampled soils, water, sediment, and benthic invertebrates in 36 burned and 21 reference (unburned) headwater watersheds across three states in the northwestern U.S. The changes in Hg resulting from wildfire were examined in different media using burn severity indices and a paired watershed approach. In soils, increased burn severity corresponded with depleted total Hg (THg) concentration in the organic layer, suggesting loss through volatilization. Burn severity had no effect on THg in the mineral layer. In water, concentrations of particulate THg and methylmercury (MeHg) concentrations increased with increasing burn severity, with means ~2-fold greater in burned compared with reference watersheds. In contrast, concentrations of filter-passing THg and MeHg in water, and MeHg in benthic invertebrates, were similar in burned and reference watersheds. Increased erosion in burned watersheds is likely the primary driver of changes in particulate concentrations, as evidenced by a comparable increase in suspended particulate material concentrations. Therefore, although wildfires resulted in increased concentrations of particulate THg and MeHg, we saw no evidence for corresponding increases in dissolved Hg transport or bioaccumulation in headwater streams. The increased supply of particulate THg and MeHg may, however, result in increased bioaccumulation in downstream environments. These results suggest that minimizing erosion and capturing particles in wildfire-affected areas will be important to minimize downstream effects.

4.15.P-Th-107 The Influence of Evergreen Forest Cover on Fish Mercury Concentrations in Western U.S. National Parks

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The global prevalence of mercury (Hg) contamination and its complex biogeochemical cycling in the environment has resulted in elevated Hg concentrations in biota from some of the most remote and pristine environments. However, uncertainty exists regarding the relative importance of Hg deposition or landscape factors that control Hg cycling and bioaccumulation. To address this, we examined total mercury (THg) concentrations in 1,344 fish across 60 subalpine lakes from 12 national parks that represent three distinct high-elevation regions across the western U.S.: Cascades and Olympic Peninsula, Sierra Nevada and Great Basin, and Rocky Mountains. This study aimed to: (1) assess the magnitude of mercury contamination in a collection of remote, small catchment lakes; (2) quantify the variability of fish THg concentrations among and within parks; and (3) test the relative importance of landscape versus input factors on lake-specific fish THg concentrations. Preliminary findings indicate that the spatial variability in fish THg concentrations was 18-fold higher than in wet Hg deposition, suggesting that landscape factors may be particularly important determinants of mercury risk in these environments. When accounting for species and size, least-squares means (\pm standard error) fish THg concentrations (ng/g ww) were significantly lower in the Rockies (46.2 ± 5.0) and Sierra (56.5 ± 5.8) than in the Cascades (67.8 ± 6.1). Additionally, fish THg concentrations increased with increasing conifer density ($P < 0.0001$, $R^2 = 0.432$) but were not influenced by wet Hg deposition across the catchment. In fact, fish THg concentrations differed by more than 400 percent across the forest density gradient, highlighting the importance of land cover in controlling Hg bioaccumulation in fish. These findings suggest that forest composition is an important aspect of Hg delivery to lake food webs, and although the mechanisms are unclear, could be tied to some combination of forest influences on catchment organic carbon, or increased surface area for dry Hg deposition. Results highlight the importance of obtaining accurate measurements of dry Hg deposition and throughfall deposition in coniferous forests, which unlike wet Hg deposition, are not currently assessed on a landscape scale.

4.15.P-Th-108 An Assessment of How Climate Change May Alter Mercury Loads and Sources in Tributaries and Sediments of Lake Superior

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Lake Superior has a vast and largely undeveloped watershed in comparison to the other Great Lakes, which makes it challenging to study how climate change will affect sources and tributary fluxes of mercury (Hg). Changes in snowpack, drought, rainfall will shift the timing and magnitude of Hg delivery to the lake. Ongoing work from the Great Lakes Sediment Surveillance Program (GLSSP) and Cooperative Science and Monitoring Initiative (CSMI) for Lake Superior enables us to assess how climate change will alter Hg source portfolios within the region and connect watershed and atmospheric Hg sources to nearshore and offshore sediments. Lake sediments record Hg loads through space and time whereas tributary measurements provide a contemporary look at Hg loading. In addition, Hg stable isotope ratios can be leveraged as a fingerprinting tool to identify the primary Hg sources (e.g., atmospheric, watershed, and industrial) in sediments and tributary waters. To examine the relative contributions of each of these sources to Lake Superior, surface sediments were collected at 31 locations (26 open water and 5 nearshore sites) and cores at three additional locations. At open water sites, Hg isotopes indicated precipitation was the primary Hg source to sediments, but within nearshore sites Hg originated from watershed runoff. Industrial Hg was a significant source within nearshore sites close to the St. Louis River and Thunder Bay, areas of known historical Hg contamination. Given how important watershed fluxes were to the nearshore zones of Lake Superior, we also leveraged tributary measurements to assess regional differences in total Hg (THg) and methylmercury (MeHg) concentrations and loads. We conducted an

expansive binational assessment within 42 watersheds that represent a diverse range of landcover types including forested, wetland, agricultural, and urban. THg concentrations in tributaries from the remote, heavily forested Pukaskwa National Park were higher than tributaries on the south shore of Lake Superior and Thunder Bay region. THg loads were highest in the spring, driven by the combination of elevated concentrations and discharge during spring snowmelt and rain. MeHg concentrations lacked consistent seasonal patterns, reflecting the significant ecological and hydrological variability among these watersheds. Pairing these datasets allows us to assess how Hg sources to Lake Superior may shift over time with site remediation and changing hydrologic regimes.

4.15.P-Th-109 Switching to Marine Prey Leads to Unprecedented Mercury Concentrations in a Population of Coastal Alaska Wolves

Benjamin D Barst¹ and Gretchen H Roffler², (1)University of Alaska, Fairbanks, (2)Alaska Department of Fish and Game

Wolves are renowned for their dietary plasticity and consume a broad diversity of prey including marine species, especially in coastal areas. Coastal wolves in Southeast Alaska (*Canis lupus ligoni*) consume many diet items spanning terrestrial and aquatic food webs including salmon seasonally, and other fish and marine mammals opportunistically. In some populations, especially on small islands lacking herbivores, wolf diets can consist almost exclusively of marine resources. Sea otters (*Enhydra lutris*) were once nearly extirpated throughout their North Pacific range but have rapidly recovered in some areas, including in and around Glacier Bay National Park, due to reintroduction efforts and legal protection. This recovery has led to recent spatial overlap of wolf and sea otter populations in Southeast Alaska. Intensive monitoring efforts in Southeast Alaska from 2015–2020 revealed that sea otters were the primary prey of wolves on Pleasant Island and were less significant in the diets of wolves from the adjacent Gustavus forelands on the mainland. Given their reliance on marine resources and methylmercury's propensity to biomagnify in aquatic food webs, we investigated whether marine resource use led to elevated mercury concentrations in wolf tissues. Additionally, we analyzed the carbon and nitrogen stable isotope ratios of amino acids from wolves and sea otters to fingerprint their basal carbon sources and estimate trophic positions. Results indicate that most of the essential amino acids from Pleasant Island wolves were sourced from aquatic plants, demonstrating a strong connection to an aquatic food web. Total mercury concentrations in the livers of Pleasant Island wolves ranged from 4.9 up to a maximum of 64.3 parts per million wet weight, which far exceeds liver total mercury concentrations previously reported for wolves, including those with access to marine resources. Our findings suggest that certain wolves in Southeast Alaska are exposed to high levels of mercury through their marine diets, which puts them at severe risk for toxic effects. Results of our work benefits the efforts of the Arctic Monitoring and Assessment Programme to identify wildlife populations with elevated mercury exposures.

4.15.P-Th-111 Re-examining Mercury Exposure and Risk Assessment Through the Lens of Invasive Species

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Biogeochemical and ecological factors are primary drivers of bioaccumulation of mercury (Hg) in the environment, but co-occurring stressors can greatly alter Hg cycling and risk. The impact of invasive species on both Hg cycling and energetic regimes is an understudied issue which can have profound effects on the Hg burdens in native species and potentially impact consumption advisories. We examine the effects aquatic invasive species (AIS) have on fish Hg burdens and the associated changes to human exposure risks. We will cover how dreissenid mussels have altered Hg bioaccumulation within game fish from the Upper Midwest and Great Lakes regions of the United States. Assessments of dreissenid mussel invaded lakes in Minnesota have shown a demonstrable increase in Hg concentrations in adult game fish, on average 72% higher, when

compared to individuals collected in uninvaded lakes. AIS driven changes to Hg cycling are also observed in Lake Michigan, where temporal analysis of the Great Lakes Fish Monitoring and Surveillance Program archive highlighted key food web and Hg source shifts associated with dreissenid invasions. These examples demonstrate how Hg exposure can be altered by bottom-up food web changes. In the Florida Everglades, many different invasive species impact the local food web, but Burmese pythons are one of the highest profile invaders. While the presence of pythons has drastically altered the ecosystem, the popularity of removal programs has resulted in python hunting and increased consumption by local populations. Pythons within this region sit at a unique trophic niche and can reach levels exceeding 10 mg kg^{-1} , higher than top trophic level marine fish, resulting in a new Hg exposure pathway, absent of consumption guidelines. This example demonstrates how Hg exposure can be altered by top-down food web changes. These case studies highlight how the presence of invasive species can alter our understanding of bioaccumulation as well as result in unexpected Hg exposures based on how we manage invasive populations.

4.15.P-Th-112 Demethylation of Methylmercury by Selenocysteine in Aquatic Environments

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Mercury (Hg) contamination in aquatic environments poses a significant risk to both ecosystems and human health, mainly through the consumption of fish containing neurotoxic methylmercury (MeHg) that biomagnifies in aquatic food webs. MeHg risk to wildlife and humans is influenced by the conversion of inorganic Hg(II) to MeHg by microorganisms and MeHg uptake into the food web (bioaccumulation and biomagnification). The interaction between Hg and selenium (Se) in the environment, broadly termed 'Hg-Se antagonism', has been long-observed to influence these processes. Further, the biomineralization of MeHg to mercury-selenide (HgSe) has been observed in high trophic level organisms. Despite these observations, the mechanisms underlying Hg-Se antagonism in natural environments are not well understood and require further investigation. In aquatic environments with elevated Se, we hypothesize that Hg-Se antagonism is primarily driven by the demethylation of MeHg by selenocysteine, which may prevent the MeHg bioaccumulation in plankton and subsequent biomagnification in fish. In this study, we aimed to investigate the mechanism of Hg-Se interactions in lake water through a combination of field and laboratory experiments. Water samples were collected from four lakes (Deer, Goose, Goose Lake South Bay, and Teal lakes) with differing inputs of Hg and Se and analyzed for dissolved organic carbon (DOC), primary anions (SO_4^{2-} , NO_3^- , Cl^-), selenium species (selenate, selenite, selenomethionine, and selenocysteine), inorganic sulfide ($\text{H}_2\text{S}/\text{HS}^-$), and total Hg and MeHg. To further examine mechanistic controls, laboratory experiments quantified the kinetics of selenocysteine mediated demethylation of MeHg under environmentally relevant concentrations of MeHg and selenocysteine (1:100 molar ratio). This presentation will compare results from laboratory experiments and water quality data collected from the four lakes, to elucidate how Hg-Se interactions affect the uptake of MeHg into the food web. We anticipate the study results will provide new mechanistic insights that help unravel the complex mechanisms underlying Hg-Se antagonism in aquatic environments.

4.15.P-Th-113 Field-Based Methyl Mercury Bioaccumulation Model from Sediments Through the Food Web in a River/Estuary System in the Northeastern United States

Jeffrey M. Morris, Fabrizio Bonatesta, Andrew McFadden, Matt Rissing, Claire Lay and Jamie Holmes, Abt Associates

Our study focused on a river/estuary system in the Northeastern United States that has been impacted by releases of mercury and contaminated for several decades. We developed a methyl mercury (MeHg) uptake model using field-collected sediment and tissue samples. Our uptake model relates sediment MeHg concentrations to the aquatic food web in local habitats to derive trophic magnification slope (TMS) values across a range of sediment MeHg concentrations. The TMS values include fish and invertebrate tissue concentrations across a range of trophic levels. This model allows us to predict uptake into different trophic

level organisms across a broad spatial extent based on interpolated sediment MeHg concentrations. Our presentation will focus on model development, trophic level assignments across varying habitats using stable nitrogen and carbon isotope data, and model outputs. We will also evaluate model applicability to the riparian food web based on predicted concentrations of MeHg in spiders and other insectivores.

4.15.P-Th-114 Seasonal Variation in Mercury Concentrations in Tetragnathid Spiders

Jessica L. Landaverde¹, Ryan R Otter¹, Connor Olson² and Mario Montesdeoca², (1) Middle Tennessee State University, (2) Syracuse University

Riparian spiders are used in ecotoxicology as sentinels of bioavailable contaminants that may be transferred from aquatic to terrestrial habitats via the insect mediated contaminant pathway. Spiders in the family *Tetragnathidae* are particularly of interest because of the high proportion of emergent aquatic insects in their diet. These spiders have become widely used in ecotoxicological studies in recent years, however, the changes in retained contaminant concentration that occur within a sampling year has yet to be investigated. In this study, our objective was to determine if variation in total and methyl mercury concentrations occurred in tetragnathid spiders throughout a sampling year within a river with a fish consumption advisory for mercury. Spiders were sampled within the same 100m reach of the East Fork Stones River in Cannon County, TN twice a month between April and November 2021. Results showed that spider mercury concentrations varied throughout the year, with an overall increasing trend over time. This work adds to the growing literature on the use of tetragnathid spiders as sentinels of bioavailable contaminants, such as mercury. This research implies that seasonality may be a significant factor in the transfer of mercury from aquatic to terrestrial habitats via tetragnathid spiders.

4.15.P-Th-115 Effects of Lifetime Hypoxia Exposure On Fish Mercury Uptake And Food Web Structure

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Climate warming is producing large impacts on aquatic ecosystems, including oxygen loss. Hypoxia (operationally defined as $< 2 \text{ mg O}_2/\text{L}$) can alter habitat for fish and thereby affect trophic interactions. Additionally, anoxia favors microbial methylation of mercury (Hg) to methyl Hg. Me-Hg can persist in hypoxia, resulting in potential for increased Hg transfer up the food web. Using mass spectrometry, we analyzed fish ear stones (otoliths), eye lenses, and muscle tissues to assess the long-term effects of hypoxia exposure on food web relationships by tracking changes in fish ecology and Hg exposure through time in Lake Erie. Lake Erie is one of the Great Lakes that experiences extreme seasonal hypoxia, primarily in its Central Basin. Lake Erie Walleye (*Sander vitreus*), Yellow Perch (*Perca flavescens*), and Round Goby (*Neogobius melanostomus*) were collected to assess a gradient from most heavily dependent on localized, benthic habitat (Round Goby) to least (Walleye). Manganese to magnesium ratios (Mn:Mg), were used as a hypoxia proxy in otoliths of all otoliths, and this ratio was significantly higher in the hypoxic Central Basin compared to the more normoxic Western Basin only in our most hypoxia tolerant species (i.e., Round Goby). Sulfur stable isotopic ratios ($\delta^{34}\text{S}$) also served as an indicator of hypoxia due to a large negative fractionation from sulfate reduction. We measured $\delta^{34}\text{S}$ in eye lenses and muscle tissues and found significantly lower $\delta^{34}\text{S}$ in Central Basin Round Goby; this result was in agreement with our otolith hypoxia proxy. Also, we found that fish with higher hypoxia exposure history showed significantly higher eye lens [Hg]; this trend was not detectable by muscle tissue [Hg]. However, species that had less or no exposure to hypoxia showed distinct outcomes when subjected to the polluted conditions of the Western Basin, particularly in the case of Yellow Perch. Similarly, factors such as age and migration patterns play a significant role in Walleye. As a result, these factors contribute to [Hg] in the eye lens of both species. Overall, our results showed that eye lenses can complement the chemical data from otoliths and can be used to study fish Hg and hypoxia exposure, in addition to trophic history. This novel study demonstrates the strength of combining complementary analyses of different chronometric body parts to explore the influence of hypoxia exposure on food web relationships and Hg accumulation patterns.

4.15.P-Th-117 Mercury Analysis in the Tissues of Stranded Bottlenose Dolphin (*Tursiops truncatus*) in Northeast Florida, 2013-2021

Gretchen K. Bielmyer-Fraser, Julia Courville and Ashlen Ward, Jacksonville University

Bottlenose dolphins are long-lived marine mammals, upper-level predators in the marine trophic web, and inhabit near-shore environments, thereby increasing their susceptibility to land-based sources of pollution like metals. Mercury is a ubiquitous and persistent metal that can bioaccumulate and biomagnify up the food chain. Dolphins are known to accumulate mercury; however, few studies have examined the effects of metal exposure on these species. Research has shown that mercury exposure can weaken the immune system of bottlenose dolphins. The objectives of this study were to assess mercury concentrations in tissues of stranded bottlenose dolphin and to compare tissue mercury levels in dolphins that have stranded during the 2013-2015 morbillivirus Unusual Mortality Event (UME; immunosuppressed individuals) with those that stranded at a normal rate (2016-2021). This study will provide new data about the influence of multiple stressors on these sentinel species and has implications for management strategies and necropsy procedures.

4.15.P-Th-118 Relationship Between MeHg Concentrations in Two Spider Taxa, Emerging Aquatic Insect MeHg Concentrations, and MeHg Flux: Implications for Using Spiders as Sentinels

Maddy Pratt Hannappel¹, Lexi Freeman², Matthew M Chumchal², Jim Kennedy¹, Benjamin D Barst³ and Ray Drenner², (1)University of North Texas, (2)Texas Christian University, (3)University of Alaska, Fairbanks

Widespread atmospheric deposition of Mercury (Hg) has contaminated all waterbodies on Earth. In aquatic systems, bacteria convert inorganic Hg to toxic and bioavailable Methylmercury (MeHg). Emergent aquatic insects (e.g., mosquitos and midges), that accumulate MeHg while in the aquatic system, can transport MeHg to terrestrial consumers (e.g., spiders and birds). Because spiders can accumulate MeHg from emergent aquatic insect prey, spiders have been proposed as sentinels for monitoring MeHg. Sentinels are organisms that accumulate contaminants in their tissues without significant adverse effects, and whose tissue concentrations reflect the amount of a contaminant biologically available. An important part of developing spiders as sentinels is understanding how tissue concentrations relate to levels of MeHg in the aquatic systems. In this study, we compared spider MeHg concentrations to MeHg concentrations of small emergent aquatic insects at the base of the aquatic food web (a proxy for the level of bioavailable MeHg), and MeHg flux from small emergent aquatic insects (a proxy for the amount of MeHg transported to the terrestrial system). We sampled 2 taxa of spiders (Tetragnathidae and Pardosa) from the shorelines of 11 human-made ponds at the LBJ National Grasslands in Texas. Small emergent aquatic insects were collected from each pond using floating emergence traps then analyzed for MeHg to determine their MeHg concentrations and quantify MeHg flux from the insects (calculated as ng MeHg/m²/day). To account for an effect of spider size on spider MeHg concentrations, all spiders were measured (tibia + patella length of leg I) and size was included as a covariate in statistical models. We found that spider size had a significant effect on spider MeHg concentrations in both taxa. Methylmercury concentrations in Tetragnathidae were positively related to small emergent insect MeHg flux, where Pardosa MeHg concentrations were positively related to MeHg concentrations in small emergent insects. Our results suggest that spiders in the families Tetragnathidae and Pardosa may act as sentinels for different measures of aquatic MeHg contamination.

4.16.A.T Microplastics on the Planet: Input Sources, Transport Pathways and Eco-Environmental Impacts

4.16.A.T-01 Introduction

Charles S. Wong, Southern California Coastal Water Research Project

4.16.A.T-02 Effects of Different Aging Treatments on the Adsorption of Lindane and Alachlor by Polystyrene Particles

Rui Cai, Mark Krzmarzick and Jorge Gonzalez Estrella, Oklahoma State University

The aging processes of microplastics can significantly influence their adsorption behavior and potential risks as environmental contaminants. This study investigates the adsorption behaviors of lindane and alachlor onto PS microplastics (MPs) under different aging processes: UV light exposure only, UV light exposure combined with peroxide oxidation, and non-aging. Kinetics experiments reveal that both aging processes reduce the adsorption capabilities of lindane and alachlor, indicating that hydrophobic interaction is the main mechanism in this adsorption experiment. The Freundlich model provides a good fit for the adsorption isotherm data, suggesting the presence of heterogeneous adsorption sites on the surface of the PS MPs. These findings emphasize the importance of considering the aging effects of microplastics when assessing their fate and potential risks as adsorbents of organic contaminants in the environment.

4.16.A.T-03 Sea Anemones Extract Tin from Polyvinyl Chloride Preproduction Pellet Consumption

Zoie Diana^{1,2}, Megan Swanson², Nelson Rivera², Heileen Hsu-Kim² and Daniel Rittschof², (1)University of Toronto, (2)Duke University

Marine plastic pollution is projected to grow in the coming years. We investigate polyvinyl chloride pellet consumption in sea anemones, which are model animals for corals. Results indicate that anemones consume up to 83% of plastic pellets readily and will consume all of the pellets offered with the addition of 10 µl of aqueous shrimp extract. Anemones retained consumed plastic pellets for 222±53 minutes. Feeding retention time decreased with repeated feedings of the same pellet to new anemones. In one of the three experiments where tin was measured, anemones appeared to extract tin from plastic pellets after feeding on one pellet daily for 10 days. Anemones did not extract measurable amounts of lead from pellets. To reduce harm to marine animals, stakeholders should aim to reduce global plastic production and pollution, reduce harmful additives in necessary plastics, and elucidate the mechanisms underlying plastic consumption by marine animals.

4.16.A.T-04 Benthic Fallout: Quantifying Microplastic Accumulation in Rockfish Across Coastal California

Jordan Colby, Francine Anne De Castro and Christina Pasparakis, University of California, Davis

Of the 108 species of rockfish, 76 reside in the waters off of coastal California. These top benthic predators are long lived and sedentary, which makes them ideal to use as bioindicators for localized microplastic pollution. These fish act as a cultural and commercial cornerstone for communities across the pacific coast for generations, and it is our goal to evaluate the body burdens that they are facing due to anthropogenic pollutants. Since the 1950's, the use of microplastics for commercial and personal use has increased from 1.7 million tonnes to 299 million tonnes as of 2014. With the rate of plastic use showing no signs of considerable decrease, understanding the sinks of plastic pollution is paramount in future management actions to preserve benthic ecosystems. In order to track the current state of California coastal ecosystems, we collaborated with the California Collaborative Fisheries Research Program (CCFRP) and the National Oceanographic and Atmospheric Administration (NOAA) to measure the biomagnification of plastics in rockfish within 12 sampling locations across the state. We investigate the composition, dispersal, and accompanying leachates present in benthic environments from across the state by analyzing multiple fish tissues from all six sampling locations. In collaborating with multiple governmental and academic institutions we can link the age of the fish

utilizing otolith data to measure the species specific rate of bioaccumulation across a large range of latitudes. Our endpoints utilize a high efficiency extraction protocol, GC mass spectroscopy and FTIR spectroscopy to capture the full range of present pollutants both quantitatively and qualitatively.

4.16.A.T-05 Microplastics and Plastic-Associated Contaminants in the Canadian Arctic Snow

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Plastic pollution produces adverse impacts caused by plastic pieces, plastic additives, and chemicals adsorbed on plastics in the environment. Atmospheric deposition (e.g., snow) is a pathway for airborne microplastics (1 µm - 5 mm) and plastic-associated contaminants to enter the soil and aquatic environments. However, little is known about how microplastics and plastic-associated contaminants deposit into the Canadian Arctic via atmospheric deposition. To address this knowledge gap, this study investigated the occurrence and distribution of microplastics and three groups of plastic-associated contaminants: organophosphate esters (OPEs), per- and poly-fluoroalkyl substances (PFASs), and benzotriazole ultraviolet stabilizers (BZT-UVs) in Canadian Arctic snow. Samples were collected between February 2020 and May 2021 from Little Fox Lake (Yukon; n=6), Yellowknife (Northwest Territories; n=5), Cornwallis Island (Nunavut; n=9), and Alert (Nunavut; n=15). For microplastic analysis, we employed filtration (>20 µm), microscopic visualization, and Fourier transform infrared imaging techniques. Plastic-associated contaminants were extracted by solid phase extraction and analyzed by ultra-high performance liquid chromatography-tandem mass spectrometry and gas chromatography coupled to triple quadrupole mass spectrometry. The results showed a microplastic average abundance of 26 ± 25 n/L (counts per L, mean \pm S.E.) in melted snow. Snow samples from Cornwallis Island had a higher abundance (47 ± 10 n/L), followed by Little Fox Lake (22 ± 8 n/L), Alert (12 ± 4 n/L), and Yellowknife (12 ± 3 n/L). Prevalent shapes were fibers (53%) and fragments (38%), with a length of 692 ± 56 µm (mean \pm S.E.). Twenty plastic polymers were found, and polypropylene (29%) and polyester (28%) were the dominant plastics. Yellowknife and Cornwallis Island snow samples were analyzed for OPEs and PFASs. Seventeen OPEs and 12 PFASs were frequently detected (>75%) in the snow samples (mean concentration: Σ_{26} OPEs = 97 ± 5 ng/L; Σ_{23} PFASs = 3.5 ± 0.3 ng/L). These values are higher than those reported in other Arctic studies. Polymer identification of some samples and the analysis of BZT-UVs are currently underway. Once all data are obtained, the relationships among microplastics, OPEs, PFASs, and BZT-UVs will be examined. The results of this study will increase baseline knowledge on understanding the transport of microplastics and plastic-associated contaminants into the Canadian Arctic.

4.16.A.T-06 Assessing Environmental Fate of Floodwater-Associated Tire Road Wear Particles along Roadway-Salt Marsh Interfaces

*Dana Norton*¹, *John E. Weinstein*¹, *Jonathan Barr*¹ and *Bonnie M. Ertel*², (1)The Citadel, (2)University of South Carolina

In Charleston, South Carolina, nuisance flooding occurs at higher frequencies than those recorded in the 1950's, with nuisance flooding occurring during extreme high tides and heavy rain events. In addition to public health concerns related to nuisance flooding, increased frequencies can put unexpected strain on city infrastructure and increase public exposure to various microplastic particles. Studies in Charleston Harbor have shown that >90% of total microplastics in sediments in certain locations are TRWP and that stormwater and floodwater, which have high concentrations of these particles, may be important pathways by which they are transported to estuaries. The overarching goal of this project is to characterize TRWP fate by quantifying TRWP levels in salt marsh habitats adjacent to roadways subject to frequent nuisance flooding to characterize exposure of these particles 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, and Rosemead Road in Mount Pleasant was used as a control site. Sediment samples were collected using a transect design in triplicate. Tire road wear particles and other 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 (TRWP, fragment, film, or fiber) were recorded. Suspected microplastic and TRWP were confirmed using a hot needle test, and a subset of particles were tested for polymer type using Fourier Transform Infrared Microscopy (FTIR). 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.

4.16.B.T Microplastics on the Planet: Input Sources, Transport Pathways and Eco-Environmental Impacts

4.16.B.T-01 Withdrawn - Microplastic Extraction Methods in Complex Water Samples- Effects on Aged and Pristine Microplastics

Razegheh Akhbarizadeh¹, Jessy Xu¹, Freya Boerner¹, Miriam L. Diamond¹ and Paul Helm², (1)University of Toronto, (2)Ontario Ministry of the Environment, Conservation and Parks

Harmonized methods are needed for optimal extraction of microplastics from surface waters containing natural organic matter are needed that consider aged and pristine microplastics. We report on a systematic study of extraction procedures to effectively remove unwanted organic matter while balancing the retention of aged and pristine microplastics. Sequential combinations of oxidative-alkaline protocols (e.g., potassium hydroxide, hydrogen peroxide, Fenton's reagent) were applied to particles comprised of polymers (olefins, polystyrene, polyamide, polyesters, tire rubber) from 300 to 1000 μm and various shapes with and without organic matter. We found that the recovery of microplastics from digested water samples depends on the polymer-type, aging, and shape. Recoveries of easily degraded aged particles (e.g., polyurethane and polystyrene foams, polyester fibers) were up to 6 times lower than that of their pristine version after applying the same digestion method (2-35% vs. 12-79% after alkaline-oxidative digestion without organic matter). The Response Optimization Model showed that for organic matter $< 2\%$ in concentration, digestion with Fenton's reagent minimizes losses of microplastics; decreasing the strength of the digestion reagents, reducing exposure time, and maintaining the solution at room temperature also increases recovery rates. For samples with organic matter $> 10\%$, a sequential combination of two or more digestion solutions can balance efficiency and effectiveness while minimizing microplastic losses. Adding appropriate amounts of organic matter and aged (weathered) microplastics to the lab spikes is highly recommended as a best practice.

4.16.B.T-02 Laser-Based Spectroscopy for Automated Identification of Microplastics

Louis Tisinger, Agilent Technologies, Inc.

Recognition of microplastics as a significant environmental pollutant is a fairly recent development, and the means by which to analyze them are quickly developing. Larger plastic fragments, those greater than a millimeter, can be easily interrogated by physical and spectroscopic methods, e.g., visible microscopy, density, melting point, macro-infrared spectroscopy, etc. The latter, infrared spectroscopy, is a very good way to analyze particles, owing to the specificity of IR spectra; an IR spectrum is a molecular "fingerprint". Microplastic particles, i.e., those that are less than a millimeter in diameter, require more complex methods. In order to measure them, they need to be found in a field of view, and then, using an appropriate spectrometric method, be measured/identified. Fortunately, there are now available technologies, including automated single-point infrared microscopes and imaging systems - both linear array and focal plane array - which streamline the process of collecting particle spectra. However, identification, via library searching, tends to be a distinct step in the microplastic analysis workflow of these systems. Also, emphasis has tended toward only identifying the microplastics; the following are also important in understanding the source potential environmental impact: morphology, count, size, and size classifications - these properties might typically require manual usually

measurements (the Southern California Coastal Water Reclamation Project draft guidance document for analysis of microplastics prescribes a full set of physical property measurements in addition to particles identification). To that end, a new laser-based IR analyzer has emerged which automates the entire microplastics analysis workflow, including particle identification and measurement of their physical properties. This presentation will (i) describe and show details on the operation of the system (ii) describe the simple microplastics analysis workflow, and (iii) present sample data.

4.16.B.T-03 Insight into the Eco-Corona Formation and Interaction of Environmentally Weathered Microplastics Using Fourier Transform Infrared Spectroscopy (FTIR) and Spectra Pattern Recognition Techniques

Oluniyi O. Fadare¹, Jeremy L. Conkle² and Hussain A. Abdulla¹, (1)Texas A&M University, Corpus Christi, (2)Delaware River Basin Commission

Microplastics (MPs) released into aquatic ecosystems undergo various physical, chemical, and biological processes that influence their behaviors in the natural environment. Different environmental constituents (eco-corona) adsorb on MPs' surface, including natural organic matter, organic pollutants, metals, eco-proteins, and microbe colonization. Currently, limited knowledge of eco-corona formation on MPs in the natural environment is known. To improve our understanding of microplastic corona formation and characterization under natural conditions, MPs were extracted from particulate organic matter obtained from wrack line debris using three different extraction methods. The sub-fractions of each extracted MP (NaOH-MPs, Formic acid-MPs, and n-hexane-MPs), the raw MPs (Rw-MPs, manually separated under the microscope), and pristine MPs (Pr-MPs) were characterized and compared using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). A total of 1400 samples were collected, comprising a hundred ATR-FTIR spectra for each group of three different polymer types (polyethylene, PE, polystyrene, PS, and polypropylene, PP). The spectra were analyzed by various pattern recognition techniques (principal components analysis, classification, and regression trees). PS in n-hexane-MPs sub-fraction could not be characterized due to interaction of the polymer with the solvent. The characteristic peaks of each polymer type were altered in NaOH-MPs, Formic-acid-MPs, and Rw-MPs due to eco-corona formation. In contrast, n-hexane-MPs (PE, PP) peaks appeared unaltered and similar to the Pr-MPs (reference samples). However, the spectra intensity pattern recognition revealed a reduction in n-hexane-MPs peak intensity when compared with the reference samples. The results of the study give insight into how environmentally weathered microplastics could be effectively identified and enhance our knowledge of eco-corona formation on microplastics.

4.16.B.T-04 Development of a Device for the One-Pot Isolation and Digestion of Microplastics

Matthew Ross, Tianna Groeneveld and Zach Vydra, MacEwan University

Typical workflows for the isolation of microplastics from environmental matrices utilize a combination of density flotation to separate microplastics from more dense matrix material and chemical or enzymatic digestion to remove organic material. This multi-step workflow necessitates transferring samples between multiple containers, increasing particle loss and risk of contamination. To overcome these issues, we developed a device that enables both digestion and density flotation steps to take place within the same apparatus. The device is constructed of inexpensive and widely available stainless steel components. Recoveries were assessed in sand by spiking samples with fluorescent microbeads and consumer plastic fragments, which ranged in size from 63 μ m to 1mm and in density from 1 to 1.4 g/cm³. Extractions of microbeads from sand produced acceptable recoveries (>75%) for beads >250 μ m in diameter. Compared to typical methodologies, our novel device produced significantly better recoveries for small microplastics (<75 μ m) and a reduction in contamination. The modular design also provides flexibility and adaptability to a range of sample matrices, and the technique was further applied to quantify microplastics in biosolids, river water, and sediment.

4.16.B.T-05 Impacts of Microplastics on Nitrogen Cycling in Ammonia-Oxidizing Bacteria and Wastewater Activated Sludge Communities

Mara F Walters¹, Bongkeun Song¹, Meredith Evans Seeley², Michele Cochran¹, Christopher D Burbage³ and Kevin Parker³, (1)Virginia Institute of Marine Science, (2)National Institute of Standards and Technology, (3)Hampton Roads Sanitation District

Microplastics are a contaminant class of concern that pervades myriad organisms and ecosystems worldwide. Evidence for the widespread impacts of microplastic pollution includes studies that have demonstrated microplastics can affect nitrogen (N) cycling processes, such as those mediated by sedimentary microbial communities. Wastewater treatment is another environment in which N cycling by microbes is imperative to system health. One of the microbial groups central to N cycling is the ammonia-oxidizing bacteria, which carry out the first step of nitrification by oxidizing ammonia to nitrite. Efficient nitrification is essential for downstream processes such as denitrification, which removes excess N from systems. Previously, we demonstrated that microplastics composed of polyvinyl chloride (PVC) and polyurethane foam (PUF) can inhibit the ammonia oxidation of *Nitrosospira* sp. AV. Here, we show evidence that in the case of PVC, leaching of plastic additives (including flame retardant compounds), rather than the polymer type or physical characteristics, may have caused the toxic impacts we observed. We also investigated the impacts of PVC and PUF microplastics on the nitrification and denitrification activities of activated sludge obtained from sequencing batch reactors (SBRs) at the Hampton Roads Sanitation District. Activated sludge contains the essential microbes that carry out nitrification and denitrification during the wastewater treatment process, thereby removing excess N from wastewater. Our results suggest that microplastics composed of these two common household polymers may inhibit nitrification, but not denitrification, during wastewater treatment. Overall, we conclude that PVC and PUF microplastics can negatively impact important N cycling microbes in the environment and during wastewater treatment and that their additives may play a central role in this effect.

4.16.B.T-06 Quantifying Microfiber Emissions from Clothes Dryers

Monica Arienzo¹, Meghan Collins¹, Emily Frey² and Marilee Movius³, (1)Desert Research Institute, (2)Tahoe Regional Planning Agency, (3)League to Save Lake Tahoe

Synthetic and natural textile fiber production has increased in recent years. Studies have shown that washing synthetic clothing is a source of synthetic microfiber contamination to the environment when microfibers are released with wastewater. However, only a few studies to date have focused on the clothes drying process as a source of microfiber contamination to the environment. Prior studies have shown that tumble dryers may generate more microfibers than washing machines for the same amount of clothing. To better understand dryer emission of microplastics, we recruited League to Save Lake Tahoe community scientists to install a fine mesh filter on their dryer vent for three weeks. During that time, the League to Save Lake Tahoe volunteers recorded their general drying habits using the Citizen Science Tahoe mobile application including type of material dried (e.g., pants, shirt, etc.), composition (e.g., cotton, acrylic, silk), date, and time. The dryer vent mesh was returned to the laboratory, the accumulated material was removed from the mesh, weighed, and analyzed using microscope-enabled Fourier Transform Infrared Spectroscopy (FTIR). Results showed on average 138 +/- 77 mg of material was emitted per dryer event. The composition of the material dried was primarily cotton, followed by polyester which agreed with the FTIR results of the dryer vent material. These results indicate that clothes dryers are potentially an important source of synthetic microfibers to the environment.

4.16.P-Tu Microplastics on the Planet: Input Sources, Transport Pathways and Eco-Environmental Impacts

4.16.P-Tu-158 Quantifying Suspended Microplastics in the Water Column with the Urbanized Patapsco River, Maryland

Owen Bradley, Stephanie Phan and Jeffrey Ashley, Thomas Jefferson University

Microplastics, tiny plastic particles less than 5 mm, are found globally, especially in the water columns of lakes, rivers, estuaries and oceans. Microplastics may pose serious risks and dangers to aquatic organisms such as affecting their ability to feed, grow, and ultimately survive. In this study, the extent of microplastic levels in the water column was assessed in the highly urbanized section of the lower Patapsco River around Baltimore, one of the most polluted urban waterways in the United States. Water column samples were collected from 9 different locations in the Northwest Harbor and the Middle Branch of this estuarine ecosystem. At each site, a 32-ounce water sample was collected (triplicate samples were performed), filtered, and assessed using a digital microscope to identify the number, type and colors of microplastics. Every water sample analyzed had microplastics present with an average of 9.3 microplastics being found per sample from the 9 sites. Black and clear microfibrils were most common (mean=3.7 black fibrils and mean=3.4 clear microfibrils). The sites that had the greatest highest levels of microplastic accumulation were Fort Armistead (mean=18 particles/sample), West Covington Park and Inner Baltimore Harbor (means=12 particles/sample for both). Although this was the first study to quantify microplastics in this region, it remains difficult to assess sources of these microplastics from our limited data set. Future research could include greater spatial assessments as well as identifying sediment microplastics in addition to water column levels.

4.16.P-Tu-159 Bioactivity of Microplastic Containing Environmental Debris and Laboratory Produced Plastic Particle Mimetics

Sarah Morgan and Lisa DeLouise, University of Rochester

Microplastics (MPs, $1 \mu\text{m} < \text{MP} < 5 \text{ mm}$) are an emerging contaminant of concern due to their persistence and pervasiveness. Environmental MPs are a complex mixture of various plastic polymers and sorbed chemicals that have been shown to have a wide range of toxic effects across numerous species. Since exposure to unknown quantities of MPs is unavoidable for most species, it is necessary to characterize the bioactivity of MPs present in the environment as well as supplement these results with studies using reproducible laboratory synthesized plastic particle mimetics in order to characterize the health risks associated with MP exposure. Our contribution to this knowledge gap is two-fold: first we are collecting water samples from the nearshore waters of Lake Ontario near Rochester, NY and analyzing the debris using novel silicon nitride nanomembranes and various in vitro toxicity endpoints, and second, we are generating various plastic particle mimetics and monitoring both sorption and bioactivity. In both approaches the initial endpoints being investigated in various mammalian cell lines are cell viability, aryl hydrocarbon receptor (AhR) activity, and estrogen receptor (ER) activity. Initial mimetics were created using various combinations of polystyrene (PS), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and bisphenol A (BPA) which were selected for their commercial availability, potent AhR activity and well-documented ER activity, respectively. We hypothesize that MP-contaminant mixtures produce toxicity profiles that are unique from those of both MP and contaminant single exposures. Analysis is ongoing, but thus far we have found that water samples from Lake Ontario contain isolatable debris including Nile Red positive plastic particles and that this debris is not cytotoxic but does have some AhR activity that increases with increased volume filtered and is decreasing over time. Additionally, we have found that soaking with both TCDD and BPA alters PS morphology, and these changes are reflected in decreasing AhR activity with increasing time for TCDD-PS co-exposures but may not be reflected in changes in ER activity for BPA-PS co-exposures. Overall, our ongoing work highlights the need for additional research into the effects of polymer and chemical identity on the risks associated with exposure to various MP mixtures.

4.16.P-Tu-160 Effect of Microplastic on the Interspecific Competition between Exotic and Domestic Species of *Daphnia*

Changhae Kim, Zhihan Cao and Jinho Jung, Korea University

Microplastic (MP) has gained increasing attention as a significant threat in the aquatic environment, prompting extensive research into its potential harm in recent years. Concurrently, the ecological disruption caused by exotic species has emerged as another pressing environmental concern, receiving growing recognition. The influence of environmental factors, such as temperature and food availability, on interspecific competition has been well-established. However, the impact of microplastics (MP) and plastic additives on this dynamic remains elusive. Here, we aimed to evaluate the effect of polyethylene MP fragments and the UV filter BP-3 on the competition between an exotic species and a domestic species of *Daphnia* in South Korea. The model species *Daphnia magna* was chosen as the exotic species, and *Daphnia pulex* was chosen as the domestic species for its wide distribution. Populations of 10 individuals of both species were exposed together to polyethylene MP fragments ($18.23 \pm 8.02 \mu\text{m}$) containing benzophenone-3 ($2.89 \pm 0.20\%$ w/w) for 21 days. Growth and reproduction were measured to evaluate how MP and BP-3 affect interspecific competition. Bioconcentration of MP and BP-3, feeding rate, and behavior changes were analyzed to explain how the two species were affected differently. To our knowledge, the present study is the first to consider the effect of plastic additives in interspecific competition under MP exposure. We suggest that MP and plastic additives may significantly influence competition within the aquatic environment. It is imperative that we recognize the ecological impact of these factors to gain a comprehensive understanding of their implications.

4.16.P-Tu-161 Establishing a High Efficiency and Practical Method for Analysis of Microplastics in Various Matrices

Wenjian Lao, Sydney Dial and Charles S. Wong, Southern California Coastal Water Research Project

Microplastics are ubiquitous on our planet. Analysis of microplastics is a tedious task because they have a diversity of polymer types, size fractions, morphologies, and colors. The steps of an analytical procedure usually include particle extraction, sample cleanup, size fraction, filtration, particle counting and characterization, polymer identification, and data reporting. Currently, there is a lack of a generic sample cleanup approach and data reporting protocol. The goal of the present study is to establish a simple but effective cleanup method and provide guidance for handling analytical data for reporting. Our cleanup method is based on a combination of acid digestion and alkaline digestion. Effects of both digestions on the plastic particles were qualitatively and quantitatively evaluated with a set of commercially available standard plastic materials. The results demonstrated that there is essentially no effect of these reagents on most types of plastics. The efficiency of the cleanup method was further demonstrated with environmental samples (water, sediment, and fish) collected from wastewater treatment plants and rivers. By applying the Poisson distribution for microplastics, a proper detection limit, i.e., minimum detectable amount (MDA), was established using counts from laboratory procedural blanks. Guidance for using the MDA in handling the data for reporting are proposed and validated with a dataset of an interlaboratory calibration exercise. Adoption of our method (e.g., by the State of California via legislation) may enhance throughput in sample preparation and be beneficial to submitting high quality data.

4.16.P-Tu-162 Effects of Microplastic Ingestion on Dengue Virus Serotype 2 Infection and Dissemination in *Aedes aegypti* and *Aedes albopictus* Mosquitoes

Gabriella H. McConnel, Corey Brelsfoard and Jaclyn Canas-Carrell, Texas Tech University

As vectors of Chikungunya virus, dengue virus, yellow fever virus, and Zika virus, *Aedes aegypti* and *Aedes albopictus* are commonly considered the most important vectors of arboviruses impacting human health. In close association with humans and urban environments, these species have adapted to breeding in discarded containers and urban litter, including tires, animal troughs, plastic tubs, and planters. Specifically, Dengue virus is maintained in an endemic-epidemic cycle involving humans and predominantly these two species. To

determine effects of microplastic ingestion on dengue virus 2 infection and dissemination in *Aedes aegypti* and *Aedes albopictus* mosquitoes, first instar larvae were dosed with four different concentrations (0 MP/mL, 50 MP/mL, 100 MP/mL, and 10,000 MP/mL) of 1.0 µm polystyrene microplastics and moved to cages through emergence. Mosquitoes were left to mate for five days and provided infectious bloodmeals of dengue virus serotype 2. After feeding, engorged females were caged and incubated for 14 days. Individuals were collected and stored at -80 °C, and segmented prior to RNA extractions and virus quantification by qPCR. Results from mosquito tissues will be used to calculate infection rate, dissemination rate, and potential for transmission for each species and each treatment group. Data analysis is underway.

4.16.P-Tu-163 Microplastics in Gomti and Saryu Riverine Systems, India: A Baseline Assessment Study

Amit Kumar Mishra and Jaswant Singh, Avadh University

Accumulation of microplastics (MPs) in aquatic ecosystems is an emerging concern. Rivers are efficient carriers for transferring MPs from terrestrial to aquatic areas, and ultimately to oceans. A root cause study can provide an effective solution to this problem; hence, tracing MPs in riverine systems can illustrate long-term microplastics pollution. This study aimed to investigate the occurrence and distribution of microplastics in the surface water and sediments of two major tributaries (the Gomti and Saryu Rivers) of the Ganga River, India. The Gomti River meanders through an urban network region, whereas the Saryu River courses through a semi-urban area. Water and sediment samples were collected from different selected sites in the catchments of both rivers and analyzed for their physical characteristics, such as size, number, type, and color as well as polymeric composition. Digestion, density separation, and filtration using a self-designed setup were suitable for microplastics extraction. MPs were detected in all samples, with an average abundance of 6.07 and 2.34 items/L, for water whereas 445 and 224 items/kg for sediment (dw), in the Gomti and Saryu Rivers respectively. Various colored MPs with more than five different shapes (fibers, fragments, films, foams, and pellets) were observed in the collected samples. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy confirmed that polypropylene, polyethylene, and Acrylonitrile Butadiene Styrene were the most abundant polymers, with the majority being fibers and fragments. Population density, urbanization rate, and land use were identified as the key differences between the MPs abundance of the two riverine systems. The present study is a pioneer in counting MPs in the Gomti and Saryu River systems. Assessment of contamination levels and types of MPs present in river sediments. These findings offer a valuable understanding of the current state of MPs within the surface water and sediments of the Gomti and Saryu Rivers. This information can aid in the development of effective regulations aimed at reducing MPs and curbing contamination in rivers, ultimately contributing to environmental protection.

4.16.P-Tu-164 Determining the Drivers of Spatial and Seasonal Microplastic Characteristics in Narragansett Bay, Rhode Island Surface Water

Sarah Davis, Coleen C. Suckling and Andrew J. Davies, University of Rhode Island

Microplastic (MP) pollution is a critical but absent metric in the majority of water quality analyses conducted in New England's largest estuary, Narragansett Bay. Recent research indicates high variability of MP concentration and characteristics within estuarine systems, suggesting that the observed composition of MPs is dependent not only on each sampling site's geography, physical processes, and pollutant sources, but also on sampling time relative to seasonality. Seasonal sampling is necessary to establish a strong local baseline across a significant temporal pattern within Narragansett Bay that can be used to direct and measure future interventions at the waste and ecosystem management level and move towards comparative future monitoring efforts. A two year multi-site seasonal sampling effort using surface water trawling in Narragansett Bay was completed and analyzed for MP concentrations and particle characteristics. The observed results indicate similar particle characteristics (morphology, color, polymer type) but significant variation in particle concentrations (particles/m³ of seawater) across sampling sites and seasons. Key drivers of site- and season-

specific MP yield fluctuation within the study system are hypothesized to include stormwater runoff and freshwater river inputs, which will be evaluated for their relationship to the observed outcomes and detailed further within this presentation.

4.16.P-Tu-165 Non-Targeted Analysis of Organic Chemical Contaminants on Microplastics

Stefanie Landeweer and Piero Gardinali, Florida International University

Microplastics are widely recognized as a ubiquitous emerging contaminant in the environment. The chemical structures of plastic polymers give microplastics unique properties and allow for environmental contaminants to interact differently with them than with naturally-occurring surfaces. The purpose of this study is to analyze and characterize the chemical space that is formed on these unique environmental surfaces. Samples were prepared for analysis using a high pressure and temperature solvent extraction using water and methanol. Analysis of the extracts was performed using both excitation-emission matrix (EEM) spectroscopy and liquid chromatography-high resolution mass spectrometry (LC-HRMS). HRMS data was subjected to non-target analysis (NTA) in order to categorize compounds extracted from the environmentally exposed plastics. Preliminary results using standard solutions show that the extraction procedure is able to collect compounds from environmental plastics that are not present in pre-production plastic pellets and which compounds are extracted depend on which solvent is used. Preliminary experiments using pre-production pellets and a standard solution showed that the partitioning to the microplastics seems to be dependent on the compound's octanol-water partition coefficient (K_{ow}). This study will provide a better understanding of whether microplastics, which are frequently consumed by organisms, can act as environmental sinks for certain organic contaminants as well as allowing the use of microplastics as a tool to better understand the organic chemical loads in the environment.

4.16.P-Tu-167 Determining the Presence and Impacts of Microplastic Fibers in *Crassostrea virginica*, the Eastern Oyster

Amara Kathleen Pouv, Coleen C. Suckling and Andrew J. Davies, University of Rhode Island

Plastic is a ubiquitous part of daily life and has contributed to huge advancements in innovation and convenience. However, the waste and pollution associated with ever increasing plastic usage is a growing concern for environmental health. Microplastics, like synthetic microfibers, are commonly found in the oceans but the effects, retention, and accumulation within marine organisms are still largely unknown. To address the knowledge gap, this study employs two approaches: 1) a broad spatial sampling of shellfish in a major US estuary; and 2) examining the retention time and short to medium-term accumulation of polyester microfibers in the Eastern Oyster (*Crassostrea virginica*), an important food source and commercially valuable shellfish. To accomplish aim one, wild shellfish populations in Narragansett Bay will be sampled and tested for microplastic presence and abundance. Aim two will involve a high frequency sampling approach, where an experimental population of *C. virginica* will be exposed to environmentally relevant concentrations of polyester microfibers then tested for microplastic presence and abundance over time. Results of this study will help advance our understanding of how environmental microplastics are affecting marine organisms and inform future experiments that investigate the combined effects of microplastics and other ocean pollutants.

4.16.P-Tu-168 Microplastic and Nanoplastic Risks in Dredged Sediments: From Databases to Strategic Responses

Justin Wilkens¹, Alyssa Calomeni², Jonna Boyda¹, Alan Kennedy¹ and Andrew D McQueen², (1)U. S. Army Corps of Engineers, (2)U.S. Army Engineer Research and Development Center

There is international focus on global plastic pollution and the subsequent weathering and distribution of microplastics (MPs) and nanoplastics (NPs) in the environment, including human health and ecological concerns. The US Army Corps of Engineers is responsible for dredging federal navigation channels and disposing or beneficially utilizing several hundred million cubic yards of sediments annually. This research intends to begin development of a strategic response to address potential public concerns regarding the plastic

risks in these sediments. Therefore, data are needed to characterize background concentrations and potential exposures of MPs and NPs as well as sensitivities of aquatic species. The objectives of this research were to conduct a literature review on 1) the occurrence and abundance of MPs and NPs in sediments relevant to dredging operations, and 2) the ecotoxicity data associated with MP and NP exposures to organisms of interest for dredged sediment evaluations. The reviewed literature revealed median MP sediment concentrations ranging from lowest to highest as items per kilogram dry weight as follows: natural lakes and reservoirs (89) < estuarine (196) < riverine (273) < marine (357) < Great Lakes (1,716). To better understand the relative sensitivities of organisms commonly used in dredge sediment evaluations, ecotoxicological data such as lowest observed effect concentrations (LOECs) and lethal median concentrations (LC₅₀s) were analyzed to calculate species sensitivity distributions for five invertebrates and one green alga. Based on the species sensitivity distributions, the least to most sensitive organisms were identified as *Daphnia magna* (Cladoceran Crustacean) < *Raphidocelis subcapitata* (green algae) < *Daphnia pulex* (Cladoceran Crustacean) < *Hyaella azteca* (Amphipod Crustacean) = *Ceriodaphnia dubia* (Cladoceran Crustacean) < *Palaemonetes pugio* (grass shrimp). However, notable data gaps exist for MPs and NPs, including limited ecotoxicity data for MPs and marine species as well as lack of information on NP concentrations in sediments and NP ecotoxicity data. This study contributes to addressing the crucial aspect of understanding the potential exposure scenarios associated with dredged sediments. These data can be used to help develop decision frameworks, tools and approaches to proactively address detection, exposure, and hazards associated with MPs and NPs in dredged sediment and effectively communicate relative risks.

4.16.P-Tu-169 Microplastics in Your Microgreens? Assessing How Microplastics Impact Agriculture, Soil Function, and Plants

Rachel Zajac-Fay, Tina Liu, Krista Thrasher, Alexandra Ambrosino and Jason M. Conder, Geosyntec Consultants, Inc.

Microplastics, an emerging contaminant of concern, are ubiquitous in the environment due to the widespread use of plastics. While microplastics research has intensified in the past decade, the focus up until the last couple of years has been on aquatic environments. However, microplastics have been detected in terrestrial environments, including on agricultural land. The use of plastics in agriculture, or plasticulture, is a common practice dating back to the 1950s. Greenhouses, tunnels, plastic mulch, seed coatings, and other methods have since been developed to increase crop production. Plastics can enter the soil environment through other pathways such as biosolids generated at wastewater treatment plants. Studies have shown that these biosolids often contain high levels of microplastics, and biosolids are commonly land-applied and used as fertilizer in the agricultural industry. A critical review of existing literature was performed to understand the sources of microplastics in agriculture, the impact on soil function, assess what plant species have been evaluated in toxicity tests, and the resulting effects on plant physiology. The research team conducted a review of existing peer-reviewed literature on the topic of microplastics in agriculture and soil environments with a focus on six specific critical review papers. The key findings of this review found that microplastics in agriculture come from a variety of sources; microplastics can alter physical soil properties; microplastics can enter and translocate in certain plants; microplastics can inhibit seed germination and reduce plant growth and biomass. Key data gaps will be discussed, such as limitations of the toxicity studies conducted. This presentation will also discuss next steps that can be taken to further refine our understanding of microplastic impacts in agricultural systems.

4.16.P-Tu-171 Variation in Microplastic Distribution in a Background Headwater Lake, Canada During the Ice and Ice-Free Period

Brittany Welsh¹, Erin R. Bennett¹, Andrew Paterson², Ken Drouillard³ and Julian Aherne¹, (1)Trent University, Canada, (2)Ontario Ministry of the Environment, Conservation and Parks, (3)University of Windsor

Microplastics are pervasive contaminants of concern owing 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 demonstrated 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 background 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. Monthly lake samples were collected from the centre of the lake, 1 m below the surface during the ice-free period, with surface grab samples collected once. During the ice-covered period, under-ice water samples were collected from the surface and 1 m below the surface, in addition to snow and ice samples (divided into upper and lower layers) from the surface of the frozen lake. A sub-set of the snow, ice, and under-ice lake water samples were also collected for dissolved organic carbon content to assess the source contribution of atmospheric deposition. Preliminary results show that microplastic concentrations in both the lake surface and 1 m are two times greater during the ice-free period than the ice-covered period. Furthermore, microplastic concentrations appear to be slightly higher in the lake surface than at a depth of 1 m. It is expected that microplastic concentrations would be highest in the snow and ice as they prevent microplastics in atmospheric deposition from reaching the lake thereby acting as a temporary microplastic sink during the ice-covered period and microplastic source once melted during the ice-free period.

4.16.V Microplastics on the Planet: Input Sources, Transport Pathways and Eco-Environmental Impacts

4.16.V-011 Microplastics in Pelagic Fish and Surface Water from the St. Lawrence River, Montreal, Canada

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To determine the risks that microplastics may pose to wildlife and the ecosystem, it is crucial to examine their abundance and characteristics in the environment. However, in the St. Lawrence River (SLR), a distinctive aquatic system that links the Great Lakes of North America and the Atlantic Ocean and serves as a source of drinking water for humans, such knowledge is still very limited. Although microplastic contamination has been documented in the water and sediments of the SLR in a few studies, no research has yet reported the presence of microplastics in pelagic fish from the SLR. In addition, wastewater treatment plants (WWTPs) are recognized as substantial vectors/sources of microplastic release into aquatic ecosystems. Nonetheless, it remains uncertain how the large WWTP in Montreal, QC, Canada might impact the microplastic contamination in downstream fish. To this end, this study aims to investigate the contamination of microplastics in pelagic fish from the upstream and downstream of the Montreal's WWTP point of discharge in the SLR. Northern Pike (*Esox Lucius*) (n=20/site) and their prey, the yellow perch (*Perca flavescens*) (n=5/site), as well as surface water (n=7/site) were sampled upstream (Iles de Boucherville) and downstream (Ilet Vert) of the Montreal primary WWTP between June and August 2022. Microplastics were analyzed in the gastrointestinal tract (GIT) and the gills of these fish to elucidate both active and passive ingestion. Preliminary results (n=11 fish/sampling site) indicate a mean abundance in the Northern pike of 2.8 ± 0.9 (S.E.) and 3.7 ± 1.0 microplastics candidate particles/fish upstream and downstream of the WWTP, respectively. For the yellow perch (n=5/site), abundance was higher upstream than downstream with a respective mean of 1.4 ± 0.2 and 0.54 ± 0.4 microplastics candidate particles/fish. Thus, results suggest that this WWTP may not significantly affect the microplastic accumulation in pelagic fish. Although non-significant, Northern pike displayed a higher abundance (3.2 ± 3.3 particle/fish) than their prey, the yellow perch (0.9 ± 1.0 particle/fish). For all species and sites combined, fibers represented between 71 % and 84 % of the particles found. In addition to the assessment of particle color, abundance and

shape, polymer (>53 μ m) identification will be conducted using Fourier transform infrared spectroscopy and/or Raman spectroscopy. Preliminary results will be presented in this poster.

4.17.P-Tu Point-of-Use Drinking Water Exposome and Potential Human-Health Effects

4.17.P-Tu-172 Drinking-Water Exposome Research: Bottled Water

Paul Bradley, Kristin M Romanok and Kelly L. Smalling, U.S. Geological Survey

Bottled water consumption in the United States and globally has increased amid heightened concern about environmental contaminant exposures and health risks in drinking water supplies, despite a paucity of directly comparable, environmentally-relevant contaminant exposure data for bottled water. This study provides insight into exposures and cumulative risks to human health from inorganic/organic/microbial contaminants in bottled water. Bottled water from 30 total domestic United States (23) and imported (7) sources, including purified tapwater (7) and spring water (23), were analyzed for 3 field parameters, 53 inorganics, 465 organics, 14 microbial metrics, and *in vitro* estrogen receptor bioactivity. Health-benchmark-weighted cumulative hazard indices and ratios of organic contaminant *in vitro* exposure-activity cutoffs were assessed for detected regulated and unregulated inorganic and organic contaminants. No enforceable chemical quality standards were exceeded, but several inorganic and organic contaminants with maximum contaminant level goal(s) (MCLG) of zero (no known safe level of exposure to vulnerable sub-populations) were detected. Precautionary health-based screening levels were exceeded frequently. The results indicate that simultaneous exposures to multiple drinking-water contaminants of potential human-health concern are common in bottled water. Improved understandings of human exposures based on more environmentally realistic and directly comparable point-of-use exposure characterizations, like this bottled water study, are essential to public health because drinking-water is a biological necessity and, consequently, a high-vulnerability vector for human contaminant exposures.

4.17.P-Tu-173 Drinking-Water Exposome Research: Private-Wells in an Intensive Agricultural Landscape

Paul Bradley¹, Kelly L. Smalling¹, Kristin M Romanok¹, Dana Kolpin¹ and Darrin A. Thompson², (1)U.S. Geological Survey, (2)University of Iowa

In the United States and globally, contaminant exposure in unregulated private-well point-of-use tapwater is a recognized public-health data gap and an obstacle to both risk-management and homeowner decision making. To help address the lack of data on broad contaminant exposures in private-well tapwater from hydrologically-vulnerable (alluvial, karst) aquifers in agriculturally-intensive landscapes, samples were collected in 2018-2019 from 47 northeast Iowa farms and analyzed for 35 inorganics, 437 unique organics, 5 *in vitro* bioassays, and 11 microbial assays. Twenty-six inorganics and 51 organics, dominated by pesticides and related transformation products (35 herbicide-, 5 insecticide-, and 2 fungicide-related), were observed in tapwater. Heterotrophic bacteria detections were near ubiquitous (94% of the samples), with detection of total coliform bacteria in 28% of the samples and growth on at least one putative-pathogen selective media across all tapwater samples. Health-based hazard index screening levels were exceeded frequently in private-well tapwater and attributed primarily to inorganics (nitrate, uranium). Results support incorporation of residential treatment systems to protect against contaminant exposure and the need for increased monitoring of rural private-well homes. Continued assessment of unmonitored and unregulated private-supply tapwater is needed to model contaminant exposures and human-health risks.

4.17.P-Tu-174 Exposures and Potential Health Implications of Contaminant Mixtures in Public-Supply Drinking Water

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Continued improvements in drinking-water quality characterization and treatment/distribution infrastructure are

required to address the expanding number of environmental contaminants. To better understand the variability in contaminant exposures from the drinking water resource (surface and groundwater), through the treatment/distribution process, to the point-of-use (tapwater, TW), a synoptic assessment of broad chemical exposures was conducted in 2019 using system-specific source waters, pre-distribution and service-area TW from 10 drinking water treatment plants in the greater Minneapolis/St Paul area of Minnesota, United States. Source and TW samples were analyzed for 465 unique organic compounds (e.g., pesticides, pharmaceuticals, volatile organic compounds, disinfection byproducts, etc.), 34 inorganic constituents, and 3 field parameters as well as *in vitro* estrogen, androgen, and glucocorticoid bioactivities. Mixtures of organic and inorganic contaminants were prevalent in source water and TW samples, indicating the continued need for broad assessments of mixed contaminant exposures to characterize potential drinking-water human health outcomes. Contaminant concentrations were similar among drinking water sources and no exceedances of Environmental Protection Agency maximum contaminant levels (MCL) were observed in any treated TW sample in this study; the latter indicating broad regulatory compliance and effective treatment. No TW sample contained estrogenic, androgenic or glucocorticoid activity at concentrations that may cause potential adverse human-health effects. However, multiple exceedances of non-enforceable MCL goals (MCLG) and other health advisories combined with frequent exceedances of benchmark-based hazard indices indicated potential aggregated risks to vulnerable subpopulations and emphasizes the continued need to assess contaminant mixture exposures at the drinking-water point of consumption using a broad analytical scope.

4.17.P-Tu-175 Effects-Based Bioassay Screening Approaches Applied to Residential Tapwater to Inform Consumer Point-Of-Use Decisions

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Effects-based bioassays are expeditious and economic non-targeted chemical analysis tools frequently used in preliminary stages of water quality monitoring efforts, or as part of effects-directed analysis. Using *in vitro* effects-based methods, we previously detected biological activity, indicative of endocrine disrupting compounds, in surface and source waters. However, there is a lack of contaminant occurrence data in unmonitored private-supply tapwater, and unknown adverse health effects from contaminant mixtures exposures. Therefore, USEPA and U.S. Geological Survey scientists collaborated with universities, Tribal, and non-profit partners to produce directly comparable occurrence data sets of public and private tapwater supplies, as well as of bottled water, to better inform consumer risk-management decisions at the point-of-use (POU). Community volunteers (POU water samples) across seven case studies in the US and Puerto Rico were selected based on exposure susceptibility (e.g., impacted source waters, geographical location, and aging distributions systems, among others). Using *in vitro* cell-based tools we developed a tiered screening process and tested solid phase-extracted water samples for biological activity. Estrogenic (T47d-KBluc; detected most often and in highest concentrations; ng Estradiol equivalents L-1) and androgenic activity (CV1-chAR; ng Dihydrotestosterone equivalents L-1) were detected in impacted source and private supply samples. Only one public-supply sample contained estrogenic activity above method detection limits; glucocorticoid (CV1-hGR) and antiandrogenic activity were not detected in any sample. Overall, estrogenic activity ranged 0.01 – 2.97 ng E2Eq L-1 (median: 0.05 ng E2Eq L-1), androgenic activity ranged 0.07 – 0.17 ng DHTEq L-1 (median: 0.15 ng DHTEq L-1). All estrogenic (3.8 ng E2Eq L-1) and androgenic (11 ng DHTEq L-1) detections fell below previously reported human-health Effects-Based Trigger values (for similar *in vitro* method) for drinking water. Consistent with our previous case studies, biological activity was detected most often in source waters, but contemporary treatment systems typically eliminate activity to below *in vitro* methods detection limits. However, private supplies sourced from ground water have potential to exhibit endocrine activity, especially when heavily impacted by local agriculture or anthropogenic waste. *Abstract does not reflect Agency views or policy.*

4.17.P-Tu-176 Health Risk Assessment, Water Quality Indices and Reproductive Hormone Profile Among Abakpa Citizens in Enugu Metropolis, Nigeria

Prince Ozioma Emmanuel¹, Olawale Otitoju² and Joseph Ikwebe², (1)New BB Bread (2)Federal University Wukari

This study was carried out to determine the health risk assessment, water quality indices and reproductive hormone profile among citizens of Abakpa in Enugu metropolis. Ten (10) water sampling points comprising of wells and streams were analyzed for chemical properties such as BOD, COD, DO, pH, TSS, TDS, TH, and Alkalinity and Heavy metal composition such as Zinc (Zn), Lead (Pb), Chromium (Cr), Cadmium (Cd) and Mercury (Hg) using the American Public Health Association's (APHA) guidelines and Atomic absorption spectrophotometer (AAS) respectively. Five (5) blood samples comprising males and females was collected across the sampling areas and assayed for reproductive hormone profile such as Testosterone, Follicle Stimulating Hormone, Progesterone, Prolactin, Estradiol and the enzyme Aromatase was using the ELISA kit method. The result of chemical properties such as TSS, TDS, COD, Total hardness and Alkalinity were all within World Health Organization (WHO) stipulated limits, whereas DO, BOD and pH were higher than WHO limits across all sampling points from the sampling area. Some parameters showed significance difference while some sample did not at different sampling locations at 95% confidence level. The result of heavy metals showed very high concentrations of analyzed metals with Hg present in five water samples from Abakpa at concentrations ranging from 0.01 to 1.96ppm. Pb concentration ranged between 0.06-0.35ppm; Zn 24.93-33.65ppm; Cr 0.00 – 0.23ppm; Cd. 0.11 – 0.17 ppm across all sampling points. All the result of reproductive hormone showed lower concentration of testosterone in men (1.99 to 2.19ng/ml) and high concentration in females (2.03 to 2.06ng/ml). The concentration of FSH was in the range of 0.67 to 0.77ng/ml in males, while the level was between 0.71 to 0.83ng/ml in female. Prolactin results was 3.49 to 3.86ng/ml for males and 3.44 to 3.59ng/ml in females. Estradiol levels were 8.48 to 9.54ng/ml and 9.61 to 9.84ng/ml for females and males respectively. However, progesterone level were between 34.08 to 37.45 ng/ml for females and 36.48 to 39.56ng/ml. The aromatase enzyme was averagely below 4.0 Pmol/mg in both male and female. The risk assessment showed low risk exposure but mercury had high risk exposure tendencies of 16.333 when compared to other heavy metals from all samples. Consumption of water in sampled area's may pose a serious health challenge; high concentration of heavy metals could be attributed to the hormonal imbalance.

4.17.P-Tu-177 Cumulative Health Risk Assessment of Private Well Water Consumption Across Montana

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There is no federal oversight of home well water quality and safety. To assist homeowners, our team is conducting cumulative health risk assessments of Montana home well water quality by HUC8 level watershed, using data from Montana State University Extension Water Quality (MSUEWQ) Well Educated Program (Well Ed) and from the Montana Groundwater Information Center (GWIC). In addition to quantifying percentages of home wells that exceed EPA health benchmarks (MCLs) for various inorganic contaminants, the USGS's methodology for assessing cumulative risk is being employed, and the results of these two methods are being compared. The latter method sums the ratios of the median of each contaminant concentration, divided by an established health benchmark for that chemical, calculated for each watershed. The percent MCL exceedance method is being employed by Well Ed, administered by MSUEWQ, which guides private well owners through testing and understanding their water quality. The initial analysis of 2015-2020 Well Ed data found that nitrate-N and arsenic concentrations exceed their respective health standards (MCLs) in 3.3% and 9.8% of wells, and 11.2% of wells exceed the state's child health advisory of 0.1 mg/L for manganese. In comparison, initial cumulative risk analyses of statewide groundwater data in GWIC found that of the 67 (out of 97) HUC8 watersheds with sufficient data for analysis; more than 50% of wells in 40 of these watersheds have water unsafe for lifetime consumption (based on median values of each contaminant with an EPA MCL or Health

Advisory). Statewide, uranium and arsenic are the largest contributors to health risks, followed by strontium and manganese, with smaller contributions from other metals and nitrate. Community-based well testing clinics are being piloted. Our team is creating and disseminating watershed-level GIS maps of the cumulative risk data as well as watershed specific flyers about contaminants for which to test, the associated health risks and options for well owners to mitigate their unsafe water. These results will be compiled by county and made available through the Well Ed program website. This framework for conducting cumulative health risk assessment in partnership with university Extension programs could be a useful roadmap for analyzing other statewide databases, disseminating the results with resident well owners as well as increasing the rates of home well water testing.

4.17.P-Tu-178 Disinfection By-Product Formation Potential in Drinking Water: Variability at the Household Level and Impact of Source Type

Berkley N. Anderson, Gabrielle Black and Thomas Michael Young, University of California, Davis

Disinfection by-products (DBPs) are known contaminants of concern due to their carcinogenicity and correlation with adverse reproductive and developmental effects, but household level variability in DBPs and systematic investigation across diverse source water types is generally lacking, especially for unregulated DBPs. The purpose of this study was to investigate DBP formation potential (DBP-FP) in household tap water collected from over 120 randomly selected participants supplied by eight community water systems using four distinct source types, two types of disinfection processes, and across two seasons, as well as in various bottled waters. A total of 268 samples were analyzed using two methods, liquid-liquid extraction and thin-film solid phase microextraction to (1) quantify DBP-FP concentrations (2) assess spatial variability, temporal variability, and impact of source water type and (3) identify compounds contributing most significantly to oxidative stress using previously reported AREc32 bioassay potencies. Study findings confirm the importance of unregulated DBPs; unregulated DBPs accounted for 5.7% of total detected concentrations across all 268 samples but contributed 98.9% of all calculated total oxidative stress (TOS). The results of this study reveal that regulatory methods may not be capturing household DBP exposure due to high variability and toxicity associated with unregulated compounds including haloacetonitriles and iodoacetic acid. In addition, regions in southern California that use complex water sources and chloramination had significantly higher unregulated DBP-FP and TOS. This study adds to the growing body of literature emphasizing the need to address unregulated DBPs, especially in regions with water sources that are increasingly impacted by anthropogenic activities (indirect and direct potable reuse, higher salinity waters, increases in DBP precursors).

4.17.T Point-of-Use Drinking Water Exposome and Potential Human-Health Effects

4.17.T-01 Drinking-Water Exposome Research: Northern Plains Nations

Paul Bradley, Kristin M Romanok and Kelly L. Smalling, U.S. Geological Survey

In the United States, private-supply tapwater is rarely monitored. This data gap undermines individual and community risk-management decision making, leading to increased probability of unrecognized contaminant exposures in rural and remote locations that rely on private wells. We assessed point-of-use tapwater in three Northern Plains Tribal Nations, where ongoing tapwater arsenic interventions include expansion of small community water systems and point-of-use adsorptive-media treatment for remote homes participating in the ongoing Strong Heart Water Study. Tapwater samples from 34 private-well and 22 public-supply sites were analyzed for 3 field parameters, 476 organics, 34 inorganics, and 3 *in vitro* bioactivities.

Health-benchmark-weighted cumulative Hazard Indices and ratios of organic-contaminant *in vitro* exposure-activity cutoffs were assessed for detected contaminants. Sixty-three organics and 30 inorganics were detected in tapwater. Arsenic, uranium, and lead were detected frequently. Concentrations equivalent to public-supply Maximum Contaminant Levels were exceeded only in untreated private-well samples. Precautionary health-based Hazard-Index screening levels were exceeded frequently and attributed primarily to inorganics in

private supplies and chlorine-based disinfection byproducts in public supplies. The results support ongoing interventions to reduce tapwater arsenic exposures and indicate that simultaneous exposures to other co-occurring tapwater contaminants are common, warranting consideration of expanded source, point-of-entry, or point-of-use treatment(s). This study supports the need for increased monitoring of private-well tapwater employing a broad, environmentally-informative analytical scope to reduce the risks of unrecognized contaminant exposures and to identify possible additional targets for simultaneous tapwater risk mitigation (e.g., uranium, lead, disinfection byproducts in this study).

4.17.T-02 Cumulative Health Risk Assessment of Home Well Water Consumption, Crow Reservation, Montana

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On the Crow Reservation in Montana, there was widespread concern among Tribal members about apparent cancer clusters. Tribal stakeholders and a Tribal college science faculty member, with help from federal volunteers, conducted a community-wide environmental health risk assessment. This led to a consensus that extremely poor home well water quality and contaminated river water were the priority concerns, and to subsequent formation of the Crow Environmental Health Steering Committee (CEHSC). Funding was secured in 2010 to test home wells for metals, nitrate and fecal contamination, and by 2014 the CEHSC had completed an initial cumulative risk assessment of exposure to these contaminants in Tribal home wells, following the EPA Hazard Index methodology. About 39% of home wells were unsafe for lifetime consumption due to metals and nitrate, and an overlapping ~ 40% showed risk of fecal contamination. A GIS map was developed to show the distribution of risks across the Reservation. This presentation addresses steps taken since 2014 to further understand contamination issues and importantly, provide affordable safer drinking water sources for Tribal members. Home wells continue to be tested, project staff explain test results to families, and free home water coolers which dispense water from refillable five-gallon jugs are being distributed. A collaboration with the Tribal non-profit Plenty Doors Community Development Corporation (PDCDC) to raise funds for home plumbing repairs and upgrades has been successful, and PDCDC is working to implement a home repair program. Crow youth from fifth graders to graduate students are being mentored in researching and learning about local water resources from both scientific and cultural perspectives, through summer camps, research internships, and new courses at the local Tribal college. Impacts of climate change on water resources and health was researched both through western science and Elder interviews. Research results are being shared locally through community meetings and nationally through peer reviewed publications. Most recently, the CEHSC is collaborating with the USGS' Environmental Health program to conduct far more thorough assessments of Tribal home well and river water contamination, with initial sampling completed in August 2022. Existing Tribal home well water data is being reanalyzed employing USGS cumulative risk assessment methods and health benchmarks, and comparative results will be presented.

4.17.T-03 Community-Based Participatory Research Methods to Understand Spatial and Seasonal Variation in Drinking Water Disinfection Byproducts in Eastern Kentucky

Jason Unrine¹, Yogesh Gautam¹, Nina McCoy², Madison Mooney², Debi Sexton³, Shristi Shrestha¹ and Lindell Ormsbee¹, (1)University of Kentucky, (2)Martin County Concerned Citizens, (3)Kentucky Headwaters

Drinking water disinfection byproducts (DBPs) are the most common cause of health-based regulatory violations for drinking water in Kentucky and have been a source concern among community members. 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 remote mountainous landscape and declining resources available for maintaining and operating infrastructure.

Furthermore, there are complex seasonal and spatial patterns of DBP exposure within drinking water networks. In response, we have established a university-community partnership to help reduce exposure to disinfection byproducts in eastern Kentucky, focusing on Martin and Letcher Counties. This project uses citizen science to better understand how seasonal variation in source water chemistry, distribution network position, and home characteristics influence DBP exposure. Among the findings to be discussed are seasonal changes in bromide concentrations in source water as a driver of DBP formation as well as significant differences in bromide concentrations and bromide:chloride ratios among river basins. We will also discuss results from hydrologic models using KYPipe pipe flow software that are able to predict DBP concentrations in specific distribution network locations based on water age. These preliminary results have already yielded a better understanding of drivers of exposure in the region and provided utilities with tools that will enable them to optimize processes to help reduce DBP exposure.

4.17.T-04 Advances in Predicting Occurrence of Per- and Polyfluoroalkyl Substances (PFAS) in Groundwater Used for Drinking Water

Andrea Tokranov, Katherine Ransom, Bruce Lindsey, Miranda Fram, Laura Bexfield, Paul Stackelberg, Stefan Voss, Danielle Dupuy and Elise Watson, U.S. Geological Survey

Per- and polyfluoroalkyl substances (PFAS) have been associated with a range of human health effects. While extensive PFAS monitoring efforts are underway at both the state and federal level, analysis is expensive, time consuming, and not conducive to continuous monitoring. There is a need for predictive models to identify areas of concern for PFAS in drinking water resources. This study leverages a United States Geological Survey dataset on groundwater PFAS concentrations to build machine learning models to predict PFAS occurrence. Since 2019 the United States Geological Survey has monitored for PFAS through ongoing sampling by the National Water Quality Network groundwater (NWQN-GW) and California Groundwater Ambient Monitoring and Assessment Program – Priority Basin Project (GAMA-PBP). Wells are grouped by networks targeting drinking water supplies within a principal aquifer or target specific land uses, and largely consist of public-supply, domestic, or shallow monitoring wells. These long-term, established networks provide consistent and comparable information on groundwater quality and are used to assess groundwater quality trends and natural and human impacts. To date, over 1,200 groundwater samples covering a broad range of geographic regions, aquifer types, and land uses have been collected for PFAS using consistent sampling methods and analytical protocols. Between 24 and 28 PFAS compounds were measured for each sample. The total PFAS sum was converted to a binary dependent variable (detected or not detected) for modeling, as any PFAS detection indicates anthropogenic influence. The study goal is to build machine learning models to predict PFAS occurrence using only mappable factors to enable predictions in unmonitored locations where water quality data is unavailable. Over 40 potential predictor variables are being evaluated and include land use, well depth, depth to water, precipitation, population density, soil characteristics, and potential PFAS point sources on the landscape like fire training areas and airports. Preliminary results indicate that boosted regression tree models are one of the best performers of the models evaluated, and that urban land use (a proxy for sources of PFAS) and well depth (a proxy for the fate of PFAS in the subsurface) are top predictors for PFAS occurrence. This study aims to provide information to water resource managers and may be used to guide sampling to areas of predicted concern.

4.17.T-05 Contaminant Exposures and Estrogen Receptor Agonism Vary Strongly Within Community Drinking Water Systems and Across Bottled Water Sources

Thomas Michael Young and Gabrielle Black, University of California, Davis

Different water source types and varied land uses near those sources have been repeatedly shown to affect the nature and level of contaminants present in drinking water supplies, but far less is known about household level variability in either contaminants of concern or associated bioactivity measurements. In this study contaminants in eight California community water supplies representing four distinct source types (local surface water, distant

surface water, groundwater, and a mixture of those types) were characterized by collecting tap water from fifteen households in each community on two sampling dates each. Fifteen brands of packaged water were also tested twice for a total of 270 samples. All samples were analyzed using multiple instrumental techniques including both gas and liquid chromatography with high resolution mass spectrometry. Extracts from all samples were also measured for estrogen receptor agonism using the CALUX-ER α bioassay. This presentation focuses on the variability of GC-HRMS nontarget analysis and CALUX-ER α data. Although systematic community level variations in water quality were observed, a key finding of the study was that both ER α agonism and a number of organic compounds exhibited high levels of variability among in-group samples and across sampling date. Estrogen receptor agonism, for example, had within community coefficients of variation exceeding 200% for 6 community/time combinations, between 100-199% for 10 community/time combinations and below 100% for just two cases. This level of variability far exceeds that observed for triplicate analyses of each sample, which averaged less than 70% across the study. For the nontarget GC-HRMS data, between 7% and 22% of molecular features exhibited coefficients of variation in excess of 100%. Several of the compounds exhibiting the greatest variation within systems appear to be associated with distribution system components or household plumbing systems. Compounds of potential concern in this class include legacy compounds like polychlorinated biphenyls and organochlorine insecticides that may have accumulated in particular areas of the distribution system or plasticizers like acetyl tributyl citrate or phthalates that might be introduced by flexible piping components. Collectively, these results stress the need for household level measurements to accurately assess contaminant exposure and health risks associated with drinking water supplies.

4.17.T-06 Per- and Polyfluoroalkyl Substances in United States Tapwater

Kelly L. Smalling, Kristin M Romanok, Paul Bradley, Matthew Morriss, James Gray, Stephanie E Gordon, Brianna Williams, Sara Breitmeyer, Daniel K Jones, Laura DeCicco, Collin Eagles-Smith and Tyler Wagner, U.S. Geological Survey

Drinking-water quality is a rising concern in the United States (US), emphasizing the need to broadly assess exposures and potential health effects at the point-of-use. Drinking-water exposures to per- and poly-fluoroalkyl substances (PFAS) are a national concern, however, there is limited information on PFAS in residential tapwater at the point-of-use, especially from private-wells. We conducted a national reconnaissance to compare human PFAS exposures in unregulated private-well and regulated public-supply tapwater. Tapwater from 716 locations (269 private-wells; 447 public supply) across the US was collected during 2016-2021. Concentrations of PFAS were assessed by three laboratories and compared with land-use and potential-source metrics to explore drivers of contamination. The number of individual PFAS observed ranged from 1 to 9 with corresponding cumulative concentrations (sum of 16 detected PFAS) ranging from 0.348-346 ng/L. Seventeen PFAS were observed at least once with PFBS, PFHxS and PFOA observed most frequently in approximately 15% of the samples. Across the US, PFAS profiles and estimated median cumulative concentrations were similar among private wells and public supply tapwater. We estimate that at least one PFAS could be detected in about 45% of US drinking-water samples. These detection probabilities varied spatially with limited temporal variation in concentrations/numbers of PFAS detected. Benchmark screening approaches indicated potential human exposure risk was dominated by PFOA and PFOS, when detected. Potential source and land-use information was related to cumulative PFAS concentrations, and the number of PFAS detected; however, corresponding relations with specific PFAS were limited likely due to low detection frequencies and higher detection limits. Information generated supports the need for further assessments of cumulative health risks of PFAS as a class and in combination with other co-occurring contaminants, particularly in unmonitored private wells where information is limited or not available.

4.17.V Point-of-Use Drinking Water Exposome and Potential Human-Health Effects

4.17.V-031 Health Risk Assessment, Water Quality Indices and Reproductive Hormone Profile Among Abakpa Citizens in Enugu Metropolis, Nigeria

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This study was carried out to determine the health risk assessment, water quality indices and reproductive hormone profile among citizens of Abakpa in Enugu metropolis. Ten (10) water sampling points comprising of wells and streams were analyzed for chemical properties such as BOD, COD, DO, pH, TSS, TDS, TH, and Alkalinity and Heavy metal composition such as Zinc (Zn), Lead (Pb), Chromium (Cr), Cadmium (Cd) and Mercury (Hg) using the American Public Health Association's (APHA) guidelines and Atomic absorption spectrophotometer (AAS) respectively. Five (5) blood samples comprising males and females was collected across the sampling areas and assayed for reproductive hormone profile such as Testosterone, Follicle Stimulating Hormone, Progesterone, Prolactin, Estradiol and the enzyme Aromatase was using the ELISA kit method. The result of chemical properties such as TSS, TDS, COD, Total hardness and Alkalinity were all within World Health Organization (WHO) stipulated limits, whereas DO, BOD and pH were higher than WHO limits across all sampling points from the sampling area. Some parameters showed significance difference while some sample did not at different sampling locations at 95% confidence level. The result of heavy metals showed very high concentrations of analyzed metals with Hg present in five water samples from Abakpa at concentrations ranging from 0.01 to 1.96ppm. Pb concentration ranged between 0.06-0.35ppm; Zn 24.93-33.65ppm; Cr 0.00 – 0.23ppm; Cd. 0.11 – 0.17 ppm across all sampling points. All the result of reproductive hormone showed lower concentration of testosterone in men (1.99 to 2.19ng/ml) and high concentration in females (2.03 to 2.06ng/ml). The concentration of FSH was in the range of 0.67 to 0.77ng/ml in males, while the level was between 0.71 to 0.83ng/ml in female. Prolactin results was 3.49 to 3.86ng/ml for males and 3.44 to 3.59ng/ml in females. Estradiol levels were 8.48 to 9.54ng/ml and 9.61 to 9.84ng/ml for females and males respectively. However, progesterone level were between 34.08 to 37.45 ng/ml for females and 36.48 to 39.56ng/ml. The aromatase enzyme was averagely below 4.0 Pmol/mg in both male and female. The risk assessment showed low risk exposure but mercury had high risk exposure tendencies of 16.333 when compared to other heavy metals from all samples. Consumption of water in sampled area's may pose a serious health challenge; high concentration of heavy metals could be attributed to the hormonal imbalance.

4.18.P-We QSAR Models and Tools in Environmental Toxicology and Chemistry

4.18.P-We-099 External Validation and Characterization of Ecotoxicity Prediction QSAR Model KATE2020

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With growing concern for animal welfare, expectations are mounting for the use of alternative methods to animal testing such as quantitative structure-activity relationships (QSAR) in risk assessment. National Institute for Environmental Studies, Japan has developed "KAshinhou Tool for Ecotoxicity" (KATE), which predicts the acute and chronic toxicity of organic chemicals to fish, daphnid and algae by classification based on chemical structure and linear regression of toxicity values based on octanol/water partition coefficient. In KATE2020 ver. 4.0, released in March 2023, several improvements were made to achieve higher reliability, especially in the prediction model for fish and daphnid acute toxicities. The objective of this study is to externally validate KATE2020, not only to demonstrate its predictivity but also to characterize the model. Since many of the chemicals in KATE2020 training set are industrial organic chemicals (IOCs), we collected measured toxicity data of chemicals from the OECD Screening Information Dataset, which also contains IOCs, as test set for the external validation. Using the test set, the acute toxicity values for fish and daphnid were predicted using

KATE2020 ver. 4.0 and US EPA's ECOSAR ver. 2.2 to check that residuals between predicted and measured toxicity values were within a factor of 10. Only prediction results within the applicability domain (AD) regarding both a descriptor (log P) and structure were used for KATE2020. KATE2020 predicted 64% (fish) and 60% (daphnid) of the test set chemicals within AD, while ECOSAR does not determine the AD and was able to predict most of the test chemicals (fish: 94%, daphnid: 93%). In terms of prediction potency, KATE2020 showed 87% (fish) and 72% (daphnid) of the residuals were within a factor of 10, and ECOSAR showed 73% (fish), 64% (daphnid). It was revealed that KATE2020 predicts toxicity values closer to measured values within AD by ensuring interpolation in terms of log P and structure. On the other hand, KATE2020 could predict lower number of test set chemicals by setting stringent AD. Since KATE2020 further classifies chemicals in each representative structure class into "reactive" or "unreactive" class, results of the prediction potency for the both "reactive" and "unreactive" classes are shown and discussed in the meeting.

4.18.P-We-100 QSARs for Biodegradation of Chemicals in the Environment: Insights From New Data

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Environmental biodegradation rates are key data required for assessing the persistence (P), chemical fate, and exposure of organic chemicals. Despite its importance, a broadly applicable Quantitative Structure-Activity Relationship (QSAR) for predicting primary, aqueous environmental biodegradation rates is lacking. Existing prediction tools typically have one or more shortcomings such as: (i) only providing pathways and products but not rates, (ii) only use data from wastewater treatment plants and are not representative of the environment, (iii) they have narrow applicability domains (AD), and (iv) provide only qualitative rate information. Developing relevant and reliable QSARs for predicting biodegradation rates and half-lives are challenging because measured biodegradation rates are highly variable depending on the environmental and test conditions, and there is too much uncertainty in the data to allow for the development of adequate QSARs. A large database of existing biodegradation rates published up until ca. 2010 has been collected and used to calibrate QSARs, but the predictive power of these models is poor. More recently, several research groups have begun to re-evaluate testing methods using new knowledge and tools with a focus on controlling for and understanding sources of variability in the experimental data. A database of these new data has been compiled in this work, and new QSARs have been developed using the data. Preliminary comparisons to the QSARs based on the older data show that the new QSARs have improved predictive power. Environmental pH and chemical pKa have been identified as key factors which are important to consider for both the experimental measurement of biodegradation rates and the development of QSARs. The new QSARs currently have relatively narrow AD because of the limited data, so new knowledge gained will be applied to the older biodegradation data to identify reliable measurements and expand the AD of the new QSARs. These methods provide strategic guidance for future testing and addressing uncertainty in chemical assessments.

4.18.P-We-101 QSAR Evaluation and Development for the Prediction of Acute Responses in Fish to Exposure to Pesticides and their Degradates

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When little or no observed toxicity data are available for ecotoxicological hazard assessments of pesticides or their degradates, predictive toxicity tools such as Quantitative Structure Activity Relationship (QSAR) models may be used to assess the potential acute toxicity of chemicals in fish. While existing models for several chemical classes are available, the domain of data used to develop these tools often do not represent the broad range of pesticidal structures and modes-of-action that contribute specifically to pesticide toxicity. We evaluated the predictive accuracy of acute (LC50s) responses in fish for three existing models (ECOSAR, TEST, FishTox) specifically for pesticide parent structures and their degradates. Through the course of evaluation, we developed a fourth model using a random forest (RF) machine learning algorithm using strictly

pesticide data for 266 chemicals and 17 fish species. Candidate predictors for the RF model included a targeted set of physicochemical properties, three levels of the ClassyFire structure-based taxonomic classification system (superclass, class, subclass), pesticide target species, and test fish species and exposure type. A minimal set of predictors needed to maintain top RF prediction accuracy was: solubility, ClassyFire subclass, *n*-octanol/water distribution coefficient (LogD), pesticide target species, and test fish species. The RF model outperformed the other models across predictive metrics (mean absolute error [MAE], mean squared error, bias), whether summarized across individual chemical-species observations (MAE=4.3-fold cross validation [CV] error), or averaged across fish species response for each chemical (MAE=3.9-fold CV error). FishTox had the second-best performance but exhibited substantial bias (predicted > observed LC50 on average). ECOSAR outperformed TEST when assessed on the full dataset, but TEST had the best performance across models when the data were restricted to TEST's target species, fathead minnow. We further evaluated the top performing RF model on independent sets of 17 parent chemicals (MAE=6.4-fold error for mean response) and 32 degradates (5.0-fold error for mean response). The RF model likely had top predictive performance because: 1) the model was trained using only pesticide data, 2) the algorithm accounts for predictor interactions and non-linear relationships between predictors and toxicity, and 3) the model produces species-specific predictions.

4.18.P-We-102 Prospective Optimization, Evaluation, and Application of In Vitro Methods to Study Biotransformation of Organic Chemicals in Birds

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Avian species have a critical role in aquatic and terrestrial ecosystems but are understudied in the realm of bioaccumulation assessment and toxicokinetic studies. Regulatory frameworks such as the European REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) regulation require the provision of high-quality data from standardized bioaccumulation assays to inform their prospective risk assessment. Whole animal studies are often limited in throughput and raise ethical and economic concerns. Thus, *in vitro* biotransformation assays based on hepatocytes or liver sub-cellular fractions in combination with *in vitro-in vivo* extrapolation (IVIVE) models have been proposed as an alternative. In fish, such assays have been recently standardized. However, the development and adoption of avian methodologies have been slow to date. The present study seeks to bring avian biotransformation assays to a similar level as fish by optimizing and expanding the use of *in vitro* substrate depletion assays, focusing on isolated hepatocytes and liver subcellular fractions. Initial optimization efforts are focused on methods of isolation and cryopreservation, as well as the feasible assay conditions in both mallard duck and herring gull. These optimized assays will then be applied to ten organic chemicals, including PAHs, PCBs, bisphenols, organophosphates, and neonicotinoids, to generate high-quality substrate depletion data. This work also provides an opportunity to identify metabolites and evaluate biotransformation pathways and enzyme metabolism activities. Finally, at least two additional species will be selected for the application of optimized assays. This selection will take into account ecological relevance, practicality, as well as trait-based selection to contribute to a robust database of high-quality data. The prospective goal of this study is to lay the groundwork for a potential future ring trial, furthering the adoption of avian *in vitro* biotransformation assessment.

4.18.T QSAR Models and Tools in Environmental Toxicology and Chemistry

4.18.T-01 OPERA: Open-Source QSAR Models for Regulatory Support

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OPERA is a suite of over twenty QSAR models for toxicity endpoints and physicochemical, environmental fate, and ADME properties that follows the five OECD principles for QSAR modeling. It is a freely accessible

application that is based on the open-source/open-data concept. OPERA provides scientifically valid, high accuracy models with minimal complexity that support mechanistic interpretation, when possible. The models are built on thoroughly curated experimental data and standardized QSAR-ready chemical structures. OPERA has recently added models for ADME related parameters such as fraction unbound to plasma protein (Fu), hepatocyte intrinsic clearance (Clint), and Caco2 permeability (logPapp). The suite also includes three consensus models developed through international collaborative projects predicting estrogen and androgen pathway activity (CERAPP and CoMPARA), and acute oral systemic toxicity (CATMoS). In the latest version, most OPERA models including physicochemical properties and ADME parameters were updated with the latest publicly available datasets to improve their predictivity and applicability domain coverage, as well as to account for highly investigated groups of chemicals such as polyfluorinated substances (PFAS). The suite can generate predictions for single chemicals or in batch mode, and the chemical structure inputs can be processed using its internal QSAR-ready standardization workflow or provided via structure identifiers (CASRN, DTXSID, InChI Key) from its internal database of over 1M curated chemical structures from EPA's DSSTox. OPERA provide detailed prediction reports with accuracy estimates, applicability domain assessments, confidence ranges, and experimental values when available. Technical and performance details are described in OECD-compliant QSAR model reporting format (QMRF) reports. OPERA predictions are available through EPA's CompTox Chemicals Dashboard, the National Toxicology Program's Integrated Chemical Environment, and recently through FDA's Precision Platform. OPERA can be downloaded as a standalone command-line or graphical user interface for Windows and Linux operating systems from the NIEHS GitHub repository. It can also be run as a plugin within the OECD's QSAR Toolbox, and is provided as Python, C/C++ and Java libraries that can be embedded in other applications. *The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of any federal agency.*

4.18.T-02 EPI Suite™ Models and Applicability Domains: Cozying Up With Confidence and Exploring Estimates on the New Website

Lauren Cassidy, Mary Kawa and Laura Morlacci, (1)SRC Inc.

Module specific enhancements were made to the EPI Suite™ graphical user interface (GUI) to facilitate evaluation of each model's domain of applicability and provide data for read-across or analogue selection. The incorporation of Analog Identification Methodology (AIM) fragments is especially important as it allows users to see the robustness of a given estimation model based on the available data sets. By displaying analogues in conjunction with fragment counts, the model user can bridge the uncertainty gap for data-poor chemicals. These updates were made under guidance shareholders to. Given the large number of chemicals submitted each year for PMN creation and evaluation, EPI Suite™ program is one of the key programs used to assist in developing assessments of the hazards and risks of these materials under time constraints. Now, with the newly deployed web version of this software package under development, scientists can convey the logic and rationale of the modeled results and applicability domain considerations with a simple approach.

4.18.T-03 Addressing Uncertainty in Chemical Partitioning Properties and Prospects for Improvement

Trevor N Brown, Jon A. Arnot and Alessandro Sangion, Arnot Research and Consulting Inc.

Physical-chemical properties are a fundamental aspect of environmental toxicology and chemistry, and chemical evaluations. Commonly required properties include the octanol-water partition ratio (KOW), the air-water partition ratio (KAW) and the octanol-air partition ratio (KOA). These properties are key determinants of chemical fate in the environment, in organisms and in experimental test systems. When reliable experimental values are not available, physical-chemical property predictions are required and ideally the corresponding applicability domain (AD) and uncertainty estimates for the prediction of various properties for thousands of new and existing chemicals. Quantitative Structure-Activity Relationships (QSARs) and Quantitative Structure-Property Relationships (QSPRs) have been developed using the Iterative Fragment Selection (IFS) method for chemical degradation, partitioning, and other properties. IFSQSARs provide explicit estimates of AD based on

three complementary methods, and estimates of prediction uncertainty derived from external validation. We describe the general approach to developing and validating the IFSQSARs and compare their performance with other commonly used QSPRs (e.g., EPA's EPI Suite, OPERA models) and empirical data.

Uncertainty and AD are vital considerations when considering measured or predicted properties to inform decision making. For the majority of more than 100,000 chemicals in commerce, experimental data are not available for chemical evaluation. In these cases, QSAR predictions and their associated AD and uncertainties are the only tools available to assess how well we understand their partitioning, environmental fate, and the reliability of exposure modelling and risk estimation. We propose a systematic method to address uncertainty in existing experimental and predicted chemical properties by comparing the predictions of different QSARs and their AD to identify key knowledge gaps that can guide future experimental testing.

4.18.T-04 How Confidently Can Current Quantitative Structure-Property Relationships and Empirical Relationships Evaluate Chemical Properties to the Myriad of Chemicals in Commerce?

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More than 350,000 chemicals and mixtures registered in national and regional chemical inventories are awaiting assessments of their potential hazard concerns posed to human and ecological receptors such as persistence (P), bioaccumulation (B), mobility (M), and long-range transport potential (LRTP). According to the current regulatory criteria, chemicals' hazard potentials are assessed based on their intrinsic properties such as if a chemical with $\log K_{ow} > 2$, $\log K_{oa} > 5$, biotransformation half-life greater than 1200 hours, this chemical is flagged as bioaccumulation. Since the limitation in experimental measurement data of chemicals' intrinsic properties, quantitative structure-activity relationships (QSARs) are commonly used to predict chemicals' intrinsic properties. In addition to QSARs, semi-empirical relationship is commonly applied to predict hardly predicted properties from easily predicted properties such as Karickhoff relationships is user-friendly tools to predict K_{oc} from K_{ow} . In this presentation, it is imperative to evaluate the applicability domain (AD) of QSARs and semi-empirical relationships to ensure their suitability for the assessment purpose. Here, we investigate the extent to which the ADs of commonly used QSPRs and semi-empirical relationships cover seven partitioning and reactivity properties of 112,000+ chemicals registered in five comprehensive chemical inventories. The results from this study show that more than half of investigated chemicals are hydrophilic, lowly volatile and labile. The current QSARs can cover ~80% of chemicals in AD in terms of predicting K_{ow} , K_{oc} and biotransformation half-lives. However, K_{oa} medians for chemicals in and out of AD differ by 6 orders of magnitude. In addition, current QSARs exhibit a narrower AD coverage of air hydroxylation rate constant and biodegradation half-lives, compared to other properties. The current regulation applies biodegradation half-lives as criteria to assess persistent chemicals and applies air hydroxylation rate constant to assess chemicals' long-range transport potential. Thus, it is important to exercise caution when utilizing current QSARs for persistence and long-range atmospheric transport assessment. The AD coverage for semi-empirical relationship depends on how the AD is defined. For the first time, this presentation systematically informs academia and regulatory agencies on the applicability and uncertainty of the QSARs and semi-empirical relationships.

4.18.T-05 Development of Species-Specific Alcohol Ether Sulphate and Alcohol Sulphate QSARs for Use in Species Sensitivity Distributions

Kristin A Connors, Brian Morris and Jessica Brill, Procter & Gamble

Alcohol ether sulphates (AES) and alcohol sulphates (AS) are widely used anionic surfactants used in personal care products. AES/AS materials contain a distribution of alkyl chain lengths and number of ethoxylate groups. AES acute toxicity has been shown to increase with increasing chain length (CL) and decrease with increasing ethoxylates (EO). Historic AS/AES data encompasses a range of CL and EO distributions. A data normalization approach is needed to allow for the development of Species Sensitivity Distributions (SSDs) for a given CL/EO of interest. Previous work established *D. magna* and *C. dubia* acute AES/AS QSARs. It is unknown if other

trophic levels (e.g., fish) have parallel changes in sensitivity to variations in CL/EO. In this work, a series of highly characterized, pure-CL and EO materials were tested in an OECD 236 fish embryo toxicity test. These results were used to develop highly-local acute structure-activity relationships for AS and AES to support environmental effects assessment of AES as a mixture. Invertebrate and FET QSARs will be leveraged for data normalization and SSD generation.

4.18.T-06 In Silico Molecular Docking Simulations and Affinities for the Metabolism of Methoxylated Polybrominated Diphenoxybenzenes by Human CYP1B1, 3A4, 1A1/4, and Herring Gull CYP1A4/5 Enzyme Proteins

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Methoxylated polybrominated diphenoxybenzenes (MeO-PB-DiPhOBzs) are novel bioaccumulative contaminants that have been measured in both herring gulls (*Larus argentatus*) and their eggs from nesting sites in the Laurentian Great Lakes basin. MeO-PB-DiPhOBzs accumulate in soil, and herring gulls are exposed to them via the diet. While MeO-PB-DiPhOBzs have been shown to accumulate in herring gulls, it is unknown if they can demethylate to hydroxy-PB-DiPhOBz congeners, which would likely be an important for physiological elimination. The present study examined *in silico* docking simulations and revealed the potential mechanisms and relative kinetics of MeO-PBDPB metabolism. Simulations employed human cytochrome P450 enzymes including CYP1B1, 3A4, 1A1, and 1A2. Using available partial amino acid sequences for herring gull CYP1A4 and 1A5, complete protein homology was modelled using *h*-CYP1A1/2 as templates and docked with the same suite of up to 100 MeO-PBDPB congeners. Human CYP450 and active site templates were built from crystal structures from the Research Collaboratory for Structural Bioinformatics Protein Data Bank. Docking was performed using the internal coordinate mechanics software by MolSoft. Ligands were docked into each model using a flexible ligand and rigid protein structure. Congener binding positions were predicted by a Monte Carlo global optimization procedure, with a score produced by the virtual ligand screening scoring function. 3D quantitative structure activity relationships (3D-QSARs) were created using the MolSoft Atomic Property Field method wherein 3D pharmacophoric potential is generated based on empiric physico-chemical components including hydrogen bond donors/acceptors, Sp² hybridization, lipophilicity, size, electropositive/negative and charge. Mean scoring values for MeO-PB-DiPhOBz docking in each human CYP isoform ranked in the order CYP3A4 > CYP1B1 > CYP1A1 > CYP1A2. This is consistent with the affinity of CYP1A enzymes for coplanar dioxin-like substrates when compared to CYP3A4 and 1B1 which bind more globular ligands. Relative scores for MeO-PB-DiPhOBz congeners were greater in congeners with lower degrees of bromination. *In silico* docking simulations estimated that MeO-PB-DiPhOBz congeners have differing affinities for human CYP isoforms, which were different than for herring gulls. These results suggest that metabolism is influenced by MeO-PB-DiPhOBz structure and thus the fate and persistence in exposed humans and gulls.

4.19.P-Mo-124 Time-course of Physiological Changes in Brook trout (*Salvelinus fontinalis*) During Exposure to Lethal Concentrations of 6PPD-quinone

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Recent studies have implicated a tire wear leachate, *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone), to be responsible for the mass mortality of Coho salmon (*Oncorhynchus kisutch*) exposed to urban stormwater runoff. Of the species that have been tested, only select salmonids were sensitive, and Brook trout (*Salvelinus fontinalis*) are second only to Coho salmon in their sensitivity. The mechanism of action of 6PPD-quinone is not well understood, studies suggest that metabolic, respiratory, cardiovascular, and/or neurological toxicity all playing a role in the observed effects. To address this data gap we conducted 3.5 hr exposures to the 6 hr LC₉₅ concentration of 6PPD-quinone with juvenile brook trout (175.8 +/- 47.8g) and sampled individuals after 1, 2, 3, and 3.5 hrs of exposure to measure changes in the blood chemistry and gill tissue prior to mortality. Blood parameters were measured using an i-STAT blood analyzer CHEM8+ and

CG4+ cartridges, which measure a suite of analytes. The cartridges and analyzer have previously been used with fish blood samples to measure ion dysregulation. Hematocrit was also measured in a larger subset of fish with glass capillary tubes, and gill structure changes were examined using histology. Exposure concentrations were measured at the start and end of each sampling duration. Changes in blood chemistry were evident in asymptomatic fish within 1 hr of exposure, with significant increases in hematocrit, calcium, potassium, and decreases in glucose, chloride, sodium, bicarbonate, total carbon dioxide concentrations, blood pH, and oxygen saturation. After 2 hrs of exposure select individuals began to show behavioral symptoms associated with 6PPDq exposure including gill flaring and erratic swimming. There were also additional increases in blood lactate and an even more pronounced increase in blood potassium concentrations. By 3 hrs of exposure morbidity and mortality was beginning to be observed in the exposed fish and the blood parameters measured were similar to the effects observed at 1 and 2 hrs. Histological examination of the gill filaments revealed morphological changes consistent with mounting a response to osmotic dysregulation. Ion dysregulation and gill inflammation appear to be contributing factors in 6PPD-quinone toxicity to juvenile Brook trout. The changes in blood chemistry we observed give insight into the possible mechanism of action of 6PPD-quinone in sensitive salmonid species.

4.19.P-Mo-125 Analysis of 6PPD-Q and Other Rubber-Derived Chemicals (TRCs) in Fish Bile and Plasma

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Several chemicals are used during tire manufacturing process to protect tire rubber from oxidizing and fast wearing. They eventually leach out and can be widely present in urban stormwater runoffs. These so-called tire rubber-derived chemicals (TRCs) can compose 10-14% of the tire composition. 6PPD [*N*-(1,3-dimethylbutyl)-*N'*-phenyl-*para*-phenylenediamine] is one TRC found in urban waters with a particular concern. In 2021, it was determined that a quinone transformation product of 6PPD was one of the key compounds responsible for the pre-spawn mortality of coho salmon (*Oncorhynchus kisutch*). This finding triggered a number of studies worldwide, developing analytical methods for measuring 6PPD and its quinone derivative 6PPD-Q, as well as determining their toxicity in different fish species. We developed a method for analysis of fish plasma and bile for a number of TRCs commonly found in stormwater runoffs, including 6PPD-Q, 6PPD, N,N'-diphenylguanidine (DPG), N,N'-dicyclohexylmethylamine (DCA), and others. Our analytical method includes a solid-phase extraction approach and final analysis by liquid-chromatography coupled with tandem mass spectrometry. This method proved to be fast, relatively inexpensive and sensitive, with a limit of quantitation around 10-100 pg/mL of sample and efficiency recovery ranging between 60-120%. We applied this new method in bile and plasma of English sole (*Parophrys vetulus*) collected from urban, near-urban and non-urban areas of Puget Sound, WA. Our data showed 6PPD to be one of the primary TRCs measured in both bile and plasma samples reaching up to 80ng/mL of bile and 0.7ng/mL of plasma. 6PPD-Q was also measured in several samples primarily detected in urban sites. The levels of TRCs measured were positively correlated with land percent of impervious surfaces in the adjacent areas to the sampling sites. In addition to the different levels of TRCs, their individual distribution in both bile and plasma samples were crucial to provide site. To the best of our knowledge this is the first set of environmental data for this selection of TRCs in fish bile and plasma samples and represent baseline levels for Puget Sound, WA for a benthic fish species.

4.19.P-Mo-126 Chronic Effects of Dietary Administered Tire Particles in Common Carp

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Since tires account for 30–55% of terrestrial microplastics emissions, exposure and toxicity assessments of tires

and tire additives have been conducted. Although there are many reports on their acute toxicity, little has been reported on their chronic toxicity. In this study, we examined the growth and hematological and histological changes in common carp (*Cyprinus carpio*) after oral administration of tire particles. Cryo-milled tire tread (CMTT) samples were obtained from USTMA. We set a 28-day exposure period and a 7-day recovery period. Nine glass tanks were prepared, and seven carp (mean body weight: 1.86 g) were placed in each tank. CMTT was supplemented to the diet at 0%, 2.5%, and 10%, and one of these diets was fed at 3% of the fish weight per day. Thus, the doses were 0 (control), 0.75 (low), and 3.0 (high) mg/g-b.w./day. After the exposure period, the body length and weight of all the fish were measured. Blood samples were taken from five out of seven fish in each tank for hematological analysis. The digestive tract, hepatopancreas, spleen, trunk kidneys, and heart were also fixed for histological observations. The remaining two fish were pooled in one tank per treatment, and they were fed tire-free diets for one week. After a recovery period, these fish were dissected for the same analyses as above. No mortality of test fish was observed during the whole experimental period. CMTT administration had no effect on carp growth or condition factor. Hematological analysis showed a significant decrease in hemoglobin concentration (control: 6.1, low: 5.1, high: 4.9 g/dL) and hematocrit level (32.6%, 27.9%, 27.5%). On the other hand, total blood cell counts did not differ between treatment groups. Histological observations revealed that tire exposure caused slight swelling of hepatocytes and a decrease in the cytoplasmic vacuoles (probable lipid droplet), but no significant histological features were observed in the other organs examined. Tire particles were observed only in the intestinal tract and not found elsewhere, suggesting that the hematological changes were due to the tire additives rather than to the effect of tire particles. However, this hematological change tended to recover after a 7-day recovery period, indicating that the effect was reversible. Although the effects observed in this study were less severe, there is concern that chronic hematologic changes may cause energy metabolism and other physiologic consequences.

4.19.P-Mo-127 Effects to Marine Amphipod Exposed to Particles and their Dissolved Components Derived From New Or Used Tires

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The tire particles have been generated by the friction between tire and road surface, suspended in the atmospheres, and then settled land and aquatic environment. A part of them achieves to sediments in the coastal areas, and is residue for long period. The tires are made from the rubber, antioxidant, compound and reinforcing agents, and others, and contained over 100 kinds of substances. Recently, possible toxicities of 6-PPD quinone as an oxide of 6-PPD in the tire to fish was reported. However, other information for the toxicities to aquatic organisms is limited, and required to be revealed urgently. Although the most of particles could be in the sediments at the coastal areas, the various organisms inhabit there. The materials from settled tire particles on sediments have any effects to aquatic organisms, the pollutions could seriously impact even to the food chains composed the complex in sediments. The marine amphipods such as *Ptilohyale barbicornis* are a typical benthic invertebrate and an important feed to a lot of organisms. In the present study, the effects to *P. barbicornis* by the exposures of tire particles or their effluents into the sea water were evaluated with preparing 2 tires produced the different tire makers. Additionally, we also prepare both a new and a used one of individual tires. Each tire particles settled to the bottom of glass vessels with the gradually increasing the particles for individual tires. In a glass vessel, an amphipod was kept, and exposed to the particles for 2 weeks. As a result, we observed that the each LC50 was 0.01 and 0.02 mg/mL for 2 new tire particles, respectively, while LC50s for used tires were 0.03 and 0.2 mg/L, respectively. Additionally, each effluent was also resulted 0.9 and 1.0 mg/mL for new, and 0.8 to 1.0 mg/L for used, respectively. The toxicity variations of particle derived from new tires depending on the exposure time tended to remain lower than those from used. However, LC50s derived from effluents varied similar with new and used with exposure time. These results suggest that the particle

toxicities were reduced depending on the driving distances, however those of effluents did not depend on the distances. A tire has several layers made from many components, and, therefore, the several different layers were continuously emerged to the tire surface.

4.19.P-Mo-128 Gaining Traction: The Investigation of Alternatives to 6PPD and Their Toxicity to Coho Salmon (*Oncorhynchus kisutch*)

Caitlin Lawrence and Jenifer McIntyre, Washington State University

6PPD is a critical component of automobile tires that reacts with ozone in the environment to prevent rubber from cracking. The recent identification of 6PPD-quinone, a transformation product of 6PPD, as the causal toxicant of urban runoff mortality syndrome in coho salmon (*Oncorhynchus kisutch*) has led to increased efforts to find an alternative for 6PPD use in tires. Although several alternative chemicals to replace 6PPD have been proposed, there is little to no information on if the alternatives and their transformation products are toxic. 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, but they also have the potential to produce quinones that have unknown toxicity. This project will investigate the toxicity of proposed alternatives in the PPD family, including both the toxicity of the parent compounds and their potential transformation products. Acute toxicity testing will be performed on coho salmon before and after ozonation of the alternative PPDs to assess toxicity relative to that of 6PPD and its transformation product, 6PPD-quinone. The goals of this project are to: (1) determine the LC50s of 6PPD and the alternative parent compounds for coho salmon; (2) determine if the potential transformation products of the alternatives are less toxic to coho salmon than 6PPD-quinone; (3) and to identify the transformation products formed during ozonation of the alternatives.

4.19.P-Mo-129 Rolling Out Guidance: What Environmental Officials Need to Know About 6PPD and 6PPD-quinone

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In 2020, scientists at the University of Washington-Tacoma and Washington State University identified the 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 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, the tire antiozonant, 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 first product (a focus sheet in prep, anticipated issuance 9/2023) presents the latest science on toxicity, fate and transport, and assessment and mitigation strategies for the emerging contaminants of concern, 6PPD and 6PPD-quinone. The ITRC 6PPD Team will assemble a panel consisting of the nation's leading researchers and experts that will utilize the ITRC's 6PPD and 6PPD-quinone focus sheet 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.

4.19.P-Mo-130 When the Rubber Meets the River: An Assessment of 6PPD-Quinone Acute and Sublethal Toxicity on San Francisco Bay Delta Species of Conservation Concern

Jackie Lang, Louise Cominassi, Andrea Chandler, Luann Wong, Nann Fanguie, Thomas Michael Young, Florian Mauduit, Richard Connon and Amelie Segarra, University of California, Davis

Tire and road wear particles are an inevitable consequence of tire use in automotive traffic and are of particular concern in near-urban ecosystems. 6PPD-quinone, a toxic transformation product of a common tire additive, has been detected in water samples from San Francisco region creeks at concentrations near and above the LC50 for coho salmon. The presence of 6PPD-quinone in the San Francisco Bay Delta region thus presents a potential risk to local aquatic species. The goal of this project is to assess the toxicity of 6PPD-quinone to five species of conservation concern: Coho Salmon, Chinook Salmon, Steelhead, Longfin Smelt, and Delta Smelt. Larvae and juveniles of each species were tested for acute toxicity with ongoing investigations of sublethal toxicity. Sublethal endpoints include behavior, thermal tolerance, and swimming performance to assess potential neurotoxicological and cardio-respiratory effects of 6PPD-quinone exposure. Results suggest interspecies differences in susceptibility, with coho salmon and steelhead showing acute sensitivity while Chinook Salmon, Longfin Smelt, and Delta Smelt are acutely insensitive. The lack of acute sensitivity in Chinook Salmon suggests intraspecies differences in susceptibility, given a recent report of susceptibility in a different population from the Harrison River in British Columbia, Canada. The results from this study will directly inform ongoing restoration efforts for these critically endangered species.

4.19.T Shedding Light on Tire Wear Microplastics, From Transport and Fate to Toxicity and Management

4.19.T-01 Best Practices in the Analysis of 6PPD-Quinone

Million Woudneh, Bharat Chandramouli, Coreen Hamilton and Connor Taggart, SGS AXYS Analytical Services Ltd.

6PPD-quinone (6PPDq), an oxidation product of a common tire antioxidant 6PPD, has been identified as one of the most toxic compounds to fish, particularly Coho salmon with an updated LC-50 (juvenile) of 41 ng/L. The acute toxicity of 6PPDq and its entry into ecosystems has raised significant concern. Analytical methods have appeared in refereed journals for the quantification of this analyte in the environment. However, various aspects of the sampling and analytical conditions useful for accurate and reproducible quantification of 6PPDq are not standardized leading to significant concerns on data quality and benchmarking. In this study, we investigated best practices in the analysis of 6PPDq. We performed sample adsorption experiments with multiple container types and sample types to identify best practices for sample collection. We performed a 35-day stability study at multiple storage temperatures and in the presence/absence of multiple preservatives including ascorbic acid. We studied the effect of sample pH and sample filtration. Our study also included a 28-day study on the stability of analysis extracts. All these experiments were performed using an ultrahigh-pressure liquid chromatography-tandem mass spectrometric method with whole sample extraction, silica-based extract cleanup and isotope dilution quantification that was validated in aqueous, soil and sediment samples. We also used this method to study 6PPDq and 6PPD occurrence and seasonality. Results from this study show that 6PPDq is stable in samples for 35 days at 4°C, and that certain container materials can adsorb 6PPDq possibly leading to reduced reported concentrations. The data derived best practices proposed in this study can be used by other laboratories and researchers to generate accurate and reproducible data that policy makers can rely on.

4.19.T-02 Occurrence of 6PPD-Q in Aquatic Biota from Puget Sound, WA

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The tire manufacturing process includes chemicals that are used to reduce oxidation and premature tire wear.

These chemicals can account for 10-14% of a tire's total composition and have been identified in urban stormwater runoff. Currently, greatest concern is for the tire rubber anti-oxidant 6PPD [*N*-(1,3-dimethylbutyl)-*N'*-phenyl-*para*-phenylenediamine] and its quinone environmental transformation product, 6PPD-Q. The latter has been directly associated with pre-spawn mortality of coho salmon (*Oncorhynchus kisutch*). Our lab has developed methods for analyzing 6PPD-Q in a variety of matrices including surface water, shellfish, salmon and other finfish and marine mammal tissues. Both GC-MS/MS and LC-MS/MS are used with several types of extraction and clean-up methods. Initial results from monitoring three urban creeks in the Seattle, WA metropolitan area found 6PPD-Q levels are often greater than 20 ng/L with peak levels near 80 ng/L. Highest levels were observed in the fall and winter months during storm events. Ongoing analysis of transplanted caged mussels (*M. trossulus*) deployed in the winter throughout Puget Sound has shown 6PPD-Q at levels between 0.6 ng/g and 3.1ng/g wet weight (w/w). Preliminary studies of 6PPD-Q in juvenile Chinook salmon (*O. tshawytscha*) whole bodies suggest detection frequency below 15% with measured levels below 1 ng/g w/w. We have also attempted to quantify 6PPD-Q in archived killer whale (*O. orca*) blubber, which so far has not been detected. These studies are designed to aid exposure assessments to 6PPD-Q and better understand the potential for tire rubber compounds to bioaccumulate in marine food webs.

4.19.T-04 Life Stage and Environmental Conditions Affect Toxicity of 6PPD-Quinone to Coho Salmon

Garrett Foster¹ and Jen McIntyre², (1)Washington State University, Puyallup, (2)Washington State University
Tire rubber and the microplastics that break off tires on roadways contain up to 2% of an added antidegradant called 6PPD. 6PPD reacts with atmospheric ozone, creating over 30 byproducts including 6PPD-quinone, which kills coho salmon in just hours at concentrations as low as the parts per trillion. The discovery of this fact in late 2020 spurred a rapidly growing body of research into the effects of 6PPD-quinone on other fish species, techniques for 6PPD-quinone pollution mitigation, alternative antidegradants for tires, and risks from human exposure to 6PPD-quinone. However, there's still much to learn about the toxicity of 6PPD-quinone to coho salmon. Interactions with water quality and life stage could give us clues to 6PPD-quinone's metabolism and toxicodynamics in coho. Better understanding of what makes 6PPD-quinone so toxic to coho could show us how tire rubber and urban stormwater runoff infrastructure need to change to protect this valued species and could inform us about whether 6PPD-quinone is dangerous to other species, including our own. To study life stage sensitivity, we exposed coho embryos episodically to 6PPD-quinone from fertilization through hatching into alevin. We found that coho embryos were far less sensitive to 6PPD-quinone than parr, probably due to their thick protective chorions, but are comparable to parr immediately upon hatching. In our experiments to understand how 6PPD-quinone toxicity is affected by water quality, we exposed coho salmon parr to 6PPD-quinone at lethal concentrations at environmentally relevant ranges of temperature, pH, ionic strength, and natural organic matter. Our primary response variable was mortality, but we also collected livers for mRNA expression analysis by quantitative polymerase chain reaction. Temperature was the only water quality parameter that had a strong observable effect on the toxicity of 6PPD-quinone to coho parr. Concentration-response data was closely aligned for temperatures from between 5°C and 18°C, but at 20°C 6PPD-quinone was significantly less toxic. We're still gathering results on mRNA expression but hypothesize that higher detoxification gene upregulation at the warmer temperature is what leads to the reduction in toxicity. Understanding the genes and enzymes involved in this detoxification pathway could help us identify what chemical characteristics of 6PPD-quinone make it so toxic to coho and other sensitive species.

4.19.T-05 Toxicity of 6PPD-Quinone Across Fishes of Commercial, Cultural, and Ecological Importance

Markus Brinkmann¹, Catherine Roberts¹, David James Montgomery¹, Summer Selinger¹, Hannah Mahoney¹, Francisco da Silva¹, Justin Miller², Eric Stock², Alper James Alcaraz¹, Jonathan Karl Challis¹, Neil Mochnacz³, Andrew Chapelsky³, Lynn Weber¹, David M. Janz¹, Steve Wiseman⁴ and Markus Hecker¹, (1)University of Saskatchewan, Canada, (2)University of Lethbridge, (3)Department of Fisheries and Oceans, Canada,
Stormwater runoff from urban landscapes has recently been linked to mass mortalities of coho salmon in the

U.S., also dubbed urban runoff mortality syndrome (URMS). The chemical responsible has been identified as N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q), a transformation product of the rubber tire antioxidant 6PPD. This presentation summarizes a series of studies that assessed the acute toxicity of 6PPD-Q across eight other fishes of commercial, cultural, and ecological importance in North America, and to characterize the specific mechanisms that drive toxicity using acute and sub-chronic experiments with select fishes. Acute toxicity differed greatly among species: No mortality occurred for Arctic char (AC), brown trout, bull trout, westslope cutthroat trout, and white sturgeon even at the highest measured concentrations (>13 µg/L), while lake (LT), brook, and rainbow (RBT) trout were sensitive to 6PPD-Q exposure with LC50 values between 0.33 and 0.59 µg/L. Furthermore, sub-chronic exposures of RBT and LT demonstrated teratogenic effects in both larval RBT and LT. Initial experiments with primary cultures of RBT gill cells suggest that the mechanism of toxicity may be related to uncoupling the mitochondrial electron transport chain. Comparative cardiac ultrasound, electrocardiography and blood gas analysis revealed significant decreases in hemoglobin oxygenation and sympathetic stimulation in sensitive RBT but not insensitive AC, further supporting this hypothesis. Whole transcriptome analysis in RBT identified several molecular toxicity pathways that may explain the apical effects described above. Further research is underway to develop a comprehensive toxicity pathway model supporting risk assessment of 6PPD-Q across fishes.

4.19.T-06 Investigating the Modes of Action for 6PPD-Quinone Toxicity in Salmonids

Justin Greer, Ellie Dalsky, Rachael Lane, Prarthana Shankar and John Hansen, U.S. Geological Survey
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 PNW. Induction of 6PPDQ mortality at low concentrations has only been demonstrated in select teleost fish belonging to the Salmoninae subfamily. Confoundingly, other Salmoninae species are not sensitive, and overall species sensitivity does not appear to have a discernable pattern with established species phylogenies. Investigations into the mechanisms of action are critical to better understand this highly variable species sensitivity that creates significant obstacles in evaluating the large-scale environmental impacts to biota. Our lab is currently investigating the modes of action for 6PPDQ using a suite of *in vitro* cellular assays and *in vivo* exposures in coho salmon to better elucidate the underlying mechanisms of toxicity. *In vitro*, immortalized cell lines have shown promise in recapitulating species-specific toxicity of 6PPDQ, including metabolic and cytotoxic effects in cells derived from coho salmon. Similar effects are not elicited in Chinook or sockeye salmon cell lines, similar to *in vivo* toxicity studies. A comprehensive survey the transcriptional changes associated with exposure *in vivo* has also been performed using whole-transcriptome sequencing in coho salmon alevin following brief 6PPDQ exposure. Molecular responses indicated that pathways governing organismal development and endothelial signaling may be substantially altered by 6PPDQ. Alterations in endothelial signaling are consistent with macromolecule accumulation in the brain of exposed animals, and therefore may represent a prominent mechanism of toxicity. Further refinements of mechanism of action can be used to identify other potentially susceptible species and further our understanding of the impacts of 6PPDQ on global aquatic health.

4.19.V Shedding Light on Tire Wear Microplastics, From Transport and Fate to Toxicity and Management

4.19.V-012 Within and Across Generational Effects of Tire Wear Micro- and Nanoparticles in the Model Estuarine Species Fish *Menidia Beryllina*

Clarissa Raguso^{1,2}, Susanne M Brander² and Lauren Miki Kashiwabara², (1) Milano-Bicocca University (2) Oregon State University

Tire wear particles (TWP) represent one of the most commonly detected microplastic types in the environment. They are released by tires undergoing due to friction on the road and enter the environment by being transported

by wind, gravity, and currents, causing direct or indirect adverse impacts on marine and terrestrial organisms. Considering the limited available data regarding the tire particle toxicity in aquatic organisms, this study aims a) to provide information about TWP exposure impacts on survival, internalization, growth, and behavior and b) to investigate possible lasting transgenerational effects impacting future generations in the model fish species Inland Silverside (*Menidia beryllina*). Specifically, 5-day post fertilization embryos were exposed to micro (1-20 µm) and nano (< 1 µm) tire particles, created from a mixture of different tire types, at four concentrations (10, 100, 1000 and 10,000 particles/ml) and to TP leachate. After the 96h exposure, behavioral assays were performed using a DanioVision Observation Chamber (Noldus, Wageningen, the Netherlands) where each fish was subjected to a dark: light cycle stimuli. Growth measurements were assessed using a weight:length index, and organisms were cleared with CUBICTM clearing reagents (Cubic-L and Cubic-R solutions) to visualize and count the internalized particles. A subsample of exposed fish are also being reared in clean water to the larval stage of the F1 generation to assess possible indirect transgenerational effects on survival, growth, particle internalization, and behavior. Previous studies have already demonstrated tire particles to have behavioral toxicity and negative effects on survival and growth in *Menidia beryllina*. This study will provide additional important knowledge on longer term responses to tire particles, leading to a more comprehensive perspective of what organisms are experiencing in the real environment.

4.19.V-028 Investigating the Effects of Car Tire Additives to a Fresh Water Amphipod

Henry Ebele Obanya, Fay Couceiro and Alex Ford, University of Portsmouth

Behaviour in ecotoxicology is expanding as it provides a link between the biochemical and physiological effects of environmental contaminants. This has been facilitated by advancements in computational automaton and the increased prevalence of behavioural modulating compounds in the environment. The main aim of this study is to assess the effects of car tire additive compound, 6PPD quinone, 2-Mercapto Benzothiazole (2-MBT) and Diphenyl guanidine (DPG) on the behaviours of model crustaceans, *Gammarus pulex*. A developed assay on a Zantiks unit was used to measure locomotion as a kind of behaviour associated with stress on the invertebrate. The Gammarids were exposed to environmentally relevant concentrations (100µg/l, 1 µg/l, 0.1 µg/l, 0.01 µg/l) of the car tire additive compounds. Exposure to these compounds had significant impacts on the behaviour of these Gammarids. The animals moved rapidly when light came on after 2 minutes, but they get slower with time. There was significant difference found in the mean distance moved between day 7 and 14. The movement of Gammarids were affected over time with most effect found in 100 µg/l. The results obtained showed that the compounds affected the locomotion behaviour of the Gammarids after 7days and 14days even at environmentally relevant concentrations. This may then mean that the presence of these compound in the environment may affect their behaviour in the wild as impaired locomotion can hinder their capacity to escape predation, alter reproduction and generally affect population.

4.20.P-Th The Behavior, Fate and Impact of an Increasingly Complex Array of Contaminants in Changing Arctic and Antarctic Environments

4.20.P-Th-119 Trends of Perfluoroalkyl Substances (PFAS) in Seawater and Ringed Seals from Resolute Bay, Nunavut, Canada

Magali Houde¹, Derek C.G. Muir¹, Peter Amarualik², Jane Kirk¹, Christine Spencer¹, Mary Williamson¹ and Amila O. De Silva¹, (1)Environment and Climate Change Canada, (2)Resolute Bay, Canada

Per- and polyfluoroalkyl substances (PFAS) are under intense scrutiny for their environmental persistence. In this study, the trends and accumulation of perfluoroalkyl carboxylic acids (PFCA) and sulfonic acids (PFSA) were measured over time in seawater and liver of ringed seals (*Pusa hispida*) collected around Resolute Bay, Nunavut in the Canadian Arctic. Seawater was collected in Barrow Strait between 2005 and 2021 at depths of 10-100 m. Ringed seal liver samples were collected during Inuit subsistence hunting between 1992 and 2019. Seal liver and seawater were analyzed for C4-C14 PFCA and C4-C12 PFSA by LC-MS/MS. In seawater, the

majority of the chain lengths (e.g., PFBA, PFHxA, PFOA, PFNA, and PFOS) declined in concentrations between 2010 to 2021 with the most recent sum of PFAS levels at 2.0 ng/L. Under-ice depth profiles from 0 to 100m, sampled in late May showed inverse correlations of PFAS concentrations with major ions – sodium, potassium, magnesium and potassium. These results suggested surface inputs of PFAS, likely through snow and ice melt. In ringed seals, higher sumPFCAs (C6-C16) hepatic concentrations were generally observed between 1998-2004 and 2014-2019, with lower levels measured during the period of 2008-2013. Sum PFASs (C4-C12) concentrations were relatively stable in seal livers between 2005-2013 (5 to 9 ng/g ww). From 2014-2019 the sumPFASs increased with the most recent year having an average of 19 ng/g ww. Taken together, trends of mean PFCAs and PFASs over the past decade seem to be different in seawater and seal liver. Many factors may explain these differences including the influence of diet and foraging on the accumulation of PFAS in ringed seals and the input of melting permafrost and snow/ice to seawater. The pattern of PFAS also varied with shorter chain PFAS dominating seawater whereas the more bioaccumulative PFAS with ≥ 8 carbons were prevalent in pinnipeds. Together these results demonstrate the value in both abiotic and biotic sampling to track Arctic temporal trends. To our knowledge this is the first time that measurements of PFAS in water and in marine mammals at the same location over time have been carried out. Our findings highlight that though PFCA and PFSA use and emissions are declining, their persistence, mobility, and cycling in the global marine environment contribute to their ubiquitous presence in the Arctic in the present day.

4.20.P-Th-120 Influence of Climate Related Factors on the Temporal Trends of Perfluoroalkyl Substances and Polychlorinated Biphenyls in Landlocked Char in Two High Arctic Lakes

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Temporal trends and climate related parameters affecting the fate of and perfluoroalkyl substances (PFASs) and polychlorinated biphenyls (PCBs) were examined in landlocked Arctic char (*Salvelinus alpinus*) in two paired High Arctic lakes on Melville Island (Nunavut, Canada). Permafrost disturbances as well as subaqueous slumps in West Lake that began in 2008, have increased turbidity 50x, while the water chemistry of neighboring East Lake has not been drastically impacted. These lakes have similar catchment/lake area ratios (West – 8.0 km²/1.4 km²), East – 11.6 km²/1.6 km²) and, given their remote location, on uninhabited Melville Island, they receive PCBs, PFAS and other pollutants solely from atmospheric deposition. By comparing temporal trends of PCBs and PFAS in the two lakes we hoped to examine how permafrost degradation and turbidity might affect the bioaccumulation of PCBs and PFAS in Arctic char. Fish were collected in late July from 2008 to 2019, by gill netting. Fishing success in West Lake declined in 2018 and no fish were collected there in 2019. All fish were dissected on site and subsamples of muscle+skin, liver, otoliths and GI tract were kept frozen for transport (-30°C). In total, 120 samples from muscle+skin arctic char were collected and analyzed for PFAS and PCBs. Σ71PCB showed significant declining trends of concentrations in Arctic char from East Lake (-1.4 % per year) as expected due to the past national and regional bans/ restrictions on use and emissions of these chemicals in circumpolar and neighbouring countries. However, the concentrations of PCBs were found to increase significantly (+2.1 % per year) in char collected in West Lake. The increases of PCBs in char from West Lake in comparison to East Lake may be in response to greater inputs of PCBs bound due to permafrost disturbances and from lake sediments released during subaqueous slumps greatly affecting West Lake. Total perfluorocarboxylates (C4-C14) PFCA showed decreasing concentrations at rates of 7.7 %/y and 3.3 %/y in West and East Lakes, respectively. Concentrations of PFASs in West and East lakes were similar in both lake water and char muscle, despite major differences in water chemistry. These results suggest that climate disturbances due to warming in the Arctic will affect the fate and bioaccumulation of particle borne, hydrophobic, compounds differently from more water soluble pollutants.

4.20.P-Th-122 Considerations for Designing an Antarctica Monitoring Program for Cyclic Volatile Methylsiloxanes (cVMS)

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Environmental monitoring in Arctic and Antarctic remote locations brings several challenges compared to monitoring in more metropolitan and major source regions. This presentation will highlight important considerations for the design and implementation of a remote monitoring program for cVMS. In particular, the main goal is to be able to obtain accurate cVMS concentrations in air and surface media with a more thorough understanding of impact from local emission sources which may be found in polar regions and potential bias from collection and analysis. In 2021, the Silicone industry partnered with the Norwegian Institute for Air Research (NILU) and launched an Antarctica air monitoring project with a strict quality control protocol and sampling locations that allowed for an assessment of impacts from the local station compared to a sampling location upwind and isolated from the local station. These types of comparison site selections are critical for understanding the impact of these local point sources within the Arctic and Antarctic regions. Impact of local wastewater release or solid waste disposal is equally important. The types of matrices available also lead to challenges. For example, the concentration of cVMS in air is much lower than that in the Arctic; this requires highly sensitive methods. In addition, soils in Antarctica are often frozen and rocky with low organic carbon (OC); this brings challenges in sample collection and processing. The low OC can lead to difficulty with storage and collections due to high potential for loss of such volatile analytes. Use of matrix (soil, vegetation, biota) matched quality control and the use of target analyte spike addition to collected samples and following the exact sample transport, storage, processing and analytical procedures is essential to understand loss and contamination during collection, transport and analysis. With these considerations a program is being planned in Antarctica for cVMS materials with three main objectives: (1) determine whether concentrations of cVMS in air are high enough to result in significant exposure in surface media; (2) determine concentrations of cVMS in soil, vegetation, sediment, and aquatic biota in locations free of anthropogenic influence; and (3) understand the potential for local anthropogenic sources and artefacts from sample collection and analytical procedures to introduce bias into the results of the monitoring campaign.

4.20.T The Behavior, Fate and Impact of an Increasingly Complex Array of Contaminants in Changing Arctic and Antarctic Environments

4.20.T-01 Impacts of Permafrost Degradation on Metal Concentrations in Arctic Char from Melville Island, Nunavut, Canada

Karista E Hudelson¹, Derek C.G. Muir², Benjamin D Barst³, Samantha Burke⁴, Jane Kirk², Debbie Iqaluk⁵, Xiaowa Wang², Scott Lamoureux⁶ and Melissa Lafrenière⁶, (1)Hudelson Environmental Research, (2)Environment and Climate Change Canada, (3)University of Alaska, Fairbanks, (4)Minnow Environmental, (5)Resolute Bay, Canada, (6)Queens University

Climate change is destabilizing permafrost landscapes in the Arctic and this, along with other changes such as increased rainfall and longer ice-free periods, can profoundly impact lake ecosystems. Long-term monitoring at the Cape Bounty Arctic Watershed Observatory on Melville Island, Nunavut, Canada (74°50'N, 109°30'W), has documented the effects of internal stochastic disturbances which have altered the physical and chemical limnology of West Lake, increasing turbidity 50x, while milder disturbances have occurred in neighboring East Lake where limnology has not been drastically impacted. The onset of limnological divergence between the two lakes began in 2008, following one of the warmest years since 1949. The sole fish species in these lakes, Arctic char (*Salvelinus alpinus*), serve as bellwethers of lake conditions, ecosystem health, and regime shifts. Here we present Arctic char condition factors and concentrations of a suite of metals in char from East and West lakes from 2008 to 2019 in order to make inferences regarding the effects of the changes in physical

and chemical limnology. Relative condition factors (CFs) of char in East Lake averaged 1.11 (± 0.30 SD) and did not change significantly from 2008 to 2019, while the CFs in West Lake char showed a marked decline from 2008-2017 (only one adult fish was collected in 2018). There was a significant increase in length-adjusted ($R^2_{adj.}=0.59$, $p<0.01$) mercury (Hg) concentrations in West Lake char from 2009 to 2017 coinciding with the huge increase in turbidity, while Hg in East Lake char declined ($R^2_{adj.}=0.72$, $p<0.001$). Increasing concentrations of cesium (Cs) and rubidium (Rb) were also seen in West Lake char while declining in East Lake. Other metals were higher in the East Lake than the West Lake char (selenium (Se): 0.55 ± 0.13 and 0.40 ± 0.08 ; thallium (Tl): 0.011 ± 0.005 and 0.006 ± 0.005 ; iron (Fe) 11.5 ± 17.4 and 9.2 ± 12.4 , respectively) and showed no significant temporal trends in either lake. Fish growth (age-at-size) was negatively related to length-adjusted char Hg in East Lake, i.e., faster growing fish had lower Hg concentrations. Somatic growth dilution may also explain the declining concentrations of Cs and Rb. Complicating the interpretation of multielement bioaccumulation, carbon and nitrogen stable isotope ratios in char muscle in West Lake indicated a shift in diet including possible dietary starvation brought on by the profound increase in lake turbidity.

4.20.T-02 Ultraviolet Absorbents and Industrial Antioxidants in the Tissues of the Seabirds, Mammals, and Fish from the Canadian Arctic

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Ultraviolet (UV) absorbents and industrial antioxidants are used in various products, such as plastics, personal care products, and paints, among others. These chemicals have been detected in numerous matrices, indicating their widespread occurrence in the environment. Exposure to these contaminants is known to cause different types of toxicities in organisms. However, the distribution of these contaminants in the tissues of wildlife in the Arctic is poorly understood, which impedes the risk assessment and policy development of these compounds. This study analyzed ten benzotriazole UV stabilizers (BZT-UVs), six UV filters (UVFs), two synthetic phenolic antioxidants (SPAs), and six secondary amine antioxidants (Ar-SAs) in the liver, muscle, or blubber of three seabird species, four marine mammal species and five fish species collected between 2017 and 2021 from the Canadian Arctic ($n=278$ samples). Contaminants were analyzed by gas chromatography-mass spectrometry. Overall, seabirds from eastern Nunavut accumulated higher levels of BZT-UVs, UVFs, and Ar-SAs in the liver than other species. It is noteworthy that UV328, which recently was added to Annex A of the Stockholm Convention, showed higher accumulation in the liver of thick-billed murre than other species (mean concentrations of 45 ± 18 (S.E.) ng/g wet weight; $n=28$), which was higher than previously reported levels in the liver or egg of seabird species from Canadian Arctic, Norwegian mainland, and Svalbard. Data suggest that some murre in this population may have been exposed to levels of UV328 higher than in other species, which should be investigated further. The southern Hudson Bay polar bear population was more contaminated with 26DTBP (Ar-SA) and UV329 (BZT-UV) than the western population. Liver UV329 levels in these polar bears were approximately twice as high as Svalbard polar bears' blood levels. Eastern Beaufort beluga from Tuktoyaktuk (Northwest Territories) and sea-run Arctic char from Cambridge Bay (Nunavut) had relatively the lowest contamination levels of the target contaminants. For tissue distribution in mammals, the blubber of walrus and ringed seals accumulated more \sum BZT-UVs than their muscle and/or liver, whereas no trend was found for \sum UVFs and \sum Ar-SAs. In contrast, ringed seals from Sachs Harbour (Northwest Territories) showed higher levels of \sum SPAs in the liver than the blubber. These data establish a baseline for monitoring these contaminants in the Canadian Arctic food web.

4.20.T-03 Long Term Temporal Trends of Perfluoroalkyl Substances in Landlocked Char from High Arctic Lakes

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Landlocked Arctic char are the only top predators in most high Arctic lakes and serve as a sentinel species for changes in atmospheric inputs of persistent organic pollutants and their bioaccumulation in aquatic food webs. Atmospheric measurements and studies of ice cap snow/firn/ice cores over the past 10 years have shown continuing and, in some cases, increasing, inputs of poly/perfluoroalkyl substances (PFAS) to the high Arctic. We were interested to see if these trends were reflected in the landlocked char as well. PFAS (C7-C14-perfluoro carboxylates (Σ PFCAs) and C4-C12 perfluoroalkyl sulfonates (Σ PFASs) were analysed in muscle of char and results compared to published atmospheric measurements and ice core records. Data was available for char from 4 remote lakes, spanning 14 to 30 sampling years from the early-1990s to 2021, with sample sizes ranging from 3 to 10 fish per year. PFOS was the predominant PFSA in char from all lakes, averaging 59% of Σ PFASs, followed by FOSA (21%). C9, C10 and C11-congeners were the predominant PFCAs averaging 49%, 15% and 11% of Σ PFCAs, respectively. PFOS and Σ PFCAs increased in Lake Hazen, the most northerly (82°N) lake, to 2010 and 2008, respectively, and then slowly declined (4.7 and 1.4% /y, respectively). The trend for PFOS in char agreed with results for air filter particulates sampled at Alert (83 °N) where maximum concentrations were reached in 2012 followed by a decline over the period 2012-2018. However, further south, in Amituk Lake, a remote lake (75 °N) on Cornwallis Island, PFOS and Σ PFCAs in char increased over the period 2010-2019. The increase in PFOS mirrored the trend in an ice core at the same latitude on Devon Island which showed increasing PFOS from the mid-2000s to 2015. The increasing trends of Σ PFCAs and PFOS in char were surprising given the phase-out of C8-PFCAs by 2014 as well as PFOS in the early 2000s. Climate related factors could be an important influence on trends of PFAS in char. A huge increase of glacial meltwater inputs to Lake Hazen, occurred from the mid-2000s to 2013 resulting in increased fluxes of PFAS to lake sediments. Amituk Lake has a large catchment to lake area ratio (31 km²/0.38 km²) and thus increases there in could reflect remobilization of previous atmospheric deposition. Food web changes could also be important. Char collected in Amituk Lake from 2010 to 2021 have shown increases in $\delta^{15}\text{N}$ (3 ‰) suggesting that we have collected fish that are probably more piscivorous.

4.20.T-04 Persistent Organic Pollutants in Beluga Whales (*Delphinapterus leucas*) from Bristol Bay, Alaska

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Remote high latitude locations are often sinks for persistent organic pollutants (POPs), which can ultimately bioaccumulate in local wildlife, such as beluga whales (*Delphinapterus leucas*). Of those inhabiting Alaskan waters, Bristol Bay belugas represent a healthy and stable population of approximately 2000 individuals. Conversely, the Cook Inlet population has been in decline, and despite protective measures, is not recovering with less than 300 individuals remaining. To understand the decline of this sensitive population, health assessments of wild Bristol Bay belugas were conducted to obtain baseline parameters from which to compare. This highly collaborative and substantial effort (e.g., federal and state agencies, aquariums, native associations) was conducted between 2008 and 2016. Of the samples collected and cryogenically archived at the NIST Biorepository, blubber tissues from 50 belugas (n=30 male; 20 female) are being measured for POPs (e.g., polychlorinated biphenyls (PCBs), chlorinated pesticides (DDTs), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs)). The preliminary data (i.e., PCBs, DDTs, oxychlordane, Mirex and HCB) are presented here. To date, the Σ POP concentration for Bristol Bay beluga is 665 ng/g. In contrast,

∑POP median concentrations of these same compounds for belugas from Cook Inlet (n=15 male; 12 female) and the eastern Chukchi Sea (n=26 male; 14 female), two other geographically distinct populations, are reported at orders three to ten times that of Bristol Bay beluga, respectively. As such, POPs in Bristol Bay beluga are comparatively low leading to this population's viability as a baseline population for future POPs measurements.

4.20.T-05 Insights of a Temporal and Spatial Contaminant and Health Assessment of Two Alaskan Polar Bear Populations

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The coupled Arctic coastal-marine ecosystem surrounding Alaska experiences a range of natural and anthropogenic stresses. Human activities, such as fossil fuel burning and industrial processes, have significantly altered the rates of change, the magnitudes, and confounding factors of environmental stresses in the Arctic Ocean. These acute stresses ultimately affect system-wide trophodynamics and species interactions, including those in the coastal-marine ecosystems of Alaska. Trophodynamics directly and disproportionately influence apex predators, such as polar bears (*Ursus maritimus*). This project aimed to provide data on targeted health assessments of individual bears following exposure to anthropogenic contaminant stressors on two geographically diverse stocks of the Beaufort and Chukchi Seas polar bears. The North Slope Borough Department of Wildlife Management acquired blood and select tissue samples harvested over ten years from the Beaufort Sea stock, and the United States Fish and Wildlife Service provided adult, paired fat and serum samples collected in 2016 from the Chukchi Sea stock. Over 750 samples from 93 polar bears were analyzed for polycyclic aromatic hydrocarbons, organochlorine pesticides, flame retardants, polychlorinated biphenyls, lipids, immune function, gene expression, and reproductive and thyroid hormones. Results show that concentrations of individual compounds in three contaminant classes significantly differed between the two bear stocks. For the Beaufort bears, there was a spatial difference in contaminants with the Chukchi bears and a significant temporal difference in concentrations between 2011 and 2020. Lipid class compositions also differed between populations, which may be attributed to health status or prey availability. Limitations in sampling opportunities prohibited the ability to compare immune function, gene expression, and hormone levels. This research inspired recommendations for future types of assessments and collection methods for different tissue matrices for live-only, harvested-only, and live-and-harvested bear comparisons. A strategically designed research project incorporating unique sample collection methods for extreme field conditions and clearly defined evaluation endpoints, animal population studies involving difficult field collection conditions can enhance research outcomes.

4.20.T-06 Very Short-, Short-, Medium-, Long- and Very Long-Chain Chlorinated Paraffin Levels, Patterns and Time-Point Comparisons in Canadian (Hudson Bay) Polar Bears

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Chlorinated paraffins (CPs) are manufactured by the chlorination of paraffin fractions obtained from petroleum refining. CPs are a complex mixture of congeners within very short- (<C₁₀), short (C₁₀₋₁₃), medium- (C₁₄₋₁₇), long- (C₁₈₋₂₀) and very long- (>C₂₀) chain CP homologue groups (i.e., vSCCPs, SCCPs, MCCPs, LCCPs and vLCCPs). Overall, information is limited for CPs in environmental compartment across the globe and including the Arctic. According to a recent review most Arctic data exist for SCCPs and to a lesser extent for MCCPs while LCCPs have received no attention in Arctic biota. The polar bear (*Ursus maritimus*) is at the apex of the Arctic marine food web and thus has been shown to bioaccumulate and magnify an array of persistent organic pollutants (POPs). SCCPs were recently reported in a previous study in fat samples of polar bears harvested in 2013-2014 from two subpopulations in western Hudson Bay (WHB) and southern Hudson Bay (SHB), and where 24 SCCP congeners were detected in 100% of the samples. In 2017, SCCPs were listed under Annex A of the Stockholm Convention on POPs and MCCPs are currently under listing review. Presently, an established chloride-enhanced LC-APCI-QTOF-MS-based method was used to determine in samples a comprehensive suite

of 675 CP homologues (C_nCl_m , $n = 6-48$, $m \geq 2$) including 22 vSCCPs (C_8Cl_{7-8} and C_9Cl_{5-8}), 53 SCCPs, 72 MCCPs, and >500 LCCPs and vLCCPs. CPs were analyzed in $n=30$ fat samples from male Hudson Bay bears harvested by hunters in the years of 2014-2015, 2017-2018 and 2020-2021. Highlight observations include the following. Several vSCCPs ($<C_{10}$) were identified in the samples. Numerous SCCP and MCCP congeners were quantifiable and Σ SCCPs and Σ MCCPs were at comparable levels but greater than the levels of both Σ vSCCPs and Σ LCCPs. Analysis of the data shows that of the C_{10} - C_{20} CP congeners determined thus far about 80 congeners have been identified and can be quantified. Congeners of $>C_{20}$ are also present but at very low concentrations. Although semi-quantitative at the time of this abstract preparation, it generally appears that for all CP homologue groups levels are higher in SHB versus WHB bears. Also, Σ SCCP, Σ MCCP and Σ LCCP levels have decreased from 2015 to 2021 in SHB samples, whereas in WHB samples Σ MCCP levels increased over the period of 2014 to 2020. These CP data will be discussed and in the context of factors such as sources and changes over time, climate change and bear food web and dietary shifts.

4.20.V The Behavior, Fate and Impact of an Increasingly Complex Array of Contaminants in Changing Arctic and Antarctic Environments

4.20.V-013 Mercury Cycling in the Antarctic Coastal Zone (Admiralty Bay)

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The Antarctic is one of the few regions on the planet that is still considered a pristine area. However, latest research indicates a possible contamination of this region by anthropogenic pollutants, including mercury (Hg). In contrast to the Arctic, where Hg research is advancing due to relatively easy access, Hg transformations in the Antarctic are poorly studied. The aim of the research is to identify mercury sources in Antarctic coastal zone and to determine their potential for accumulation and biomagnification in the marine trophic chain. Sampling was conducted in the Admiralty Bay between December 2018 and January 2019. Terrestrial samples (mosses and lichens, animal fur, feathers and excrements) and marine samples (fish and zoobenthos) were collected. Total mercury, methylmercury and stable isotopic ratios of Hg were determined. In addition, selenium concentration, as well as isotopic analysis of carbon and nitrogen were analysed. Our research indicated that Hg in terrestrial flora is mainly of atmospheric origin (based on $D^{199}Hg$ and $d^{15}N$). Among benthic organisms, accumulation and biomagnification of Hg was observed. The highest Hg concentration in zoobenthos was measured in starfish (average 237 ng/g). However, the highest Hg concentration among all studied organisms were found in the fur of elephant seal (average 2711 ng/g). Ranges of -1.21 to 1.64‰ of $d^{202}Hg$ and -0.85 to 1.85‰ of $D^{199}Hg$ in terrestrial flora and 0.12 to 2.46‰ of $d^{202}Hg$ and 0.26 to 2.21‰ of $D^{199}Hg$ in marine fauna were observed. This research is one the first of its kind in Antarctic, therefore the wider context is hard to establish. It seems to be clear that the isotopic fingerprint is not homogeneous which might be a result of different factors. One of them seems to be the bioaccumulation within the zoobenthos-fish-penguins-elephant seals food chain. This is confirmed by an increase of $D^{199}Hg$ along the trophic chain and correlation between $D^{199}Hg$ and $d^{15}N$. Further research are necessary to understand Hg cycling in the Antarctic. However, this research gives some constraint on mercury isotopic fingerprint of polar flora and fauna that might be useful and should be taken into account to further enhance our knowledge in the field. This study was performed within the framework of a National Science Center projects No. 2019/33/B/ST10/00290 and 2017/27/N/ST10/02230. The authors also acknowledge the Slovenian Research Agency for funding the program P1-0143.

4.21.P-Th The Standardized Micro- and Nanoplastic Planet: Degradation, Fragmentation and Leaching

4.21.P-Th-123 Quantification and Qualification of Microplastics from Drinking Water Source of Beijing, Chaobai River

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Microplastic is one of the globally concerning emerging pollutants, which refers to plastic particles smaller than 5mm in size and can exert particle toxicity and various biological effects due to chemical additives. In urban water systems, microplastics can be sourced from personal care products containing micro-size plastic beads or due to physicochemical degradation of household products, tires, synthetic textiles, and road markings. Until now, microscopic techniques have been majorly used to confirm the physical properties of discrete microplastic particles, however, this method cannot characterize heterogeneous microplastics that are common in an urban environment and mixed with environmental matrices such as sediment and wastewater. In this study, a novel method of coupling pyrolysis with gas chromatography/mass spectrometry (GC/MS) was proposed to identify and characterize microplastics of major type polymers including polystyrene, polypropylene, polyvinyl chloride, nylon, and polyethylene. Synthesized plastic standards, and plastic nurdles collected from Chaobai River area were analyzed to verify the method. Results indicated the successful separation and confirmation of marker peaks of targeted polymers. Thence, pyrolysis-GC/MS was demonstrated as a powerful analytical tool in investigating microplastic occurrences in complex environmental matrices.

4.21.P-Th-124 Comparison of UV Photooxidation and Mechanical Abrasion on Polyethylene Terephthalate (PET) Micro and Nanoplastics in a Lab and Environmental Setting

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Photooxidation and environmental abrasion have been shown to degrade and modify polymers and plastics, often leading to the production of micro-and nanoplastic particles. Several studies have been conducted looking at the effects of photooxidation on plastics and polymers, however these studies were either conducted in a lab setting or in an environmental exposure setting. Within these studies, even fewer have looked at the effects that these environmental stressors have on polyethylene terephthalate (PET) in a dual exposure setting. This current study aims to compare the effects that photooxidation and mechanical abrasion will have on the size, shape, morphology, carbonyl index, and aspect ratio of PET consumer water bottles through lab UV exposure and an outside environmental exposure setting. It is hypothesized that the size, shape, morphology, aspect ratio, and carbonyl index of the PET microplastics will change considerably due to photooxidation, mechanical abrasion exposure, and exposure environment. PET bottles were exposed to three environmental conditions in both the lab and outside environmental exposure study: UV, aqueous, and a co-exposure with UV and aqueous conditions. An artificial weathering chamber was constructed using a California Lightworks SolarSystem 24W UVB light. Thirty-six 60 mL cylinders were placed in the UV weathering chamber. Another thirty-six 60 mL cylinders were placed outside to be exposed to natural UV light. Each cylinder had ~4.5 g of pre-cut mechanically abraded consumer bottle, this represents the amount of plastic that was present in the consumer plastic bottles. The weathering chamber and outdoor exposure cylinders received 8 h of UV exposure per day. Atmospheric and daily UV data was collected through the West Texas Mesonet, Lubbock 3WNW station. UV output of the weathering chamber was confirmed via a Macam 2030 Optical Radiometer. Photooxidation, morphology, size, and shape were monitored throughout the experiment via Dynamic Light Scattering (DLS), Scanning Electron Microscope (SEM), X-ray diffraction (XRD), and Fourier-transformed infrared spectroscopy (FTIR). This exposure will lead to the development of a broad size distribution and non-uniform shapes of

microplastics and nanoplastics from the respective polymer. This project is ongoing and will contribute to the growing literature regarding environmentally relevant conditions and their effects on weathering of micro-and nanoplastics.

4.21.P-Th-126 Assessing the Effects of Plastic Additives, Di-Ethylhexyl Phthalate and Nonylphenol, on Behavior and Gene Expression of Zebrafish (*Danio rerio*)

Carlos Mazariegos Ortiz and Jaclyn Canas-Carrell, Texas Tech University

Plastic additives (PAs), such as di-ethylhexyl phthalate (DEHP) and nonylphenol (NP), are essential chemical compounds used in polymer-based materials to confer specific characteristics such as shape, resistance, and durability, enhancing their market value. However, since these compounds can bond physically but not chemically, these additives can leach out when subjected to environmental and mechanical factors that affect their original structure. The presence of PAs in the environment raises concerns, as many of these compounds are highly lipophilic, allowing them to accumulate passively in organisms and through food webs. Thus, there is potential for adverse effects such as endocrine disruption, liver toxicity, spinal malformations, and kidney impairment. Despite their prevalent use, the precise mechanisms of how DEHP and NP exert toxicity on target organs and systems remain to be fully understood. Therefore, this study will evaluate the effects of individual and combined exposure to DEHP and NP on zebrafish embryos and adults. Zebrafish embryos at different developmental stages (2 to 96 hours post-fertilization, hpf) will be exposed to various concentrations of DEHP and NP to assess effects on survival rates and the occurrence of malformations. Furthermore, the swimming behavior of the embryos will be evaluated using a DanioVision chamber during a 5-minute light-dark transition. Zebrafish adult males and females will be exposed to high-toxicity concentrations of DEHP and NP (determined in the acute toxicity studies) over a period of one month. Following this exposure period, males and females will be placed together in a non-exposure environment to breed, and the resulting offspring rates will be recorded. Additionally, testis and gonad samples will be collected for gene transcription analysis, focusing on genes associated with gonad development. Studies are underway and data analysis is ongoing.

4.21.P-Th-127 Investigations of Microplastics in Surface Water at Rivers, Lakes and Bays Using a Novel Automated Microplastic Sample Preparation System

Yutaka Kameda and Emiko Fujita, Chiba Institute of Technology

Microplastics (MPs) in surface water at rivers, lakes and ocean is going to be recognized as one of water qualities to be generally monitored in various countries. Today, many researches are being conducted by pretreatment methods recommended by National Oceanic Atmospheric Administration. But it is well known that the methods are comprehensive, laborious and time-consuming. Therefore, analytical operators need several techniques which are able to reduce contamination during the pretreatment, to keep properties of MPs and to extract all MPs from water samples. Moreover, they must also revise their procedures in some cases. For example, they must add optional treatments such as Fenton reaction or digestion by sodium hydroxide to analyze MPs in organic-rich water samples. To analyze MPs sample with high precision for operators with little experience, a novel automated microplastic sample preparation system has been developed in Japan. The system can extract fragment-type MPs greater than 300µm from suspended matters in water samples. After collecting MPs in surface water by nets such as neuston nets, operators collect suspended matters and remove matrices greater than 5 mm by sieves. Operators put the suspended matters smaller than 5 mm in a small strainer of the system. Then, the system can automatically perform digestion by hydrogen peroxide and density separation by sodium iodide in one tube continuously. In recovery tests by adding commercial polyethylene microplastics greater than 500 µm to ultrapure water and actual suspended matters in river, recovery rates were 99.0 % and 93.3 %, respectively. Investigations of microplastics in contaminated rivers, eutrophicated lakes and Tokyo Bay were also conducted by using the system and micro-Fourier Transformed Infrared spectroscopy. This presentation shows the system in detail and the application to surface water samples.

4.21.P-Th-128 Relations of Surface Hardness and Elasticity to Carbonyl Indexes of Plastics Collected from Japanese Beaches and the Seas of Asia

Yutaka Kameda and Emiko Fujita, Chiba Institute of Technology

The degradation and fragmentation of plastics in the environment has been insufficiently understood. Especially, only a few studies reported physical properties of weathered plastics in aquatic environment though properties of Infrared spectra, such as carbonyl index were reported. To begin with, it is difficult to measure physical parameters of micro and macroplastics in the environment. The purposes of this study are to develop analysis methods for surface hardness and elasticity of micro and macroplastics in the aquatic environment, and to reveal relations of the hardness and elasticity to carbonyl index. Forty-nine macro and microplastics such as lids of plastic bottle, films, fibers and fragments collected from two Japanese beaches and three macroplastics in the ocean collected from volunteer commercial ships were used. Dynamic Ultra Micro Hardness Tester (Shimazu) were used in order to measure their surface hardness, Martens Hardness, and elasticity. Because many samples were too small, thin and soft for the instrument, methods to fix them on a glass plate and to measure them with high precision were developed. Micro-Fourier transform infrared spectroscopy was also used to identify polymers and to calculate their carbonyl indexes. This presentation shows results of only polypropylene. Fresh polypropylene fragments and films were also measured. Martens hardness values of fragments, lids and fiber collected in the environment were significantly higher than those of fresh plates. These results indicated that the surface of weathered polypropylene in the environment became harder than not weathered ones. On the other hands, elasticity values of fragments and lids were significantly smaller than new fresh ones. These results indicated that the surface of weathered polypropylene in the environment also became more fragile than new ones. Finally, their hardness and elasticity have significant relationships to carbonyl indexes. Especially their elasticity was more significant ($p=0.003$) than their hardness ($p=0.047$). Fragmentation mechanisms of plastics in the environment are very comprehensive and their fragmentation may cascade through various chemical and physical reactions. This study suggested that elasticity, which is more downstream reaction than hardening, was one of the useful indexes for fragmentation of plastics in aquatic environment.

4.21.P-Th-129 Establishment of a Semi-automatic Software to Identify Microplastics from Imaging Data by Micro-Fourier-transform Infrared Spectroscopy

Yutaka Kameda and Emiko Fujita, Chiba Institute of Technology

Smaller microplastics (MPs) smaller than 300 μm can be detected easily by Imaging technique of Micro-Fourier-transform infrared reflectance spectroscopy (μFTIR). In my laboratory, Nicolet™ iN™ 10 MX (ThermoFisher Scientific) is used to analyze microplastics greater than 20 μm in water, sediment, biota and atmosphere. OMNIC™ Series Software is also used to identify and quantify various polymers from contour diagrams of polymers. However, it is revealed that “Profiling” in the software often misidentify polymers. Therefore, many scientists must reidentify microplastics extracted by the software. This visual inspection always wastes long time and much effort. To save time and to identify microplastics more precisely, a semi-automatic software, YCALOS13 was established. The software, YCALOS 13 (You Can LOOK microplasticS) is a free software based on macro in Excel. After imaging analysis of microplastic samples by Nicolet™ iN™ 10 MX, the mapping file is converted to CSV format. The YCALOS13 can extract pixels of polymers from polymer-specific bands and the peak areas and shapes which users can decide in the configuration. It can generate each contour diagram of many target polymers simultaneously after pushing start button. Finally, ImageJ software can quantify MPs of many polymers from the contour diagrams. We compared abundances of MPs in river water samples and sea water samples between only OMNIC software, OMNIC software plus visual inspection and YCALOS13. It was revealed that YCALOS13 could detect similar concentrations to OMNIC software plus visual inspection, whereas much less MPs could be detected by only OMNIC software. YCALOS13 can be a useful tool to identify various polymers from imaging data from μFTIR .

4.21.P-Th-130 Aftermath of Microplastic Fibres being Exposed to Ultraviolet Radiation

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As it is well known, microplastics are one of the persistent environmental pollutants of concern. Numerous research works are being carried out to understand their occurrence in environmental systems and to explore their fate and transport. Of late, more importance has been paid towards understanding the fate of microplastics while they are subjected to environmental stressors such as photo radiation, thermal, tidal effects, and so on. Among the research carried out to understand the fate of microplastics, exposure of microplastic pellets to artificial Ultra Violet (UV) radiation is the widely explored area of research. However, it is to be noted that the microplastic pellets that are used in these studies are less prevalent in the environment compared to other forms of microplastics, specifically microplastic fibres, which comes out largely from laundry wastewater. However, very few studies have been carried out so far to understand the fate of microplastic fibres. Therefore, in order to augment the research knowledge in this area, the two most common polymers used for textile production were chosen for the investigation. Microplastic fibres from the two polymers were subjected to UV-A radiation in a photoreactor setup for an extended duration of 21 d and samples were taken at different times throughout the experimental duration. Microscopic and spectroscopic analyses have confirmed the aging effects of microplastic fibres caused due to photo radiation. On the other hand, as the description of this session says, it is well known that plasticizers are one of the major components added into the plastics during their production for various purposes. However, as plastic ages, plasticizers are expected to leach out. Therefore, besides investigating the microplastic fibres, the suspension medium will be analysed through mass spectrometric instruments (LC-MS/MS) to explore the derivatives of plastic additives that might have leached out during the aging process. It is also interesting that compared to the early stages of microplastic research, microplastic fibres are gaining attention nowadays as they are detected everywhere, and their source of contamination is huge (carpets, upholstery, clothes, etc.). Therefore, it is believed that the findings of this study would expand the growing understanding and research about microplastic fibres.

4.21.P-Th-131 Environmentally Relevant and Quantitative Mechanical Abrasion of Plastics and Generation of Nanoplastics Using a Novel High-throughput Weathering Reactor

Sarah Ziemann, Claire Hartwig Alberg and Boya Xiong, University of Minnesota

Mechanical degradation of plastic waste is a major source of nanoplastics and potentially unknown chemical leachate into soil and water environments. Currently, there are no available experimental methods that can quantitatively measure the inputs of mechanical degradation, deconvolute abrasion and fragmentation processes, and are based on environmentally relevant force scales. Here, we designed a novel weathering device that address all three current limitations that can be assembled into a high-throughput fashion (~100 samples). In the device, a plastic film or sheet sample was sealed at the bottom of a glass cylinder which was attached to a temperature-controlled shaker table. A monolayer of sediment was added onto the plastic samples which moved in a circular motion over the plastic to create abrasion as the entire device was moving. The physical details of sediment motion were recorded using a high-speed camera and were comparable to typical environmental sediment transport. We quantified the kinetic coefficient of friction using lateral force microscopy and the abrasive energy input of the mechanical abrasion process as a function of weathering time (~5 months). After the simulated weathering, dissolved organic carbon, nanoparticle tracking analysis, and scanning electron microscopy quantified and characterized the nanoplastic release from abrasion. X-ray photoelectron spectroscopy and atomic force microscopy were used to study morphological and chemical properties of the weathered plastic surfaces. Using the novel device, we aim to compare nanoplastic release in water and air media and evaluate the impact of pre-photooxidation on nanoplastic release. In addition, an empirical model can be developed to predict the amount of nanoplastic release based on the abrasive energy input. Preliminary results on melt-crystallized low-density polyethylene indicated that the nanoplastic release rate was found to be 7×10^5 J/day/cm² and released nanoplastics were predominantly < 200 nm in size. Our results suggest that in addition to photooxidation, abrasion induced oxidation could readily occur in air compared to in water. This

work provides a novel tool to quantitatively and mechanistically study plastic abrasion processes under environmentally relevant scales that will facilitate the development of physics-based modeling of nanoplastic release and plastic degradation.

4.21.T The Standardized Micro- and Nanoplastic Planet: Degradation, Fragmentation and Leaching

4.21.T-01 Importance of Detection, Quantification and Identification of Micro- and Nanoplastics – Does My Instrumentation Matter?

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Microplastics are now widely acknowledged as a significant environmental pollutant, and can be found in soils, natural waters, biota and animals. Microplastics are typically considered to have a size range spanning five orders of magnitude, from the sub-micron to millimetre range. To fully understand the source, distribution, transport, fate and impact of microplastics in the environment it is necessary to be able to accurately perform physico-chemical measurements. While the analysis of microplastics in the upper range of the size distribution is reasonably straight forward, as the size of the particles reduces, it becomes more challenging to characterise them appropriately. To do so accurately, it is essential to ensure that the testing instrumentation is fit-for-purpose for the sample under study and to understand the limitations of the technique being used for characterisation. Here, we will discuss some international development opportunities in various forums (regulatory, metrology, standardization) and present some recent results from investigations into limitations of instrumentation commonly used in this emerging field.

4.21.T-02 Can Fully Automated Detection and Analysis of Microplastics Be Both Time-Efficient and Accurate? An Exploration of a Novel Approach

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Scientists have demonstrated the alarming environmental ubiquity and persistence of particulate plastic in the environment. To adequately explore the issue, rapid; reliable, and harmonized characterization is essential. Suitable techniques should determine the size, shape, and polymer type of microplastic particles and provide fast quantification of each type. Existing particle characterization techniques such as visual microscopy and FTIR or Raman micro-spectroscopy have a significant limitation in speed of analysis, despite recent advances. In addition, the level of automation of these systems may lead to a corresponding decrease in accuracy of both particle detection and analysis. By combining a novel infrared source in the form of a quantum cascade laser (QCL) with rapidly scanning optics, very fast, high-automated workflows can be achieved that are, most importantly, accurate and repeatable. However, the steps performed in the analytical device are only a part of the story. Sample preparation is equally important, as is the management of potential contamination. Hence, simplifying sample preparation and limiting the number of steps required is also critical to success. Combining fast and accurate analysis with dedicated on-filter sample preparation can further enhance outcomes. The aim of this study is to explore and demonstrate combining this novel infrared technique with sample preparation and a dedicated filter suitable for direct analysis to deliver analysis that is both time-efficient and accurate. In addition, it seeks to better understand the contribution of each of these steps to achieve this goal.

4.21.T-03 Distribution and Analysis of Nanoplastics in Lake Erie Drinking Water

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Microplastics have been found in surface waters worldwide, including Lake Erie, which provides drinking water to ~11 million people. This is concerning due to the unknown risks associated with the ingestion of microplastics and the lack of knowledge surrounding the concentrations of nanoplastics (<1 µm plastic particles). Recent studies of drinking water treatment have shown that plastic particles >10 µm are effectively removed, but particles <10 µm are not effectively removed, which include the subcategory of nanoplastics.

Nanoplastics are of the greatest concern from a human health perspective since they have been shown to cross cellular barriers and accumulate in the body. Due to analytical limitations, however, previous studies of Lake Erie have been unable to effectively analyze nanoplastics leading to a potential underestimation of micro- and nanoplastic concentrations. In this study, raw and treated water were collected from six drinking water treatment plants on the Ohio shore of Lake Erie. Microplastic concentration, size, and shape were determined using scanning electron microscopy. Polymer characterization was conducted using an Optical Photothermal Infrared Spectroscopy (O-PTIR). The O-PTIR is a new instrument that allows for the identification of smaller particles (<0.5 μm) compared to traditional Raman and infrared techniques. The application of the O-PTIR provides more comprehensive information on the size distribution of micro- and nanoplastics in Lake Erie and its treated drinking water. The concentrations of micro- and nanoplastics in the raw and treated Lake Erie water were significantly higher than those previously reported in the literature. This was likely due to underestimation of the smaller micro- and nanoplastics. The results show that the majority (60-86%) of particles found in the treated drinking water were nanoplastics (size <1 μm). The raw water had a larger particle size distribution, however a significant portion (30-84%) of particles were also within the nanoplastic size range for all samples. These results demonstrate the potential for O-PTIR to increase our understanding of the distribution and characteristics of nanoplastics in both source and treated waters from Lake Erie and presumably other locations.

4.21.T-04 The Physical Abrasion of Plastic to Form Microplastics: An Experimental Approach

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Secondary microplastics (MP) are particles that result from the breakdown of larger plastic items in the environment through chemical degradation and/or physical abrasion. Weathering via UV exposure has been well studied, but much less is known about abrasion and how this results in fragmentation and MP generation. The aim of this research is to explore the physical abrasion of commonly littered plastic polymers: Polystyrene (PS), Polypropylene (PP), Polyethylene Terephthalate (PET) and High-density Polyethylene (HDPE). We tested the hypotheses that plastic items can generate microplastics through physical abrasion processes alone, whether plastics of different compositions generate MP at different rates and sizes; and whether exposure to UV light prior to physical abrasion effected MP generation rates. Laboratory experiments were designed with carefully controlled variables, using tumblers with a polymer and either water (control) or water and gravel. At sampling intervals of 2, 22 and 112 days, 7 pieces of polymer were removed, photographed, accurately weighed and examined for 3D textural analyses with an Infinite Focus Microscope. Identical experiments were conducted after exposure of each polymer type to the equivalent of 1 year of UV light in Florida. Results demonstrate that after 112 days of tumbling with gravel present there is a significant weight loss in all polymers, both in pristine and pre-weathered samples, compared with the control (two-tail t-test, $n=7$, $p<0.05$). All pre-weathered polymers, except PP, lost significantly more mass than the correspondent pristine polymers (two-tail t-test, $n=7$, $p<0.05$). This highlights how pre-weathering can influence subsequent fragmentation of polymers by abrasion. Comparison across polymer compositions demonstrates that PET (both pristine and pre-weathered) lost significantly more mass than the other polymer types at 112d with gravel (two-tail t-test, $n=7$, $p<0.05$). These preliminary results show that MP can be produced by abrasion without prior exposure of polymers to UV light and how weight loss increases significantly when polymers have been previously exposed to UV light. This experimental approach can provide useful insights on abrasion processes and microplastic fragmentation and how MP can become part of the sediment cycle.

4.21.T-05 Characterization of Extractable and Leachable Additives from Microplastics Prepared from Reference Polymer Materials

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Microplastics are pervasive in the environment and contain chemical additives that may leach under environmentally relevant aquatic conditions. In addition to the need for the creation and characterization of reference polymers for use across micro- and nano-plastic studies, there is a critical need to assess leachable

polymer additive profiles associated with these reference materials. A holistic assessment of the impacts of widespread microplastic pollution must identify and quantify leachable polymer additives and consider various polymers. We have used non-targeted analysis approaches to characterize extractable and leachable additives from NIST standard reference material polyolefins, polymers supplied in the Polymer Kit 1.0 from Hawaii Pacific University, and commercially relevant polyester fibers. All pre-production pellets and fabrics were cryogenically milled within a customized stainless-steel housing to create microplastics. The prepared microplastics underwent size characterization, additive extraction by accelerated solvent extraction (ASE), and leaching experiments in simulated freshwater media (37°C over 30 days). Solvent extracts and freshwater leachates were analyzed by HPLC with high-resolution Orbitrap MS/MS. Compound annotations were based on matches to spectral libraries and a custom, curated database of known organic polymer additives. Based on library matching and/or computational mass spectrometry evidence, a variety of known polymer additives were annotated across the range of polymer types. Multiple additives were confirmed by analytical standards and targeted for subsequent quantitative leaching rate experiments. Compounds in polymer extracts and leachate timepoints were quantified via HPLC-MS/MS. As examples, chlorinated disperse violet 93 and perfluorononanoic acid (PFNA) were quantified in the leachates of a commercial black polyester fiber. Bisphenol S was detected in tire crumb rubber as well as in nylon leachates. Comparisons of solvent extracts and freshwater leachates for polymers revealed that several common additives, such as diethyl phthalate, were shared among several plastics, while many additives were unique to specific polymer chemistries. Results from this study extend the information available for the microplastics research community and contribute to a comprehensive organic chemical characterization of polymer additive chemical space within reference materials and other common microplastic sources.

4.21.T-06 The Unusual Suspects: Screening for Persistent, Mobile, and Toxic Plastic Additives in Common Plastic Products

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Plastic additives are a chemically diverse group of substances added to plastics to impart unique physical-chemical properties. When plastic waste enters the aquatic environment, these substances can leach out of plastics, acting as a source of chemical contamination. Some plastic additives possess properties that make them persistent, mobile, and toxic (PMT). These highly polar, environmentally stable contaminants can pose a significant, long-term risk to the aquatic environment, and are expected to have low removal via wastewater treatment. Unlike their bioaccumulative counterparts (PBTs), PMTs and PMT plastic additives remain largely unregulated and under-studied, despite the threat they pose. As such, there is a need for dedicated analysis methods and leaching assessments to enable monitoring and identification of PMT sources in the environment. The presented study investigated the leaching of PMT plastic additives from 18 plastic products using a novel analysis method and optimized leaching protocol. An analytical method for PMT plastic additives was developed and optimized using high performance liquid chromatography with quantitative time-of-flight mass spectrometry (HPLC-QToF-MS). An optimized leaching protocol was also developed, with parameters including water type, UV light exposure, and time being investigated. These methods were then applied to 18 plastic products, with a suspect screening workflow used to tentatively identify PMT plastic additives. The plastics were ultimately leached in lake water with UV light applied, with leachates collected at multiple timepoints up to 34 days. A total of 486 PMT plastic additive suspects hits were measured across the 18 plastic products, within which there were 54 unique suspects including triethyl phosphate, benzophenone-3, and benzothiazole. The plastic products with the greatest numbers of PMT plastic additives measured were toy balls (20), food wrappers (16), and textile/fabric face masks (14). The most frequently measured PMTs were tris(2-butoxyethyl) phosphate (in 16 of 18 products), 2-amino-5-methyl-benzenesulfonic acid (in 14 of 18 products), and N,N'-diphenyl-guanidine (in 13 of 18 products). The results from this study provide the tools to measure

PMT plastic additives, assess their leaching in an environmentally relevant matrix, and provide a better understanding of the presence of PMTs in plastics commonly found in the environment to inform future regulatory and monitoring efforts.

4.22.P-Tu Assessing Chemicals of Concern in the Laurentian Great Lakes and Their Tributaries

4.22.P-Tu-179 Looking Back at 40 Years of Per- and Polyfluoroalkyl Substances in Great Lakes Fish

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Per- and polyfluoroalkyl substances (PFAS) are a group of thousands of anthropogenic chemicals that are used in industrial and consumer products for their unique chemical properties. Toxicological data has shown that there are potential health hazards from exposure to certain PFAS compounds. Protecting the Great Lakes watershed and ecosystem from PFAS contamination is of utmost importance, as it provides drinking water to millions of people and is home to a diverse array of aquatic species. Presence of PFAS in the Great Lakes can be measured by analyzing water, sediment, or any number of biological matrices. To analyze historical concentrations of PFAS, there are limited options. Since the hazards of PFAS were not known until decades after production began, concentrations of these compounds were not historically measured in the environment. In this study, we use archived Lake Trout and Walleye whole fish homogenates from the United States Environmental Protection Agency's Great Lakes Fish Monitoring and Surveillance Program (1978-present) to quantify PFAS taken up into the aquatic ecosystem during this period. A suite of 45 PFAS compounds from seven different classes are analyzed to determine the presence of both legacy and emerging PFAS compounds. The novel method used here was developed to analyze a wide range of compounds in particularly fatty tissue (11-18% lipids in wet weight). Detection limits of perfluorinated compounds range from 50 – 525 ng/kg wet weight. Reconstructing historical trends can help understand how previous PFAS reduction initiatives have impacted ecosystems and predict how these ecosystems might respond to future regulations and changes in industrial practices.

4.22.P-Tu-180 Leveraging Invasive Mussel Contaminant Survey Data for Stepwise Prioritization of Chemicals of Potential Concern in the Great Lakes Basin

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Historic and ongoing anthropogenic activities coupled with advancements in analytical techniques have led to the detection of large numbers of contaminants in the Laurentian Great Lakes. Consequently, identifying and prioritizing chemicals likely to cause ecological harm represents a challenge for natural resource managers. Previous prioritization efforts have focused on contaminants in sediment, water, and passive samplers, which may not be representative of bioavailable compounds that bioaccumulate in aquatic organisms. Consequently, the present study adopted a stepwise method to prioritize chemicals of potential concern detected in dreissenid mussels from samples collected across the Great Lakes basin from 2009 – 2018. The stepwise method considered environmental fate, detection frequency and exceedance of toxicity quotients based on multiple ecotoxicological benchmarks. Overall, a total of 153 compounds out of 267 analyzed were detected in dreissenid mussels, 47 of which had associated water quality benchmarks, 56 had apical benchmarks (Tier 1 ECOTOX or apical screening), 17 had non-apical benchmarks (Tier 2 ECOTOX, Cytotoxic Burst, and ToxCast) and the remaining 33 had estimated benchmarks (QSAR, estimated screening and pharmacological potency). Of the compounds with water quality benchmarks, 9 were designated as high priority including the

herbicide atrazine and 5 polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene and fluoranthene, that have previously been identified as potentially hazardous within other matrices. Across all lines of evidence (LoE), a total of 27 compounds were designated as low priority, suggesting that these contaminants do not warrant further management action or data generation based on this dataset. Similar contaminants were identified as high priority in a related study of native unionid mussels in the Great Lakes, suggesting these findings are applicable to imperiled native mussels. Overall, these findings will facilitate the development of management strategies to mitigate the effects of contaminants of emerging concern on aquatic organisms within the Great Lakes basin.

4.22.P-Tu-181 PFAS Mass Budget in the Great Lakes

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Atmospheric deposition can be an important source of PFAS to the environment, especially for the Great Lakes basin, where 10% of the U.S. population and 30% of the Canadian population live. Air (n = 50) and precipitation samples (n = 97) from five sites on the American shores of the Great Lakes and off-shore Great Lakes water samples (n = 63) from all five Great Lakes were collected in 2021-2022 and analyzed for 41 targeted PFAS. These measurements were combined with other available data to estimate the mass budget of PFAS for the Great Lakes. The Σ PFAS concentrations in precipitation across the five sites ranged between 0.16 and 20 ng/L, with the lowest levels for the site at Lake Erie. The median Σ PFAS concentration in open lake water was highest in Lake Ontario (12 ng/L) and lowest in Lake Superior (1.7 ng/L). The median Σ PFAS concentration in air samples was highest in Chicago. Overall, these trends reflect the degree of urbanization and industrialization in each watershed. Wet deposition represented 56 to 68% of the total atmospheric deposition flow, followed by gas absorption (24-30%), and dry deposition (8-14%). The net mass transfer flows were $-89 \pm 131 \text{ kg yr}^{-1}$ for Lake Michigan, $1022 \pm 413 \text{ kg yr}^{-1}$ for Lake Erie, and $-148 \pm 97.8 \text{ kg yr}^{-1}$ for Lake Superior. Despite uncertainties associated with the calculations, our estimates suggest that Lake Superior and Michigan may be closer to a steady state while Lake Erie is discharging PFAS via outflow. Here we demonstrate that wet deposition might be an important source of PFAS loadings to Lake Superior but not for Lakes Michigan and Lake Erie.

4.22.P-Tu-182 Characterizing the Prevalence of Bisphenols, Alkylphenols, Neonicotinoids, and Polycyclic Aromatic Hydrocarbons in Lake Superior Tributaries

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The Great Lakes Water Quality Agreement requires the U.S. and Canada to identify chemicals of mutual concern (CMC) that are potentially harmful to the environment or human health and to develop binational strategies to reduce CMC in the Great Lakes. Bisphenol A and alkylphenols (APE), were nominated for CMC designation but received a ‘no determination’ based on insufficient information. Previous research has identified bisphenol A and APE as being relatively widespread in tributaries throughout the Great Lakes basin and tend to be present at concentrations of potential concern. Furthermore, the Lake Superior basin has been the least studied among the lakes. In 2022, we conducted a study to fill data gaps related to the prevalence of nominated but undesignated CMC and other chemicals of interest in 18 tributaries throughout the Lake Superior basin. Surface water was collected three times and analyzed for 6 bisphenol analogues, 4 APE; 13 neonicotinoids were analyzed once. Sediment was collected one time and analyzed for the same suite of chemicals as surface water, plus polycyclic aromatic hydrocarbons (PAH). At least one bisphenol analogue was detected in water at all sites; bisphenol F was most frequently detected at concentrations ranging from 5 to 190 ng/l. Bisphenol S and bisphenol AF were detected at 8 and 4 sites, respectively. APE were detected in water at all but one site and in sediment at >50% of sites. APE signatures were dominated by 4-nonylphenol in both sample matrices, with total APE concentrations ranging from 0 to 75ng/l in water and 0 to 1500 ng/g in sediment. One neonicotinoid was detected one time in both water (imidacloprid) and sediment (clothianidin), indicating relatively low

presence of this class of chemicals. PAHs were detected in sediment at ~75% of sites, but concentrations were relatively low compared to other more developed watersheds in the Great Lakes Basin. A preliminary screening of water concentrations against bioactivity information from the USEPA ToxCast database indicates that concentrations of bisphenols AF, A, and F, and 4-nonylphenol may be at levels that warrant concern and further monitoring and research. Furthermore, the additive toxicity of these chemicals may exacerbate potential biological responses. Data gathered from this study fill gaps related to the presence of potentially hazardous chemicals throughout the Lake Superior basin and can inform future monitoring and research throughout the Great Lakes basin.

4.22.P-Tu-183 Using Multiple Taxa to Evaluate Perfluoroalkyl Substances (PFAS) in the Grand River, Ontario, A Tributary of Lake Erie

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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. The river spans 280 km in length and flows through major municipalities including Waterloo, Kitchener, Cambridge and Brantford (cumulative population ~1 million), before discharging into Lake Erie. Earlier work has characterized pharmaceuticals in wild biota in the river. Here we expand our study to examine PFAS in surface water and wild-caught fish and mussels from 6 sites sampled in 2021. Greenside darters (*Etheostoma blennioides*) were caught via electrofishing. Adult mussels (*Lasmigona costata*) were collected from the same study sites while wading. Water samples were taken during the biota collections. PFAS analysis in tissues consisted of fish whole body homogenate whereas mussel analyses were based on a composite of gonad, gill and digestive gland tissues. Health assessment endpoints were assessed in fish: condition factor, liver and gonadosomatic index and stable isotopes analysis to discern trophic status and foraging strategies. Mussel morphometrics consisting of shell length, wet weight and age were evaluated. Targeted LC-MS/MS was used to quantify C4-18 perfluorocarboxylates (PFCA) and 8 perfluorosulfonic acids (PFSA). In addition, several polyfluoroalkyl sulfonamides, fluorotelomer carboxylic acids and perfluoroalkyl ether acids were targeted. Total PFAS in water samples ranged from 10 to 50 ng/L and were largely comprised of C4 to C8 PFCA. In fish, total PFAS concentrations were on average 5.5 to 9 ng/g wet weight (w.w.) and the PFAS profile was dominated by perfluorooctane sulfonate (PFOS). In mussels, total PFAS concentrations were lower and ranged from 0.6 to 1.8 ng/g w.w. Interestingly, the PFAS composition was much more diverse in mussels and was predominantly long chain PFCAs (C12-C18) as well as the C4 and C8 sulfonamides. Spatial analysis indicated higher PFCA burdens in mussels from the Speed River tributary, compared to mussels from the 4 main stem Grand River sites. No inter-site significant differences were detected in fish. These results highlight the value in sampling multiple taxa in aquatic ecosystems to evaluate the quality and extent of PFAS exposure in aquatic ecosystems.

4.22.P-Tu-184 Method Development of Passive Samplers for the Analysis of Persistent, Mobile, and Toxic (PMT) Substances in Canadian Waters

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Plastic associated contaminants pose a significant threat to aquatic environments, one which has coincided with the global growth in plastic waste. Many of these contaminants are persistent, bioaccumulative and toxic (PBT); criteria which merit regulation under various acts and agreements such as the Great Lakes Water Quality Agreement in Canada. Unfortunately, these regulations do not account for pollutants that are *mobile* (PMT) rather than bioaccumulative. PMTs are suggested to pose an equivalent level of environmental and human health risk as PBTs, given their high polarity and low sorption potential, meaning they are expected to bypass various water treatment processes, potentially contributing to a long-term drinking water problem. Yet, they remain mostly unregulated and understudied in aquatic environments. An important challenge is the current

lack of representative sampling and robust analysis techniques for PMT plastic additives. Few studies have demonstrated the use of passive sampling (provides information on the general contamination status of an environment) for the capture of PMT substances, and none have focussed on Canadian waters such as the Great Lakes basin, which contain high amounts of plastic contamination. Here we present the use and optimization of passive sampling for the identification and prioritization of PMT plastic additives in Lake Ontario. The presented study investigated the use of polydimethylsiloxane (PDMS) strips and Polar Organic Chemical Integrative (POCIS) samplers with polyethersulfone (PES) and polytetrafluoroethylene (PTFE) membranes, and various receiving disk combinations (anion, C₁₈ and hydrophilic-lipophilic balance (HLB)) for the detection and quantification of 124 PMT plastic additives registered for use in Canada in water. The sampler extracts were analyzed using high performance liquid chromatography with quantitative time-of-flight mass spectrometry (HPLC-QToF-MS). The PDMS and POCIS sampler with PES and the HLB disk were found to retain the most PMT plastic additives and were subsequently deployed in Western Lake Ontario to investigate differences in PMT plastic additive contamination within wastewater and river plumes in the lake, as well as near drinking water intake sites. These samplers can assist in future assessments of water quality, and the results from this study aim to provide governments and regulators with a prioritization list of PMT plastic additives, to enable their regulation in Canada.

4.22.P-Tu-185 Evaluating the Prevalence of Per- and Polyfluoroalkyl Substances in Lake Superior Tributaries and Estimating Potential Bioeffects Using Risk-Based Screening Techniques

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Several per- and polyfluoroalkyl substances (PFAS) are designated as chemicals of mutual concern by the United States and Canada, yet few studies have evaluated their presence in Lake Superior, the largest freshwater lake in the world. To better understand the potential threats that PFAS pose to this valuable resource, 28 Lake Superior tributaries were investigated in 2022. Selected watersheds represented a wide range of land use characteristics and potential PFAS sources. Water samples (n=74) were collected during one event flow and two baseflow conditions, and stream bed-sediment samples (n=28) were collected once at each site. At least one PFAS was detected in 97% of water samples and 59% of sediment samples. Among the 33 PFAS analytes, 17 were detected in water and 17 in sediment, of which 13 were common to both sample types. Twelve PFAS were not detected in any samples. Total PFAS concentrations from individual water samples were generally low (near 10 ng/L), except at Newton Creek, Miller Creek, and Sargent Creek (up to 391, 230, and 121 ng/L, respectively). The 10 greatest PFAS concentrations in water were attributable to PFOS, PFHxS, and PFPeA in samples collected from Newton Creek and Miller Creek. However, across all sites and water samples PFBA was most frequently detected (93%), and the median concentration of PFBA was also the greatest among quantifiable PFAS detections in water samples. Total PFAS concentrations in the sediment samples were also generally low (near 40 ng/kg) or undetectable, except for Newton Creek and Muggun Creek (total PFAS=797 and 654 ng/kg, respectively). The most frequently detected PFAS in sediment were PFOS (n=16) and PFHxS (n=5). The relative potential for adverse biological effects among contaminants and sampling sites is being estimated in ongoing data analysis for this study by computing hazard quotients using established water-quality benchmarks and alternative benchmarks derived from available *in vivo* studies and *in vitro* assays. The concentrations documented in this study and preliminary analyses indicate that PFAS were present at concentrations which may elicit adverse effects, even in Lake Superior watersheds which have relatively low degrees of anthropogenic influence – highlighting the potency and ubiquity of PFAS in the environment.

4.22.P-Tu-186 U.S. EPA Great Lakes Fish Monitoring and Surveillance Program: Recent Trends of PCB and PBDE Congener Profiles in Top-Predator Fish

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The U.S. EPA's Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) has collected fish and analyzed trends of legacy and emerging contaminants in whole lake trout and walleye from all five Laurentian Great Lake since the 1970s. The GLFMSP represents one of the longest running environmental monitoring programs in the Great Lakes and provides a unique insight on long-term chemical trends in Great Lakes fish. Lake trout serve as a good indicator of lake contaminants due to their broad distribution and position at the top of the food web. Fish are caught from two discrete offshore sampling locations from each lake, on an alternating annual basis. Collected fish are aged, composited, and analyzed for various contaminants. Trends of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) have decreased at all sampling sites since 1992 and 2002, respectively. The GLFMSP analyzes for 143 PCB congeners, with historic reporting mainly focused on total PCB concentrations and not congener specific profiles within fish samples. Dioxin-like and non-dioxin-like PCB congeners have different toxicological effects on aquatic life, and higher and lower-chlorinated PCB congeners are also known to behave differently in the ecosystem, where highly chlorinated PCBs can readily accumulate in tissue or adsorb to sediment. In this study, comparisons of congener specific distributions of PCBs and PBDEs are explored temporally and spatially in top-predator fish across the Great Lakes basin.

4.22.P-Tu-187 Risk-Based Screening of Individual PFAS and PFAS Mixtures in Great Lakes Tributaries with Relations to Land Cover and Wastewater Effluent

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Per- and polyfluoroalkyl substances (PFAS) were found to have potential biological effects in a study using Polar Organic Chemical Integrated Samplers (POCIS) for estimation of surface water concentrations from 60 tributary sites within 20 watersheds in the Great Lakes Basin in 2018. Sites represented a range of urban to agricultural, forested, and wetland land uses and included a gradient of wastewater treatment effluent from zero to 44% of annual streamflow and several sites with airport influence. Twenty-one of 32 targeted PFAS compounds were detected in POCIS samplers, of which, 16 had available POCIS sampling rates for time-weighted water concentration estimates and risk-based screening. Risk-based screening was conducted to estimate potential for aquatic toxicity using published water quality benchmarks (available for nine PFAS), benchmarks derived from results in primary literature within the ECOTOX Knowledgebase for apical endpoints (10 PFAS) and non-apical endpoints (10 PFAS), and high-throughput screening results from ToxCast (14 PFAS). Five individual PFAS exceeded at least one of the benchmarks at multiple monitoring sites, including PFOS, PFHxS, PFBS, PFOA, and PFNA. Computation of chemical mixture effects, estimated by summation of exposure-activity ratios (EARs) for chemicals that influence common ToxCast assays and specified gene targets, indicated that EAR values increased up to 14-fold over individual chemicals with up to 14 chemicals contributing to mixture EAR values in a single ToxCast assay. Potential for biological effects from PFAS was correlated with urban land use and the proportion of streamflow contributed by wastewater effluent.

4.23.P-Mo Advances to Address Challenges in Non-Targeted Analysis for Environmental Risk Assessment

4.23.P-Mo-132 Examining Chemical Space Coverage of Solid Phase Extraction Methods on Environmental Waters for Use with Non-Targeted Analysis Methods

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Non-targeted analysis is an emerging technique for studying environmental contaminants, with the ability to

detect hundreds to thousands of unidentified chemicals in environmental samples. While the presence of a chemical detected with non-targeted analysis can be confirmed with a certain level of confidence, the absence of a chemical of interest is much more difficult to interpret with the non-targeted approach. Most current non-targeted extraction methods use broad extractions that are often not well categorized for chemical extraction efficiency across multiple classes of chemicals. However, bias from extraction is important to explore and understand; knowledge on which classes of compounds are amenable to extraction and instrumental methods provides valuable insight into the chemical space covered within a given method. In this work, over 250 reference standards, representative of chemical classes in our larger 1400 standard in-house database, were used to explore extraction efficiencies for non-targeted analysis in environmental waters. Standards cover a vast range of chemical space, ranging in LogK_{ow} from -3.4 to 8.7, with neutral masses ranging from under 100 Da to over 1400 Da. Standards were selected from multiple classes and uses: including over 100 pesticides and pesticide metabolites (ex. organochlorines, organophosphates, urea derived, carbamates, pyrethroids, triazines, neonicotinoids), per- and polyfluoroalkyl substances, personal care products, drugs of abuse, and over 100 pharmaceuticals (ex. antibiotics, antidepressants, antivirals, veterinary drugs, commonly used medications). Multiple extraction methods were examined, including a previously used and common method utilizing Oasis® hydrophilic-lipophilic balanced (HLB) SPE cartridges, as well as methods combining various SPE sorbents together to improve recovery and overall chemical coverage. Sorbents were acquired from multiple vendors, including weak anion exchange, weak cation exchange, mixed-mode cation exchange, graphitized carbon black, and reversed phase sorbents. Our goal is not necessarily to develop a single method capable of extracting all chemicals of interest, but to provide more confidence and understanding of the chemical space coverage of our non-targeted methods.

4.23.P-Mo-133 Application of Non-Targeted and Suspect Screening Workflows on Surface and Drinking Water Samples Using Reverse Phase and HILIC Chromatography

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The analysis of contaminants of emerging concern (CECs) has been an ongoing priority due to their potential for adverse effects on human health and wildlife. With many tens of thousands of chemicals in use today, prioritizing specific chemicals or mixtures of chemicals to be targeted for analysis has become increasingly difficult. Multiple non-targeted chemical screening tools were developed to look for a broad range of possible CECs present in environmental waters using liquid chromatography and quadrupole/time-of-flight mass spectrometry with electrospray ionization (LC-QTOF-ESI) in both positive and negative ionization modes. Two chromatographic techniques were evaluated: (1) reverse phase C18 and (2) hydrophilic interaction liquid chromatography (HILIC) used to capture the more polar CECs that may be present in water. Optimization of the HILIC method required more extensive method development and troubleshooting to obtain reliable separation. Using the developed workflows, a suspect screening library of roughly 1,400 possible CECs, including pesticides, pharmaceuticals, personal care products, illicit drugs/drugs of abuse, and various anthropogenic markers was made with experimentally collected data to compliment the non-targeted workflow. These standards were analyzed using both chromatographic methods and in both ionization modes, to provide insight on which classes of common environmental contaminants are best captured with these approaches. The developed non-targeted methods were also applied to environmental water samples collected with polar organic chemical integrative samplers (POCIS), including surface water receiving wastewater effluent discharge, downstream untreated drinking water, and treated drinking water. Over 30 of the suspect screening chemicals were found in surface and/or drinking water samples, and the results from the reverse phase and HILIC non-targeted analyses were compared. The applications of these methods could serve as a first step in characterizing the chemical space covered when utilizing different chromatography methods on environmental water samples.

4.23.P-Mo-134 Profiling PFAS Removal Efficiency in Drinking Water Treatment using a Non-Targeted Analysis Approach

Yong-Lai Feng, Anca Baesu and Yan Li, Health Canada

Per- and polyfluoroalkyl substances (PFAS) are a class of thousands of synthetic chemicals, many of which have been used worldwide to make products resistant to water, heat, and stains since the 1950s. Some PFAS do not break down easily and therefore stay in the environment for a very long time, especially in water. Studies show that exposure to even trace amounts of some PFAS is linked to harmful health effects. Water treatment plants in Canada and the United States do not routinely monitor for PFAS. The EPA has set a health-advisory level of 70 ppt for the combined concentration of two PFAS (PFOA and PFOS) in drinking water. The current monitoring program in Canada only targets the most researched PFAS and the number of PFAS characterized in exposure assessments is still very small compared to the total number registered for commercial use, let alone their transformation products and metabolites in the environment. This study uses a non-targeted analytical method that can rapidly map many PFAS in drinking and source water using high-resolution mass spectrometry to determine the removal efficiency of PFAS in treated drinking water. The Rapid PFAS Diagnostic Ion Search tool was compared with other data screening tools in terms of PFAS identification accuracy and false positive rate. A retention time prediction model was also used to increase confidence in the identification of new PFAS compounds in treated drinking and source water. The results showed that NTA is a powerful tool to evaluate the removal efficiency of PFAS in drinking water quality control.

4.23.P-Mo-135 Analysis of Volatile PFAS in Soil Using High Resolution GC/MS and an Accurate Mass PFAS Library

Sofia Nieto¹, Tarun Anumol¹, Matthew Giardina¹, Matthew Curtis¹, Luann Wong², Gabrielle Black², Stephan Baumann¹ and Thomas Michael Young², (1)Agilent Technologies, Inc., (2)University of California, Davis

Per- and polyfluoroalkyl substances (PFAS) are emerging contaminants that are found in water, air, soil as well as wildlife and attract growing attention. A subset of PFAS that have been detected in the environment can be volatile or semi-volatile in nature. Therefore, a variety of analytical techniques are necessary for their detection. GC/MS is typically used for detecting volatile and non-polar PFAS compounds. However, one of the challenges in PFAS analysis by GC/MS is a lack of publicly available libraries containing a comprehensive selection of the PFAS EI spectra. Therefore, an accurate mass GC/MS library for PFAS detection was created. Soil samples extracted with methylene chloride were sampled from two fields in California that have historically received biosolids. We have screened the soil samples for PFAS using an accurate mass library as well as other contaminants and identified a variety of chemicals of industrial origin, drugs, and pesticide residues using the NIST Mass Spectral EI Library.

4.23.P-Mo-137 Hydrophilic Lipophilic Balanced Retainability in the Context of Chemical Space

Anna Feerick¹, Gabrielle Black² and Thomas Michael Young², (1)University of California, (2)University of California, Davis

Hydrophilic Lipophilic balanced (HLB) cartridges are widely used in solid-phase extraction for sample cleanup and concentration for nontarget analysis. Their ability to retain a diverse set of compounds while simultaneously removing interferences makes them a preferred step in many workflows. Despite the diverse set of compounds that HLB is capable of recovering, there are inevitably many that are not successfully retained. The boundaries of chemical space, i.e., the set of known and possible compounds, covered by HLB extraction remains undefined, limiting the identification confidence of suspect and unknown contaminants annotated during nontarget analytical workflows. Defining the “HLB detectability domain”, an area of chemical space where HLB is capable of extracting versus not based on a multitude of molecular descriptors, is crucial for improving the confidence of feature annotation. We propose the use of computational machine learning-based models to predict the detectability domain of nontarget methods by examining the extraction potential of HLB sorbents. For this purpose, a dataset from a previous study consisting of 414 priority and emerging pollutants in water

was used. One- and two-dimensional molecular descriptors for each compound were obtained through PaDeL. Both classification and regression algorithms were evaluated for their potential in developing a quantitative structure-property relationship (QSPR) model that will describe which molecular descriptors most affect HLB retainability. The classification methods include classification and regression trees (CART), random forests (RF), and genetic algorithm (GA)-support vector machines. Both linear (GA-multiple linear regression) and non-linear (GA-support vector regression) regression methods were employed. RF classification models were the best performing based on their kappa value. Regression models were not successful in predicting a chemicals percent recovery, likely because the data set included a limited number of values within some recovery ranges. Knowing the range of compounds that can be readily recovered by HLB resins, confines nontarget annotations to this area of chemical space. Understanding the bounds of HLB extraction (nontarget space) is an important step in nontarget method standardization and reproducibility, both of which are necessary for improving regulatory acceptance of the methods for environmental monitoring and assessment.

4.23.V-020 Non-Target Screening of Organohalogen Compounds in Archived Tilapia Samples from Several Asian Countries

Tue Minh Nguyen¹, Junna Matsuoka¹, Pham Hung Viet², Tatsuya Kunisue¹ and Agus Sudaryanto³ (1)Ehime University, (2)Vietnam National University, Hanoi, (3)National Research and Innovation Agency, Indonesia

Nontarget screening studies have recently revealed the accumulation of typically unmonitored organohalogen compounds (OHCs) in various marine animals, but information for freshwater species is still lacking. This study investigated the accumulation profiles of known and unknown OHCs in archived muscle tissues of tilapias collected from Okinawa (Japan), Jakarta (Indonesia), Manilla (Philippines), and Hanoi (Vietnam) using nontarget analysis based on two-dimensional gas chromatography–time-of-flight mass spectrometry. The most abundant OHCs detected were legacy persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs) and PCBs. Typically unmonitored OHCs including C₁₀-, C₁₁-, C₁₅-based chlordane-related compounds, polychlorinated terphenyls (PCTs) and dichlorodiphenyldichloroethylene (DDE) homologues, were detected in a sample collected from Okinawa in 2006. However, these unmonitored OHCs seem to have declined significantly in the freshwater environments, as they were not detected in samples collected 10 years later, except for a few chlordane-related compounds.

4.23.T Advances to Address Challenges in Non-targeted Analysis for Environmental Risk Assessment

4.23.T-01 Target and Nontarget Screening of Psychoactive and Lifestyle Substances: An Exploratory Study to Support the New York State Wastewater Surveillance Network

Emily Jean Vogel, Shiru Wang and Teng Zeng, Syracuse University

Wastewater surveillance has emerged as a promising technique for tracking public health concerns such as infectious diseases, antimicrobial resistance, and trends in substance abuse. Currently, a proposal is being considered for a surveillance program throughout New York State that would leverage the existing wastewater sampling infrastructure to assess substance use. However, several operational considerations such as sample throughput, monitoring frequency, and analytical coverage must be evaluated before launching the statewide initiative. Our exploratory study sought to collect preliminary data to inform this decision-making process. Over the course of our study, flow-proportional composite influent samples were collected on a weekly or biweekly basis during 2020-2022 from ten participating wastewater treatment plants (WWTPs) within the New York State Wastewater Surveillance Network. We first developed and validated a target screening method to quantify 51 psychoactive and lifestyle substances in wastewater samples using online solid-phase extraction (SPE) coupled to liquid chromatography-high resolution mass spectrometry (LC-HRMS). Our online SPE-LC-HRMS method is anticipated to meet the throughput demands when the program eventually expands to cover 204 WWTPs across New York. Furthermore, it achieved the quantification of both acidic, hydrophobic and basic, hydrophilic substances at ng/L levels. We then implemented Monte Carlo simulations to calculate the mass

loads and consumption rates of 21 quantifiable substances at the sewershed level and to examine the effects of varying monitoring frequencies (i.e., weekly, biweekly, or monthly) on these estimates. Given the vast amount of chemical data embodied in wastewater, we further explored the use of nontarget screening to identify additional illicit drugs with a focus on fentanyl analogs. Our qualitative screening approach utilized the query of a substance list curated from government and crowd-sourced databases and the search for class-specific neutral loss fragments. Overall, our pilot study demonstrated the feasibility of applying an online SPE-LC-HRMS method for target and nontarget screening to support substance use assessment through a statewide wastewater surveillance program.

4.23.T-03 FluoroMatch Suite Software: Advancing Non-Targeted Analysis for the Comprehensive Detection and Identification of PFAS and Polymers

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PFAS are a group of highly persistent and toxic chemicals that have been detected across all environmental media, including water, soil, and air, and are found in virtually all human plasma samples. Non-targeted analysis (NTA) is a powerful approach that allows for a comprehensive and unbiased analysis of environmental samples, making it a crucial tool for the detection and identification of PFAS, especially given that the percent of total PFAS from unknown or not commonly screened PFAS continues to increase as manufactures ramp up production of alternative structures. To overcome the data-processing bottleneck in NTA we introduce FluoroMatch Suite 3, a software designed specifically for the analysis of per- and polyfluoroalkyl substances (PFAS). FluoroMatch Suite 3 covers file conversion, peak picking, blank filtering, annotation, scoring, homologous series detection, and visualization of mass spectral evidence and statistical results. Visualizations include Kendrick mass defect series normalized by CF₂ or other repeating units, retention time versus m/z, EIC visualization, annotated MS/MS and MS, violin plots, PCA, volcano plots, and filtering by statistical significance. Furthermore, interactive visual datasets can be shared online, increasing transparency and usefulness of data-sharing. This framework is not limited to LC-HRMS/MS analysis of PFAS, and examples of applications using ion mobility and to polymers such as polysorbate, polysorbides, and PEGs will also be explored. FluoroMatch 3.0 has been applied to snow, water, leachate, foams from various water bodies, aqueous film-forming foams (AFFF), dried blood spots, SRM dust, soil, and other materials, and mice dosed with AFFF to name a few. We will discuss PFAS diversity and measurement challenges across sample types. FluoroMatch was compared to targeted assays, curated datasets, and standards and annotations automatically scored as “high-confidence” were shown to have a false positive rate of 5% or less. Results show that hundreds of PFAS were detected across the samples, including uncommonly screened or previously unreported PFAS such as ether linked PFAS in blood, unsaturated perfluorosulfonic acids in AFFF, and glucuronidated PFAS conjugates in mice. *Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the US EPA.*

4.23.T-04 Using High-Resolution Mass Spectrometry DOM Characterization to Drive Nontarget Analysis of Groundwater at a Historic Crude Oil Spill Site in Bemidji, Minnesota

Gabrielle Black¹, Barbara A Bekins² and Michelle L. Hladik², (1)University of California, Davis, (2)U.S. Geological Survey

The 1979 Bemidji oil spill released over 1.5 million liters of crude oil and has since become one of the most heavily researched natural attenuation sites in the world. Previous findings have identified that high molecular weight compounds are persistent in the groundwater downgradient from the spill and that chemical

compositions are significantly different between background and downgradient wells despite the spatial distance and decades since the oil spill. To identify specific high molecular weight compounds potentially responsible for this observation, a combined approach was implemented using high-resolution mass spectrometry. Groundwater samples were collected in tandem for nontargeted analysis using electrospray and atmospheric chemical ionization techniques. Nontarget data acquisition and analyses were focused on the dominant mass ranges observed during the DOM analysis. Better understanding of the chemical speciation driving differences between background and downgradient wells could help inform environmental risk and elucidate potential compound-specific remediation techniques.

4.23.T-05 The Chemical Space Tool: Mapping and Visualization to Characterize Chemical Spaces

Charles Lowe¹, Nathaniel Charest², Adam Edelman-Munoz², Christian Ramsland², Shirley Pu¹, Jon Sobus¹ and Antony J Williams¹, (1)U.S. Environmental Protection Agency, (2)Center for Computational Toxicology and Exposure

The abstract notion of chemical space underpins much of the work we do as chemists. In this presentation, we introduce the chemical space tool. The Chemical Space Tool is intended to help researchers visualize and discover patterns within chemical data, harnessing many existing concepts and efforts within the chemistry and machine learning communities. In this presentation we demonstrate the current capabilities of the tool and discuss how it can aid the workflows of non-targeted analysis or chemical data characterization. We consider the visualization methods used, particularly the unsupervised machine learning techniques employed to capture and picture the patterns in high-dimensional data. We connect these abstract techniques to the realities of the chemical space and demonstrate how manifold learning can solve some problems that present themselves in chemical analysis. We show examples of how the application can be used to characterize chemical space and shape experimental agendas. *This abstract does not reflect U.S. Environmental Protection Agency policy.*

4.23.T-06 Chemical Space of Exposome: Where Are We and How Far We Can Go?

Denice v Herwerden¹, Viktoriia Turkina¹, Alex Nikolopoulos¹, Jake O'Brien², Tobias Hulleman¹, Leon Barron³, Kevin Thomas² and Saer Samanipour¹, (1)University of Amsterdam, (2)University of Queensland, (3)Imperial College London

The chemical space is comprised of a vast number of known and unknown possible structures. Prior to analysis of an exposome sample, the contents are unknown and could be comprised of thousands of known and unknown chemical constituents. Such samples are frequently analyzed using non-targeted analysis via liquid chromatography (LC) coupled to high-resolution mass spectrometry often employing a reversed phase (RP) column. However, it is unknown which part of the chemical space is covered by NTA studies and particularly RPLC. Knowing which chemicals are outside of the RPLC subspace can assist in reducing false positive identifications for library searching and avoid screening for chemicals that will not be present in RPLC data. From the known unknown point of view, we developed a model to assess whether a chemical theoretically can fit the chemical space of RPLC. Applying this model to the chemicals in Norman SusDat showed that ~19% fall outside of the RPLC subspace, meaning that these compounds are likely to be incorrectly identified or require another selectivity for them to be measured. Finally, the examination of recent NTA studies published between 2017 and 2023 that employed LC-HRMS revealed that only around 2% of the estimated chemical space (i.e., Norman SusDat) was covered by these studies. These results suggest the need for a paradigm shift in the NTA studies to further expand their coverage of chemical space.

4.23.V Advances to Address Challenges in Non-targeted Analysis for Environmental Risk Assessment

4.23.V-020 Non-Target Screening of Organohalogen Compounds in Archived Tilapia Samples from Several Asian Countries

Tue Minh Nguyen¹, Junna Matsuoka¹, Pham Hung Viet², Tatsuya Kunisue¹ and Agus Sudaryanto³, (1) Ehime

University, (2) Vietnam National University, Hanoi, (3) National Research and Innovation Agency, Indonesia

Nontarget screening studies have recently revealed the accumulation of typically unmonitored organohalogen compounds (OHCs) in various marine animals, but information for freshwater species is still lacking. This study investigated the accumulation profiles of known and unknown OHCs in archived muscle tissues of tilapias collected from Okinawa (Japan), Jakarta (Indonesia), Manila (Philippines), and Hanoi (Vietnam) using nontarget analysis based on two-dimensional gas chromatography–time-of-flight mass spectrometry. The most abundant OHCs detected were legacy persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs) and PCBs. Typically unmonitored OHCs including C10-, C11-, C15-based chlordane-related compounds, polychlorinated terphenyls (PCTs) and dichlorodiphenyldichloroethylene (DDE) homologues, were detected in a sample collected from Okinawa in 2006. However, these unmonitored OHCs seem to have declined significantly in the freshwater environments, as they were not detected in samples collected 10 years later, except for a few chlordane-related compounds.

4.24.P-Mo-139 Evaluation of Evidence for Enhancing Biological Pathway Conservation Across Species Generated Using a Combination of Computational New Approach Methodologies

*Peter Schumann*¹, *Claudia Rivetti*², *Jade Houghton*², *Bruno Campos*², *Geoffrey Hodges*² and *Carlie LaLone*³,
(1)Oak Ridge Institute for Science and Education, (2)Unilever, (3)U.S. Environmental Protection Agency

The ability to predict which chemicals are of concern for environmental safety is dependent, in part, on the ability to extrapolate chemical effects across many species. This work investigated the complementary use of two computational new approach methodologies to support cross-species predictions of chemical susceptibility: the US Environmental Protection Agency Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool and Unilever's recently developed Genes to Pathways – Species Conservation Analysis (G2P-SCAN) tool. These stand-alone tools rely on existing biological knowledge to help understand chemical susceptibility and biological pathway conservation across species. The utility and challenges of these combined computational approaches were demonstrated using case examples focused on chemical interactions with 3 protein targets: peroxisome proliferator activated receptor alpha (PPAR α), estrogen receptor 1 (ESR1), and gamma-aminobutyric acid type A receptor subunit alpha (GABRA1). Biological pathways in the Reactome database were identified for each of these targets using G2P-SCAN, which also inferred the conservation of those pathways across 7 common model organisms (*H. sapiens*, *M. musculus*, *R. norvegicus*, *D. rerio*, *D. melanogaster*, *C. elegans*, and *S. cerevisiae*). These mapped pathways then underwent a prioritization step through estimation of the protein target essentiality using protein-protein interaction networks. The protein target and additional critical proteins within the prioritized pathways were evaluated using SeqAPASS to generate lists of species where that pathway is likely to be conserved. Through this approach, multiple lines of evidence were generated to support hypotheses of conservation of biological pathways across species where there was taxonomic overlap between the G2P-SCAN and SeqAPASS predictions of pathway conservation. Through comparisons of relevant molecular and functional data gleaned from adverse outcome pathways (AOPs) to mapped biological pathways, it was possible to gain a toxicological context for various chemical-protein interactions. The information gained through this computational approach could ultimately inform chemical safety assessments by enhancing cross-species predictions of chemical susceptibility. It could also help fulfill a core objective of the AOP framework by potentially expanding the biologically plausible taxonomic domain of applicability of relevant AOPs.

4.24.T Placing Tools in the Hands of Decision-Makers: Novel Computational Approaches for Improved Understanding of Chemical Safety

4.24.T-01 Assessing Coastal Contamination: The Coastal Pollution Data Explorer

Arina O Morozova and *Christine Buckel*, National Oceanic and Atmospheric Administration

The Coastal Pollution Data Explorer (CPDE) will be an interactive web-based interface where users can explore

spatial and temporal trends of chemical, physical, biological, and toxicological data. The goal of the tool is to allow users to compare, analyze, graph, and download NOAA's National Centers for Coastal Ocean Science contaminant data from 1986 to the present. Moreover, users will be able to compare the data against the existing governmental regulatory values such as US FDA and EPA guidelines and detect possible drivers of higher contamination using preset supplementary data (e.g., demographic data, social vulnerability, coastal ecosystems, wastewater proximity, etc.). Contaminant data spans the coastal regions of the continental US (including the Great Lakes), Alaska, Hawaii, and Puerto Rico. The CPDE will highlight trends and measurements of various contaminants within three categories: 1) Contaminants of emerging concern which lack published health guidelines (e.g., PFAS, pharmaceuticals), 2) Legacy organic contaminants that were used historically but in most cases have been banned or strictly regulated (e.g., pesticides, PCBs, PAHs), and 3) Trace metals (e.g., arsenic, cadmium, lead, mercury). The CPDE will make it easier to target, retrieve, manipulate, and download data end users need with easy filtering, summarizing, and interacting maps, tables, and charts. This will be an online data explorer with a target audience of persons focused on water quality and human and ecosystem health (e.g., academics, state and federal organizations, and non-profit and conservation organizations). The tool will not only provide the information on chemical pollution and toxicity level of the coast but also bring awareness and communicate environmental chemical safety with the public. The CPDE is currently under development and will be demoed during the talk, a preliminary public release is targeted for 2024 with a final product planned in 2025.

4.24.T-02 EAS-E Suite: Bridging Research and Application for Chemical Assessments and Sustainability

Alessandro Sangion¹, Li Li², Liisa Toose¹, Trevor N Brown¹, James M Armitage³ and Jon A. Arnot¹, (1)Arnot Research and Consulting Inc., (2)University of Nevada, Reno, (3)AES Armitage Environmental Sciences, Inc. Scarcity of quantitative exposure data for chemicals presents significant challenges in environmental and human health risk assessments and hinders informed regulatory and business decisions. The Exposure and Safety Estimation (EAS-E) Suite platform addresses these challenges and bridges the gap between advances in scientific research and application needs for chemical assessment. EAS-E Suite is a free online platform that facilitates the application and evaluation of databases, chemical property prediction tools, and mass-balance models, supporting exposure and risk estimation by multiple stakeholders. EAS-E Suite includes curated databases of physical-chemical properties, environmental degradation and biotransformation rates, as well as production volumes for over 50,000 organic chemicals. Data queries and model predictions can be obtained by entering a chemical SMILES, CAS or name. Substances not in the database can be evaluated by using built-in quantitative structure-activity relationships. The various models that are built into the EAS-E Suite platform are automatically parameterized by the system, though users can preferentially enter alternative input parameters, if desired. One model included in EAS-E Suite (CiP-CAFE) estimates mode-of-entry and emission rates from chemical structure, functional use category and production volume. The PROduction-To-EXposure High Throughput (PROTEX-HT) model combines CiP-CAFE with the far- and near-field exposure models (RAIDAR and RAIDAR-ICE) to simulate aggregate exposures to representative ecological receptors and humans. EAS-E Suite also contains tools to evaluate Persistence (P), Bioaccumulation (B), and Mobility of chemicals. The platform also incorporates an internal Threshold for Toxicological Concern (iTTC), providing conservative, safety-based hazard screening information. EAS-E Suite offers an integrated and holistic assessment of chemical life cycles, emission rates, fate and transport processes, exposure factors, and toxicokinetics. This presentation demonstrates the capability of EAS-E Suite to screen and prioritize thousands of chemicals for hazard and risk. Holistic exposure and safety estimates are compared to P and B categorization results showing significant differences in potential assessment outcomes using hazard or risk-based information. Overall, EAS-E Suite empowers quick screening and evaluation of chemicals, facilitating informed decision-making using state-of-the-science data and tools.

4.24.T-03 EcoToxXplorer: A Web-Based Platform for Comprehensive Toxicogenomics Data Analysis

Nil Basu¹, Jessica Ewald¹, Guangyan Zhou¹, Natacha S Hogan², Markus Hecker², Doug Crump³, Jessica Head¹ and Jianguo Xia¹, (1)McGill University, (2)University of Saskatchewan, (3)Environment and Climate Change Canada

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

4.24.T-04 ExpressAnalyst: A Unified Platform for RNA-seq Analysis in Non-Model Species

Jessica Ewald, Peng Liu, Guangyan Zhou, Yao Lu, Zhiqiang Pang and Jianguo Xia, McGill University

Transcriptomics data promises to play a key role in the transition from traditional to alternative toxicity testing methods. The rich biological details that can be measured after a chemical exposure have the potential to expose mechanistic and mode of action insights that can inform *in vitro* to *in vivo*, cross-life stage, and cross-species extrapolations. However, many research groups struggle to analyze these complex datasets, especially when they are collected from ecologically-relevant species that lack high-quality genome assemblies and annotations. Here we present ExpressAnalyst, a web-tool for comprehensive RNA-seq analysis for any eukaryotic species. ExpressAnalyst has five modules that enable processing and annotation of FASTQ files, statistical and functional analysis of count tables and gene lists, and querying cross-species relationships between gene and protein orthologs. FASTQ files can be processed with Kallisto for species with a reference transcriptome, or with the Seq2Fun algorithm and the EcoOmicsDB ortholog database for species without a reference transcriptome. The raw data processing can be performed online for smaller datasets, or locally for datasets of any size using our stand-alone Docker implementation. Additionally, Seq2Fun can be used to process and integrate raw data from multiple species, enabling cross-species comparative transcriptomics analysis. The statistical and functional analysis modules support downstream analysis of count tables and gene lists produced by both Kallisto and Seq2Fun. They include steps for filtering and normalization, differential expression analysis, pathway/gene set analysis (ORA and GSEA), and visualization of results with interactive heatmaps, volcano plots, and networks. The utility of ExpressAnalyst is demonstrated in a case study involving RNA-seq data from two avian species, one with a reference genome and one without. By making RNA-seq data analysis more accessible to more researchers, ExpressAnalyst will help accelerate the adoption of toxicogenomics data in chemical risk assessment.

4.24.T-05 Species Extrapolation Using the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) Tool

*Peter Schumann*¹, *Maxwell Botz*¹, *Audrey Wilkinson*², *Wilson Melendez*², *Cody Simmons*² and *Carlie LaLone*³, (1)Oak Ridge Institute for Science and Education, (2)General Dynamics Information Technology, (3)U.S. Environmental Protection Agency

Our ability to assess the environmental risk of a chemical is, in part, limited by our understanding of toxicity across species. This presents a challenge because it is impossible to test for chemical effects in every species. Therefore, novel strategies are needed to capitalize on existing data for supporting cross-species extrapolation of toxicity data and knowledge. Bioinformatics approaches are being applied towards this challenge by making use of expanding protein sequence and structural information. The Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool utilizes these data to predict chemical susceptibility across species based on concepts derived from evolutionary biology. The assumption underlying the SeqAPASS tool is that similar, or conserved, proteins are more likely to maintain similar functions. These functions might include binding to a chemical or performing a similar role in a biological pathway. The SeqAPASS tool uses four levels of protein comparisons increasing in complexity (ranging from primary amino acid sequence to structural alignments) to determine protein conservation across species. These measures of protein similarity are relevant for extrapolation of toxicity data and generating predictions of chemical susceptibility when the protein target of the chemical is known. In this way, SeqAPASS provides a rapid means of not only predicting protein conservation across thousands of species but also generates a line of evidence for extrapolation of toxicity data to those species. Additionally, it can improve our understanding of how well model organisms or known sensitive species can serve as surrogates for other untested species through evaluation of protein target or biological pathway conservation.

4.24.T-06 The EnviroTox Platform: Update & Applications

*Michelle Rau Embry*¹, *Adriana C. Bejarano*², *Kristin A Connors*³, *Kellie A Fay*⁴, *Constance Mitchell*¹ and *Ryan R Otter*⁵, (1)Health and Environmental Sciences Institute, (2)Shell Global Solutions, (3)Procter & Gamble, (4)U.S. Environmental Protection Agency, (5)Middle Tennessee State University

The EnviroTox Platform (www.envirotoxdatabase.org) was released in 2019 with the aim of providing a fit-for-purpose, curated database of aquatic toxicity data and associated tools that would allow flexible, rapid, and predictive approaches for ecological risk assessment. The database currently contains 80,912 aquatic toxicity records representing 1,641 species and 4,267 unique chemical CAS#s and is meant to be evergreen with periodic updates scheduled based on new data sources and emerging data needs. Since its release, this platform has been cited 60 times in the peer-reviewed literature, with various uses and applications. The dataset is accessed via a web-based query system that is integrated with a predicted no effect concentration (PNEC) calculator, an ecological threshold of toxicological concern (eco-TTC) distribution tool, and a chemical toxicity distribution (CTD) tool. Although originally built with the specific purpose of exploring the utility and application of the eco-TTC, the platform has seen widespread use across the scientific community. This presentation will provide an overview of the EnviroTox Platform and showcase several applications of how it can be utilized. These examples include: Water quality standards for chlorinated benzene-like compounds. This example highlights the relationships between existing aquatic toxicity-based water quality standards (e.g., Water Quality Criteria and Standards; Environmental Quality Standards) and distribution-based approaches included in EnviroTox. Providing a risk-based context to environmental monitoring data. This example highlights a collaborative project in Brazil to prioritize additional monitoring needs using a risk-based approach for pharmaceuticals detected in surface waters by utilizing EnviroTox. Exploring taxa sensitivity across chemical classes and modes of action. The chemical-specific and species-specific information included within EnviroTox, coupled with the distribution tools allow for exploration of grouping approaches.

4.24.V Placing Tools in the Hands of Decision-Makers: Novel Computational Approaches for Improved Understanding of Chemical Safety

4.24.V-022 Modeling temperature-dependent chronic toxicity of thiamethoxam in chironomids using a DEB-based TKTD model

Josef Koch¹, Silke Claßen¹, Daniel Gerth¹, Tido Strauss¹, **Maxime Vaugeois**² and Nika Galic², (1) *gaiac* - Research Institute for Ecosystem Analysis and Assessment, Germany, (2) Syngenta

Thiamethoxam (TMX) is a systemic neonicotinoid insecticide, which is used to protect crops against insect pests. In a recent ecological risk assessment, the US EPA identified potential risk from chronic TMX exposure to freshwater aquatic invertebrates. Chironomids were identified as one of the most sensitive taxa. Given the use of TMX in different regions, the toxicity of TMX to the chironomid life cycle at variable environmental temperatures needs to be considered in risk assessment but is not yet well understood.

We developed a quantitative mechanistic effect model for *Chironomus riparius*, to simulate the species' life history under dynamic temperatures and exposure concentrations of TMX. Laboratory experiments at both constant and dynamic conditions of temperature and TMX exposure were performed for model calibration and validation. The larval dry weight and survival over time, the time to emergence, and the reproduction (as mean egg mass size per female) were assessed as endpoints. The model design was based on the state-of-the-art mechanistic approach of combining the Dynamic Energy Budget (DEB) theory with toxicokinetic-toxicodynamic (TKTD) modeling. Different assumptions about the size-dependency of damage dynamics were explored to adequately reproduce the experimental data. Furthermore, multiple hypotheses about which processes and parameters should be corrected for temperature were tested. The results showed concentration-dependent effects of TMX on the chironomids' life cycle, including slower growth, later emergence, smaller egg masses, and higher mortality rates with increasing concentrations. Besides an expected acceleration effect on the organisms' growth and development, higher temperatures further increased the adverse effects caused by TMX. With some data-informed modeling decisions (most prominently the inclusion of a size-dependency that makes larger animals more sensitive than smaller ones), the model was parametrized to convincingly reproduce the calibration data. Furthermore, the calibrated model also delivered adequate fits to independent data, which had been collected specifically for validation purposes. While the approach of combining DEB with TKTD modeling has been tested before with other species-toxicant combinations, this study is among the first to rigorously investigate the combination of toxic stress and temperature-dependency with tailor-made extensive experiments.

4.24.V-023 Simulating life-cycle toxicity of thiamethoxam in *Chironomus riparius* with realistic dynamic exposure profiles and variable temperatures using a moving-time-window approach

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Freshwater invertebrates are often considered at risk for exposure to insecticides from agricultural runoff, with chironomids representing one of the most sensitive taxa. To better quantify the chronic toxicity of the neonicotinoid insecticide thiamethoxam to chironomids, an individual-level mechanistic effect model for *Chironomus riparius* that accounts for both time-varying temperature and chemical concentration was calibrated. We used this recently developed model to simulate the life cycle of *C. riparius* with realistic exposure profiles and constant or variable temperatures using a moving-time-window (MTW) approach. In this approach, the life cycle of a species is simulated repeatedly, each time with a slightly shifted starting point in the exposure profile. This method is particularly useful when the life cycle of a test organism is shorter than the existing exposure profiles used in risk assessment. It allows determination of the worst-case exposure window resulting from a critical combination of (endpoint-dependent) life stage sensitivity and (timing and magnitude of) chemical exposure. In addition to survival, growth, time to emergence, and potential reproduction of

individuals can also be evaluated as endpoints. Because the calibration and validation studies are based on laboratory data measuring emergence success, we used the endpoint “survival at pupation” in our simulations, which also combines sublethal and lethal effects in one endpoint, as the timing of pupation is dependent on toxicity. Consequently, the MTWs were not of a predefined length as commonly used for MTWs. To quantify potential risk, exposure multiplication factors (EMFs) resulting in a pre-defined effect strength, analogous to summary statistics like ECX, can be determined. As recent research showed that in some cases non-monotonous relationships can exist between EMFs and the endpoint of interest, which can be missed by automated one- or two-sided approximation algorithms, we accounted for this by using the more robust brute-force approach for EMF calculations. Our aim was the investigation of the influence of different approaches to integration of temperature (constant temperatures vs. dynamic temperatures) on the prediction of the EMFs as risk indicators. In this study, we have contributed to a better understanding of the influence of dynamic temperatures on evaluating effects from exposure in environmentally dynamic conditions, thereby reducing uncertainty in predicting risk.

4.25.P-Tu Developments in the Era of Big Data and Artificial Intelligence in the Field of Environmental Fate and Exposure Modelling

4.25.P-Tu-188 Refining Environmental Exposure Assessments for Consumer-Use Down-The-Drain Ingredients Using Spatially Resolved Datasets and Surface Water Flow Modeling: Focus on Europe

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A key aspect to understanding the safety of consumer use down-the-drain (d-t-d) ingredients is environmental exposure assessment in receiving waters. A global framework to estimate d-t-d substance concentrations in river catchments across the globe that leverages large spatial datasets on population, wastewater treatment (WWT) infrastructure, per capita water use, river connectivity, and river flow has been developed and demonstrated for several countries. This framework was built on the well-established iSTREEM® model for the United States, and provides estimated river concentration distributions based on spatial variability of these parameters, as well as chemical-specific properties such as WWT removal and in-stream decay. We present the model expansion to Europe which leverages official European Union data on wastewater generation and WWT infrastructure and connectivity, including geographic locations of wastewater treatment plants (WWTP). The river flow data are based on a global hydrological dataset developed as part of this project, that uses high-resolution spatial data on land cover, precipitation, and river connectivity. The resulting distributions of WWT infrastructure, connectivity, and wastewater generation were consistent with values reported for Europe. Chemical case studies on common substances used in consumer d-t-d applications such as laundry detergents and personal care products were used to demonstrate the model and to compare to monitored values across Europe. Modeled values were in good agreement with monitored values and the model was able to capture spatial variation in concentration distributions. Spatially resolved exposure models are a valuable tool in prospective environmental safety assessments providing concentration distributions across modeled regions.

4.25.P-Tu-189 Fate and Persistence Estimation & Simulation Tool (F-PEST): A Comprehensive Tool for Assessing the Fate, Persistence, and Long-Range Transport of Organic Chemicals

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Evaluating the environmental fate, persistence (P), and long-range transport potential (LRTP) of chemicals is crucial for understanding potential exposures and risks to human and ecological health. Chemical assessments are challenging due to the complex behavior of chemicals in different environments, limited access to state-of-

the-science models and data, and the need to obtain chemical partitioning and degradation half-lives to parameterize multi-media environmental fate models. To address these challenges, we introduce the Fate and Persistence Estimation & Simulation Tool (F-PEST) which employs a comprehensive and integrated approach utilizing multiple metrics and assessment methods within a Weight of Evidence (WoE) framework. F-PEST includes Level I, II and III fugacity based environmental fate models consolidating information on chemical partitioning, emission, degradation, and environmental fate and transport, to evaluate neutral and ionizable organic chemicals. F-PEST is built into the freely available online Exposure And Safety Estimation (EAS-E) Suite platform that provides autoparameterization capabilities for discrete organic chemicals using SMILES, CAS or name as user input data. Moreover, a set of built-in Quantitative Structure Activity Relationships (QSARs) can estimate parameters like partitioning and distribution ratios, as well as degradation half-lives in different environmental compartments. Applicability Domain and uncertainty estimates are included with the QSAR predictions. Users can easily replace default model input parameters provided by EAS-E Suite with preferred values. Mass balance models determine the relative distribution of chemical mass across various environmental compartments, facilitating the identification of media of concern. The tool output includes Overall Persistence (Pov), Characteristic Travel Distances (CTDs) in air or water, and transfer efficiencies (TEs), to comprehensively evaluate P and LRTP. Additionally, F-PEST estimates the three emissions fractions (dispersion, transfer, and accumulation), enhancing its ability to assess the fate and behavior of chemicals in a WoE manner. A set of libraries representing diverse regional-scale environmental conditions is provided allowing users to easily compare different system conditions. Simulations can be conducted using the default conditions from the "OECD Pov and LRTP Screening Tool" or other evaluative environments such as EQC, RAIDAR, EUSES, and ChemCAN.

4.25.P-Tu-190 Using Machine Learning to Understand the Biodegradation of Polycyclic Aromatic Hydrocarbons in Sediment

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Polycyclic Aromatic Hydrocarbons (PAHs) are toxic, persistent, bioaccumulative chemicals that are often the most toxic portion of oil spills. As hydrocarbons, PAHs are biodegradable, but the rates of such degradation are often slow due to limited bioavailability and low oxygen levels. Biodegradation of PAHs is generally environmentally beneficial, but it can alter PAH fingerprints, complicating source apportionment. The extent of biodegradation of PAHs and the conditions that contribute to it were investigated at the Newtown Creek Superfund site, which is the site of one of the largest oil spills in US history, known as the Greenpoint Spill. Notably, Newtown Creek is also impacted by PAHs from urban runoff and from a Manufactured Gas Plant that used coal and other feedstocks. This analysis relied on the Remedial Investigation databases, which include measurements of 50 PAH compounds in over 1,000 samples of sediment. Conventional parameters including nutrients (ammonia, nitrate, Total Kjeldahl Nitrogen, phosphorus), as well as pH, total solids, density, total organic carbon, soot carbon, sulfide, and cyanide were also in the database. Spatial parameters (River Mile, sediment depth) were provided, and the distance to the nearest CSO outfall was calculated using ArcGIS Pro. This wealth of data was analyzed using both unsupervised (Positive Matrix Factorization; PMF) and supervised (Random Forest) machine learning techniques. Compound ratios suggested in the literature to be useful aerobic biodegradation metrics were calculated; however, these are most useful when only one dominant source of PAHs exists, which is not the case in Newtown Creek. Comparing PMF-derived fingerprints in the (presumably aerobic) sediment with the nearby (presumably anaerobic) groundwater revealed subtle differences that are consistent with aerobic biodegradation. These were used to calculate a novel biodegradation metric which was able to control for shifts in PAH sources that are not related to biodegradation. These biodegradation metrics were analyzed by Random Forest, which indicated that phosphorus was the most important parameter controlling biodegradation, followed by River Mile, total solids, and total organic carbon. Of the nitrogen measures, ammonia was most important, followed by TKN and then nitrate.

4.25.P-Tu-191 The Molecular Composition of Water-Soluble Organic Matter Improves Predictions of Potential Soil Respiration at the Continental-Scale

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Soil organic carbon (C) is one of the largest and most active pools in the global carbon cycle. Microbial decomposition of soil organic matter (SOM) – the primary constituent of soil C – releases a tremendous amount of carbon dioxide (CO₂) to the atmosphere. Climatic and soil properties such as temperature, moisture, C, and nutrient content ultimately govern SOM decomposition, but predicting soil respiration based on these variables remains error-prone. Process-based models leveraging molecular data have long been proposed as a promising avenue for resolving these uncertainties. However, the power of high-resolution SOM chemistry to explain rates of soil CO₂ efflux has not been systematically evaluated. In this study, we compared predictions of measured soil respiration using (1) a suite of standard soil physicochemical data and (2) ultrahigh resolution molecular carbon composition separately and in combination to assess the added value of molecular information to the performance of soil respiration models. We collected 66 soil cores from across the United States and measured key soil biogeochemical properties, including potential respiration and organic matter composition at two depth levels using laboratory incubations and FTICR-MS. To extract hidden information from the collected high-dimensional data, we used non-negative matrix factorization with custom k-means clustering (NMFk) to summarize molecular composition into major types. Then, the extracted signatures are mapped to soil respiration using supervised machine learning. Specifically, gradient boosting regression models were tuned and constructed on 80% of the data with 5-fold cross-validation and tested on the remaining 20% of data. The results suggest that incorporating NMFk-based molecular signatures provided better soil respiration models than with soil properties only for top-layer soils. However, there was no significant improvement in soil respiration prediction at bottom layer soils. Different biogeochemical processes and soil carbon composition likely contribute to the difference in model performance with molecular composition at different depths. Our findings support the need for integrating soil molecular measurements into future development of soil carbon models. Future studies on the critical components in molecular composition and their roles in regulating soil respiration by biogeochemical processes are needed to better understand and predict soil respiration at continental scales.

4.26.P-Mo-143 Characterization of Chemical Hazards and Environmental Risks Associated with In-Water Hull Cleanings

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Ship hull fouling is a major issue in maritime industries, which can lead to the introduction of non-indigenous species into local marine environments. In-water cleaning and capture (IWCC) systems are encouraged by some countries as a pre-requisite for ships entering local ports to remove fouling and prevent the spread of invasive species. However, not all IWCC systems can effectively treat effluents, leading to the release of contaminants into the water column, which poses a significant risk to the marine environment. In this study, effluent samples were collected during IWC of ship hulls, both through manual cleaning by divers and automated cleaning by remotely operated vehicles (ROVs). The effluents were analyzed for total suspended solids (TSS), particle size distribution, and metal concentrations. Results showed a high abundance of particles in the 8 to 10 μm size range, with a range of 23 to 80% of total particles. The study also found that effluent treatment using an 8 μm filter size was able to remove up to 93.7% of particles. The study further revealed that manual cleaning by divers and ROVs produced similar amounts of TSS, with average concentrations of 173.4 and 189.0 mg/L, respectively. However, after treatment by filtration systems, the average concentration of TSS was reduced to 55.7 mg/L. Similarly, the study found that the concentration of metals, specifically copper (Cu) and zinc (Zn), were reduced significantly after effluent treatment. For instance, average Cu concentration reduced from 161.8 to 24.5 $\mu\text{g/L}$, while average Zn concentration reduced from 1140 to 776.5 $\mu\text{g/L}$. The study also evaluated the risk associated with the release of Cu and Zn concentrations into the local marine environment by converting

the metal concentrations to release rates. The predicted concentrations of Cu and Zn showed that manual cleaning by divers had a higher impact on the local marine environment compared to ROV cleaning. However, the concentrations were subject to variation based on the surface area of ships cleaned and cleaning locations. The study concludes that IWC should only be encouraged when capture systems are available to prevent the release of contaminants back to the local marine environment. The results of this study emphasize the importance of effective effluent treatment during IWC of ship hulls and can serve as a reference for maritime industries and regulatory agencies to develop more efficient and environmentally friendly IWCC systems.

4.26.P-Mo-144 Computational Fluid and Particle Dynamics Modeling for Repeated Inhalation Exposure Assessment with OpenFOAM

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Risk assessment of a chemical substance is conducted by comparing the actual exposure level with the acceptable level. The acceptable level for humans is typically derived from repeated dose toxicity studies conducted in animals. To reduce the use of vertebrate animals in assessments, a new approach method that directly calculates the acceptable level in humans for inhalation risk assessment has been proposed based on an *in vitro* assay with human airway cells and an *in silico* dosimetry model (Corley et al., 2021, Ramanarayanan et al., 2022). This model consists of a computational fluid dynamics (CFD) model and a simple mucociliary clearance model. The CFD model covering the human airway from nose to trachea or mouth to bronchi predicts particle deposition in each airway region over wide range of particle diameters. The clearance model calculates the retained dose after repeated exposure. The model uses commercial CFD software that can easily calculate complex settings, however; there are no studies on the prediction of particle deposition in each airway region using corresponding free software that is publicly available. In addition, the clearance model is based on simple assumptions that the airway is divided into three compartments and the clearance rate is constant for each compartment. In this study, an *in silico* dosimetry model was developed using OpenFOAM, which is an open source CFD software, to predict particle deposition in each airway region. Furthermore, our model directly calculates the mucociliary clearance by simulating the mucus flow on each boundary grid to predict the retained dose in each airway region in more detail. The results of airflow and particle deposition were compared and verified with measurement data (Kelly et al., 2004, Phuong et al., 2015). It was found that the predictions from our open source software-based model generally agreed with the measurements.

4.26.P-Mo-146 Reasonable Maximum Exposure in Environmental Risk Assessment – Multiple Interpretations and Apparent Spontaneous Expansion

Francis Ramacciotti, Tamara House-Knight and Samantha Townsend, GHD

Many decisions regarding environmental remediation are made daily by regulators and the regulated community. These decisions can be site-specific or programmatic (based in statutes, rules, guidance, etc.). These remedial solutions are all typically designed to protect human health and the environment and many of the decisions in the US (and abroad) are based on risk assessment principles that go back to 1998 (Risk Assessment Guide for Superfund Part A), 1996 (Soil Screening Guidance), or further. The process and approach was historically based on a deep understanding of these documents combined with institutional knowledge regarding the quantitative and qualitative approaches used to make defensible risk-based decisions. One of the key aspects in all of these approaches, particularly in human health risk assessments, whether stated or not, is the Reasonable Maximum Exposure (RME) that provides a means of evaluating whether an assessment is appropriately conservative for a receptor population of interest. Over the past three decades institutional knowledge has been lost and when combined with other economic and social pressures, multiple interpretations of what is and is not included in the RME have emerged. Additionally, there has also been spontaneous expansion to include uncertainty regarding toxicity values in the quantification of the RME in human health assessments, when these parameters are often considered immutable, as well as historically being separate from

the RME in its original form (yet obviously part of the risk characterization). The net result of these various understandings is often disagreement over an approach simply because of “uncertainties”, where only select uncertainties that could inflate the resultant risk estimates are discussed. Rarely are all uncertainties given a fair shake and even less often is there a consistent understanding across practitioners of the conservatism inherent in RME estimates. While groups such as ITRC have attempted to restate and remind practitioners what the RME represents and includes, significant confusion continues to exist. We provide a summary of the background & basis for the RME, an understanding of what is typically included (e.g., exposures that represent in aggregate ~ 95% of the population), and an identification of a few key aspects that are imperative to risk characterization, but not included yet often associated with the RME (e.g., uncertainty tied to toxicity).

4.26.P-Mo-147 Development of Passive Sampler Rate Constants in Silicone Bands for Environmental Monitoring

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Passive samplers for environmental monitoring offer many advantages over traditional grab sampling in that they can be inexpensive, sample large volumes of water, offer lower detection limits, are easy to deploy and collect, and capture a wide range of contaminants. From an analytical standpoint, passive samplers do not directly yield contaminant water concentrations, instead they provide the mass of analyte accumulated on the sampler over the deployed time frame. The accumulated analyte mass can be converted to a time weighted water concentration using sampling uptake rate constants (R_s), however, R_s values are dependent on many factors including contaminant $\log K_{ow}$, passive sampler material and physical dimensions, salinity, temperature, and water flow rate. NOAA’s National Center for Coastal Ocean Science has been working with a local waterkeeper to deploy silicone band passive samplers around Charleston, SC, USA. While passive sampling is not a new technique, using silicone bands in marine and estuarine environments is relatively understudied and there is a lack of rate constants available to convert contaminant masses measured on the silicone bands to water based concentrations. In this study, 21 day laboratory exposures will be conducted under different environmental conditions (e.g. temperature, salinity) to study the uptake of organic contaminants (polycyclic aromatic hydrocarbons or PAHs, fipronil, and pyrethroids) onto the silicone bands. Using statistical modeling, including the Gompertz model and non-linear least squares regression, sampling rate constants will be generated for the contaminants of interest and will be used to convert measured contaminant masses on the deployed silicone bands to water concentration. Seasonal trends from the sampled sites (n=20) will be investigated. These water concentration converted data will allow for better comparison among the sampling sites especially when comparing across seasons as well as locations where flow rate and/or other environmental conditions vary. Furthermore, these water sampling rate constants will aid in future studies that use silicone band passive samplers in marine and estuarine environments.

4.26.P-Mo-148 Towards Standardization of the Biomimetic Extraction Using Solid-Phase Microextraction (BE-SPME) Analytical Method

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Biomimetic extraction using solid-phase microextraction (BE-SPME) is a passive sampling analytical method that can predict the aquatic toxicity of complex petroleum substances. The method provides a nonanimal alternative to traditional bioassays that can reduce both vertebrate and invertebrate aquatic toxicity testing. The technique uses commercially available polydimethylsiloxane-coated fibers that, following nondepletive extraction of water samples, are injected into a gas chromatograph with flame ionization detection. During BE-

SPME analysis, the solid-phase microextraction step can be conducted either manually or using an autosampler. Recent round robin research program comparing results from 10 laboratories reported that the results from the automated BE-SPME method yielded much lower inter- and intralaboratory variability relative to the results obtained using the manual method. While the source of the variability in results from the round robin was suspected to be caused by the mixing rate and type during the SPME fiber-sample equilibration, additional data from analyses of duplicate water samples at multiple laboratories using only the automated method showed significant interlaboratory variability (50-100% relative percent difference). This high interlaboratory variability hampers comparisons across studies and pooling of data to allow reproducible hazard assessments of waters containing complex petroleum substances. Additional research involving multiple laboratories has been initiated to identify other potential sources of interlaboratory variability of BE-SPME with the aim of standardizing the method. This presentation will discuss the main causes of identified interlaboratory variability and potential solutions.

4.26.P-Mo-149 A Half-Cell Reaction Approach for pH Calculation Using a Solid-State Chloride Ion-Selective Electrode with a Hydrogen Ion-Selective Ion-Sensitive Field Effect Transistor

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Autonomous pH measurement using chemical sensors in dynamic nearshore waters is encumbered by their extensive simultaneous ranges of and rates of change in pH, temperature, salinity, and other water quality parameters. Since pH controls so many biological and chemical processes, it is referred to as the “master” variable in aquatic systems. However, actionable pH data records in nearshore waters remain scarce. In this work, we define a half-cell reaction approach for pH calculation using the electrode couple comprised of a solid-state chloride ion-selective electrode (Cl-ISE) as the reference electrode and a hydrogen ion (H⁺)-selective ion-sensitive field-effect transistor (ISFET) as the H⁺-sensitive measuring electrode. This new approach splits out and isolates the independent responses of the Cl-ISE to chloride ion activity (and salinity) and the ISFET to hydrogen ion activity (and pH), calculates pH directly on the total scale (pH_{total}), and removes its chloride dependence. We also apply and compare pH_{total} calculated using the half-cell reaction approach and the existing complete cell reaction approach (which combines the electrochemical responses of the Cl-ISE and the ISFET) using data collected by two autonomous pH sensors deployed in a test tank. Salinity and pH varied between 1 and 31 and 6.9 and 8.1, respectively, over a six-day period. Our results demonstrate the rapid response of the Cl-ISE to new salinity as sensor-measured pH_{total} were $\leq \pm 0.01$ pH when compared to pH_{total} of discrete bottle samples directly following salinity dilution or concentration. Sensor-measured pH_{total} further had root-mean squared errors ranging between ± 0.0025 and ± 0.0168 pH calculated using both reaction approaches relative to pH_{total} of discrete bottle samples. Our results are notably in contrast to the few *in situ* field sensor deployments over similar environmental conditions that demonstrated pH_{total} measured with the Cl-ISE to be less reliable in nearshore waters. Therefore, additional work beyond the correction of variable temperature and salinity conditions in pH_{total} calculation is needed to examine the effects of other external stimuli on *in situ* electrode response. Furthermore, greater scrutiny of the ISFET as the H⁺-sensitive measuring electrode is needed to advance real-time pH monitoring in dynamic nearshore waters.

4.26.P-Mo-150 Fluorine Contamination in Soils at Landfill Of Phosphate-Gypsum Wastes: A New Analytical Protocol, Evaluation of Mobility, and Risk Assessment

Seok-Young Oh and Hyeongseok Kim, University of Ulsan

The new analytical method via an X-ray fluorescence analysis was evaluated to enhance the precision and sensitivity of fluorine concentrations in soils. It was hypothesized that a patent-registered XRF method may effectively analyze fluorine concentration in soil with ease and reliability. Total fluorine concentrations by XRF

were compared with those by three different types of analytical protocols, incineration/distillation, alkaline fusion, and aqua regia extraction procedures, revealing that among the three procedures, the incineration/distillation procedure did not show reliable precision and reproducibility. In contrast, total concentration from the XRF analysis is linearly correlated with those by alkaline fusion and aqua regia extractions. According to the results of the Korean waste leaching procedure (KWLP) and toxicity characteristics leaching procedure (TCLP), the leachability of fluorine from fluorine-rich soils was not directly related to total fluorine concentrations in soil. Risk assessment also revealed that fluorine-rich soil did not show a non-carcinogenic toxic effect though the total fluorine concentrations in the soils exceed the regulation level (800 mg/kg) in South Korea. Our results suggest that the XRF analysis with a newly developed pretreatment method may be a promising alternative procedure to determine the total fluorine concentration easily and rapidly in soil. Further efforts may also be needed to effectively evaluate the fluorine contamination in soil because of the leachability and risk assessment of fluorine in soil.

4.26.P-Mo-151 Synthesis of Radiolabelled Industrial Chemicals, Drugs and Crop Protection Products from Carbon-14 Building Blocks

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Carbon-14 (^{14}C) is the key radioactive isotope used in metabolism studies supporting the development of industrial chemicals, drugs and crop protection products. Carbon-14 used in radiolabelling studies across the world originates from [^{14}C]carbon dioxide which is typically stored as [^{14}C]barium carbonate. This molecule is considered as the universal starting material from which all other carbon-14 labelled compounds are prepared. The four core building blocks that stem from this universal starting material are [^{14}C]carbon dioxide, [^{14}C]cyanide, [^{14}C]acetylene and [^{14}C]cyanamide. The four core building blocks can be further expanded via a variety of different pathways to give a multitude of different families of low molecular weight ^{14}C labeled intermediates. Access to these low molecular weight ^{14}C labeled building blocks allows for complete control when designing and developing the radiolabelling of a test item. As a result, multiple synthetic routes can be designed and multiple radiolabelling sites within the target molecule proposed. This process ensures a one-stop-shop, entirely controlled radiosynthetic process from design to execution to single or multiply radiolabelled test items. The poster will show how these pathways can be utilized to provide options for the radiolabelling of industrial chemicals, drugs and crop protection products.

4.26.P-Mo-152 Effective Predictive Domain: The Hidden but Powerful Risk Assessment Variable

Samantha Townsend, Francis Ramacciotti, Tamara House-Knight and Timothy Walsh, GHD

There are countless variables involved in environmental risk assessment for contaminated sites, especially human health risk assessment. However, only a handful of these variables are actively evaluated for potential modification when a risk assessment is being performed. The remainder are usually taken as “given,” presumed to be default and/or “set”, and their implications are not always considered. One of these variables that is seldom considered is the effective predictive domain (EPD), or the range of octanol/water partition coefficients and molecular weights where the regression model can be used to predict the water permeability coefficient for organics (RAGS E, 2004). This term describes the physical/chemical properties in water that cause chemicals to not permeate the skin, i.e., it determines whether dermal contact is a relevant exposure pathway that can be modeled from water. Often this term is not evaluated because the evaluation is not directly necessary to “run numbers” and calculate risk estimates. Effectively ignoring this variable can have a significant impact on the evaluation of certain chemicals in a risk assessment. This work documents and provides examples of how the EPD can be critically used in human health risk assessments. It will also explore how this variable can be applied to classes of chemicals (e.g., emerging contaminants) and how incorporating an evaluation of the EPD

can significantly alter the results of a risk assessment. Additionally, it will explore the possibility of applying this variable in calculations for dermal contact with soil/sediment. This is intended to serve as a practical guide to using an often-overlooked variable.

4.26.P-Mo-153 Statewide Mapping of Per-and Polyfluoroalkyl Substances (PFAS) in Florida Drinking Water

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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. This has resulted in the regulatory concentration limit of PFAS to be under scrutiny. In addition to a growing list of health concerns, these chemicals are also ubiquitously used and present, they have an innate ability to be highly mobile once released into the environment, and they have an unmatched ability to resist degradation. As such, PFAS have been detected in a wide variety of environmental matrices, including soil, water, and biota; however, perhaps the matrix most closely related to human exposure is drinking water. Thus, the primary objective of this study was to focus on the detection and quantification of PFAS in drinking water from the state of Florida (United States). In this study, 465 drinking water samples were collected (via trained citizen scientists), with representation from each of the 67 counties in Florida. Well water, tap water, and bottled water, sourced from Florida, were extracted using solid phase extraction and analyzed for 32 target PFAS, using 19 isotopically-labeled internal standards, via ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). This study provided previously limited data regarding the concentrations and diverse profiles of PFAS in Florida drinking water. Furthermore, this data allowed for the comparison of drinking water contaminants geographically within the state of Florida, comparison to other US states, and evaluation with regard to the latest drinking water regulations for PFAS proposed by the US Environmental Protection Agency (EPA) in March of 2023. Furthermore, we hope that this comprehensive analysis across the state will provide more attention toward solutions and overall community awareness and hopefully, statewide regulations aimed at mitigating these harmful chemicals in our drinking water.

4.26.P-Mo-154 Assessment of Multiple Passive Sampler Techniques for the Determination of Organic Contaminants in Coastal Waters

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Passive samplers can be a useful tool to determine the presence of organic contaminants in aquatic environments. Compared to traditional passive samplers however, silicone bands and CLAM (Continuous Low-Level Aquatic Monitoring) devices are relatively new technologies that researchers are turning to because of their affordability and ease of use when conducting field monitoring. Although literature exists on research conducted in fresh water aquatic environments, there is little information regarding these two types of passive samplers in the marine environment. In this study, we examine yearly seasonal patterns of organic contaminants targeting polycyclic aromatic hydrocarbons (PAH's), pyrethroids, and fipronil 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 general water quality with the objective to determine potential differences among sites, between seasons, and year to year comparisons. The design includes deployment of silicone bands for 21 days each season for three years, as well as deployment of the CLAM samplers for four time points within the 21-day window each season for three years. Preliminary results, including 2021 and 2022 field deployments, suggest that silicone bands and CLAM samplers can be used to detect measurable amounts of organic contaminants, and that when making comparisons between years, seasons, and field sites, as well as between compound classes, noticeable differences are present. PAH 50

levels and Pyrethroid levels are more prevalent in the spring for example. The silicone band technology will be further advanced by establishing uptake rate constants for classes of contaminants that will then be applied to estimate water concentrations from the chemicals adsorbed on the field-deployed silicone bands.

4.26.P-Mo-155 Achieving More Accurate PCBs Structural Assignment Using Gas Chromatography and Mass Spectrometry “Ortho Effects” for all PCB Congeners

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The availability of PCBs commercial standards and references provided an opportunity to test all 209 PCB congeners for their mass spectrometric “ortho effect,” or the enhancement of the [M-CI]⁺ ion abundance, relative to the molecular ion for biphenyls having 2,2'-; 2,2',6-; and to a smaller degree, 2,2',6,6'- chlorine substitution. During the study, it was observed and verified that much smaller “ortho effect” were present for PCBs lacking any, or one ortho chlorine (2-), or two ortho chlorines on the same ring (2, 6-). Chromatographic runs using a 40 meter, Rtx-PCB chromatographic column, connected orthogonally with a 1-meter DB-17 column, yielded retention properties which showed previously recognized chromatographic ortho effects and ring substitution - retention order correlations. Use of the gas chromatographic “ortho effect” combined with the measured mass spectrometric “ortho effect” proved to be of value for recognizing incorrectly assigned chemical structures of PCB congeners in environmental sample analyses.

4.26.P-Mo-156 Detection of Exposure to Naturally Occurring Toxins by Liquid Chromatography High Resolution Mass Spectrometry

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Alkaloids and other non-protein toxins constitute a broad group of naturally occurring hazardous compounds. These natural toxins, originating from plants, animals, and fungi, possess diverse structures and chemical mechanisms which can result in an extensive overlap in exposure symptomology. The continued risk of accidental exposure from foraging worldwide, food contamination, and natural product use accentuates the need to identify natural toxins in clinical specimens to support poisoning investigations. A protocol was developed to detect natural toxins in biological samples. Twenty-nine toxin standards were spiked into urine samples for method development and evaluation; ten of these were selected to serve as quality control (QC) standards for validation. Samples were analyzed with reverse-phase liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Retention time, mass accuracy and fragmentation library matching were used as parameters to confidently detect natural toxins in biological samples. Validation of this method included experiments to test lowest reportable limits (LRL), ruggedness, matrix effects, stability, and reproducibility of the protocol. These experiments confirmed optimal conditions of the developed method based on detection of the ten QC compounds. Additionally, blind analysis of urine samples spiked with various concentrations of a QC mixture relative to the determined LRL was used to evaluate method performance. Validation experiments demonstrated a robust, expandable method capable of generating highly reproducible data. Importantly, this method can be expanded to include compounds as they become of interest. Ninety toxins were tested after the initial set of 29 toxins, 47 of which passed inclusion criteria to be added to the method. This will be useful for future emergency responses to rapidly identify potential natural toxins involved in an unknown exposure event.

4.26.P-Mo-157 Sediment Contaminants and Toxicity in CA Watersheds: Perspectives from 15 Years of Monitoring Data

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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 generate sediment toxicity and contaminant data for up to 100 sites across diverse watersheds.

Sediment samples are collected annually and analyzed for several contaminants as well as tested for toxicity using EPA methods. Historically, chemical analyses have included pesticides, organochlorines, flame retardants, polycyclic aromatic hydrocarbons, and metals. Toxicity has been evaluated using survival and growth for the amphipod *Hyalella azteca* and, more recently, the midge *Chironomus dilutus*. Data are analyzed in the context of watershed land use to detect contaminant trends on spatial and temporal scales. Some analytes have shown notable trends on site-specific and statewide scales. This extensive 15-year dataset has created a knowledgebase that informs future monitoring efforts aligned with new technologies that are better positioned to adequately address the goals of the program. Some of the new screening approaches currently being tested include non-targeted chemical analyses (NTA) and effects-based biological markers. These approaches provide more detailed information than standard methods typically used in monitoring programs. Preliminary NTA data show concordance with results from previous monitoring efforts including significant relationships of pyrethroid pesticides with amphipod survival. NTA enables identification of contaminants that are not routinely targeted as well as their linkages with toxicity. Assessments of sublethal effects, such as changes in amphipod swimming, are also being conducted to identify behavioral toxicity. Further, application of receptor-based assays is underway to identify relationships between toxicity trends, land use, contaminant concentrations and behavioral changes. SPoT's statewide network of sites provides diverse collaborative opportunities for testing new methods relevant to monitoring. With an increasing number and complexity of contaminants entering aquatic ecosystems, more efficient and comprehensive assessments of ecological health are required. Utilization of new diagnostic tools within SPoT paves the way for advancement of sediment and water quality monitoring and addresses expanding contamination concerns across California watersheds.

4.26.P-Mo-158 Identification of DDT+ in Southern California Bight Deep Ocean Sediment and Biota by Nontargeted Analysis

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The Southern California Bight (SCB) has some of the highest global concentrations of dichlorodiphenyltrichloroethane (DDT). The source of DDT was long believed to come from historical discharge to the Palos Verdes Shelf, a nearshore region and designated Superfund site. However, the recent rediscovery of non-containerized offshore DDT waste dumping indicates that deep ocean basins may also contain highly polluted sediments. In particular, Dumpsite Two in the San Pedro Basin is a region where known bulk dumping of DDT waste occurred. The extent of DDT+ pollution at Dumpsite Two, and its impact on the SCB, is unknown. Our study aims to compare the DDT+ profiles between the PVS with Dumpsite Two, as well as with Dumpsite Ten (a secondary deep ocean dumpsite for non-DDT waste) through nontargeted analysis using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS). Additionally, we examine the chemical profiles of four invertebrate and fish species to determine the bioavailability of DDT+ to the deep ocean food web. Preliminary chemical analysis of Dumpsite Two and Dumpsite Ten identified 16 DDT+ compounds, and 8 of these compounds were unique to Dumpsite Two, including three isomers of tris(4-chlorophenyl)methane (TCPM) and DDNU. Additionally, we detected unknown DDT+ compounds that were previously identified in SCB marine mammals. In the biota samples, 10 DDT+ compounds were detected, including two TCPM isomers, indicating bioavailability of DDT+ to the deep food web. Further comparison of the deep ocean sediment sites with PVS sediment, as well as with biota, will allow for the identification of a potential chemical signature unique to deep ocean DDT disposal. The chemical signature can be used to trace deep ocean DDT+ throughout the SCB so that its impacts may be better understood.

4.26.P-Mo-159 Modulation of Host-Guest Complexation of PFAS with pH Ionizable β -cyclodextrin Derivatives

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Poly- and perfluoroalkyl substances (PFAS) are organic pollutants of serious concern due to their adverse health effects, widespread use, and resistance to degradation. Despite significant advances in PFAS remediation, current adsorption methods for PFAS removal from water require frequent replacement of the adsorbent and the disposal or further treatment of the bound pollutants. Hence, the development of PFAS remediation methods employing an adsorbent that can be regenerated under mild conditions with pollutant recovery could be transformational. β -cyclodextrin (β -CD), a powerful PFAS complexation agent, may serve as a vehicle in the remediation strategy for PFAS contamination. Functionalization of β -CD can be used to modulate their complexation of PFAS. We herein report the incorporation of ionizable functional groups on the primary rim of β -CD, can enhance, or inhibit formation of the PFAS: β -CD complex depending on the solution pH. The extent of this effect highly depends on the functionalization of the primary rim of β -CD and the structure of the perfluorinated compound. The association constants between β -CD and PFAS were determined using ^{19}F -nuclear magnetic resonance (NMR). Attractive and repulsive electrostatic interactions seem to dominate the strength of the host:guest complexes. These results provide insight about the molecular interactions controlling complexation of PFAS with β -CD. We demonstrated complexation of PFAS can be modulated through modifications on the β -CD ring which shows promise for PFAS remediation applications.

4.26.P-Mo-160 Pesticides Screening on Surface Water and Soil along the Mekong River in Cambodia

Putheary Ngin, Umea University

The widespread use of pesticides has raised serious concerns about environmental contamination, particularly with regard to aquatic and soil ecosystems and the screening of pesticides in those environmental matrices in Cambodia has never been studied, published studies have focused on vegetables or rice. This work aimed at assessing residue concentrations in surface water and soil in four provinces along the Mekong River in Cambodia during dry and rainy seasons (276 samples in total for residues of 64 pesticides) and conducting semi-structured interviews for identifying pesticide use by local farmers. Furthermore, an ecological risk assessment of the detected pesticide residues in surface water was evaluated using risk quotients (RQs). In total, 56 pesticides were detected in surface water and 43 in soil, with individual pesticides reaching maximum concentrations of 2,600 ng/L and 1,100 ng/g dry weight, respectively. The compounds with the highest concentration were tebufenozide in the water samples and bromophos-ethyl in the soil samples. Both are used as insecticides and both were present in almost all samples. The semi-structured interview showed that the farmers tended to apply more insecticides, especially when they observed certain pests in their fields. Inappropriate pesticide use, including the timing, frequency, concentration and type of products used, is widespread. Lastly, insecticides bromophos-ethyl and dichlorvos and fungicide iprobenfos may present a very high risk to aquatic organisms in both dry and rainy seasons with RQs values of 850 for both seasons, 67 in dry and 78 in rainy and 49 in dry and 16 in rainy, respectively. Overall, this work highlights the occurrence of pesticide residues in surface water and soil along the Mekong River in Cambodia and emphasizes the urgent need for monitoring and improving pesticide use practices and regulations in the region. It is also important to do toxicity assessments and estimate the environmental and human risks.

4.26.P-Mo-162 Pesticide Screening in Urban Creeks and Ponds in Gainesville, FL

Patrick Chris Wilson and Francisca Hinz, University of Florida

Pesticides can move from sites of application into aquatic waterbodies via surface runoff and/or leaching, exposing aquatic organisms to several at any given time. Depending on the exposure duration, concentration, and pesticide mode(s)-of-action, critical species may be harmed resulting in the disruption of ecological systems. The objective of this study was to characterize the presence of individual pesticides (and mixtures) in local urban water bodies to focus future chemical fate, toxicology, and risk assessment/mitigation research.

Samples were collected in July and December of 2022 and in May of 2023 from multiple sites along Hogtown Creek, Beville Heights Creek (Cofrin Nature Park), and from five lakes/retention ponds in Gainesville, FL. These samples were analyzed for the presence of over 130 pesticides by LCMS/MS. Eleven herbicides (e.g., atrazine: $<0.33 \mu\text{g L}^{-1}$; simazine: $<0.13 \mu\text{g L}^{-1}$; clomazone: $<0.05 \mu\text{g L}^{-1}$; diuron: $<0.02 \mu\text{g L}^{-1}$), six insecticides (e.g., imidacloprid: $<0.06 \mu\text{g L}^{-1}$; dinotefuran: $<0.02 \mu\text{g L}^{-1}$), eight fungicides (e.g., propiconazole: $<0.01 \mu\text{g L}^{-1}$), and the insect repellent DEET ($<0.14 \mu\text{g L}^{-1}$) were detected. While risks of acute toxicity appear low based on available data, knowledge is lacking regarding the input sources, exposure profiles (concentrations and duration), and possible interactions between co-occurring pesticides. This project identified individual pesticides and mixtures present in these local waterbodies which will be useful for developing future research focused on the effects of environmentally relevant mixtures.

4.26.P-Mo-163 Validation of Rare Congener PCB Performance Reference Compound (PRC) Method for Equilibrium Concentration Determination of Hydrophobic Organic Chemicals in Sediment Porewater

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Passive sampling devices (PSDs) have been shown to present many advantages over conventional sampling methods for quantifying the freely-available concentration (C_{free}) of hydrophobic organic compounds (HOC) in sediment. Non-equilibrium correction protocols using performance reference compounds (PRCs) have been developed for C_{free} determination of HOCs during short-term field deployments. The inclusion of appropriate PRCs loaded into the PSD polymer prior to deployment allows the user to infer equilibrium concentrations through the application of mass transfer models. A common strategy for selecting PRCs is to use isotopically labelled compounds of the same compound class as the target HOC. The primary objective for this work was to validate non-equilibrium correction methods without the use of isotopically labelled PRCs. A series of ex situ laboratory experiments with test sediment were conducted using low density polyethylene (LDPE) PSDs spiked with two different classes of PRCs. In one set of test sediment, a standard suite of 10 rare congener PCB PRCs were used to quantify both the analyte uptake and PRC depletion for PCBs and PAHs. A second test was spiked with ^{13}C -labelled PCB PRCs to test if similar C_{free} estimations are obtained with both sets of PRCs. The experiments demonstrated that the analyte uptake and rare PCB congener PRC elimination displayed similar kinetics, regardless of analyte class tested. Furthermore, similar PCB congener fingerprint and total PCB C_{free} estimates were obtained using both ^{13}C -labelled and rare congener PCB PRCs with 83% of C_{free} for individual PCB congeners in agreement within a factor of 2 or less, suggesting that rare PCB congeners can be applied as a standard set of PRCs for C_{free} determination. This hypothesis was further tested by “switching” the PRC results from an interlaboratory ex situ passive sampling study on PCBs and PAHs which ultimately results in similar C_{free} estimation with 97% of the 70 measured C_{free} values using labelled PAH PRCs for PAHs falling within a factor of 1.5 compared to those using labeled PCB PRCs and 88% of 76 measured C_{free} using labeled PCBs for PCB congeners falling within a factor of 1.5 when using labeled PAHs PRCs. The overall effort of standardizing PRCs for PSD applications for multiple chemical classes further facilitates the wider acceptance and adoption of the technology.

4.26.P-Mo-165 New Reference Materials for Per- and Poly-Fluorinated Alkyl Substances and Other Contaminants

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Reference materials (RMs) are homogeneous, stable and value-assigned for constitutes of interest. RMs, especially matrix-based RMs, are key components for developing and validating new analytical methods and

for evaluating and harmonizing values from established methods. The National Institute of Standards and Technology (NIST) is the US's metrology agency and is responsible for providing tools, such as RMs, to support measurements in many areas including the environment and human health. NIST is continually creating new RMs both in response to evolving needs and to replace RMs that have been exhausted. NIST has several new environmental and food RMs in production or recently released that support the monitoring of per- and polyfluorinated alkyl substances (PFAS) or other organic contaminants. Materials in production specific to PFAS measurements include two soils, dairy and beef cow, pork, corn silage, spinach, cow milk and chicken eggs. A Great Lakes fish material is also in preparation that will be value-assigned for PFAS, legacy organic contaminants and select contaminants of emerging concern. Materials recently released include four PFAS-containing aqueous film forming foams (AFFF) value assigned for up to 17 PFAS. Other materials being considered for RM production specific to PFAS include drinking water and additional soil materials.

4.26.P-Mo-166 Modeling the Kinetics of Organic Carbon-Water Partitioning of Volatile Methylsiloxanes and Environmental Implications

Jaeshin Kim, Gary Kozerski and Jeremy Durham, The Dow Chemical Company

The organic carbon-water partition coefficient (K_{OC}) is an important parameter for predicting the fate and transport of chemicals in the environment. The sorption process into particulate organic matter in soils and sediments can compete with other fate processes such as volatilization, degradation, advection, sedimentation, etc. Especially for volatile methylsiloxanes (VMS), many published studies suggested selecting accurate values to predict the environmental behaviors at environmentally relevant conditions. Thus, K_{OC} values at different temperatures relevant to real world conditions are needed although most K_{OC} values have been measured at 25 °C or at room temperature. Various methods have been employed to measure or predict equilibrium concentrations of target chemicals in both phases of particulate organic carbon and water. It would be preferable to measure equilibrium concentrations in both phases at different temperatures (i.e., a batch equilibrium method), but more resources are required for the method than other indirect methods that may be used to predict equilibrium concentrations. In the latter indirect methods, only water concentrations are measured over a testing period of desorption from the chemical-laden organic phase. In this case, chemical concentrations in organic carbon phase are predicted. In another case, chemical concentrations in both water and organic phases are predicted by measuring gas-phase concentrations via volatilization from water, similar to a purge-and-trap method. The prediction can be done using a form of modeling with assumptions. We have developed a model to predict equilibrium concentrations for lab experimental conditions and performed sensitivity analysis for input parameters at different temperatures. The model suggested that desorption rate from VMS-laden organic carbon phase, mass transfer coefficient at the air-water interface, and the fraction of loss via degradation and irreversible sorption must be well characterized to predict K_{OC} values. It is also noted that the methods that predict an equilibrium concentration(s) are less precise and more variable than a batch equilibrium method. Model equifinality was possible when measured data from an indirect method were optimized with model parameters. This means that different K_{OC} values could be obtained with different model parameters.

4.26.P-Mo-167 Quantifying Exposures Assessed Using Wearable Passive Samplers: Characterizing the Sampling Behavior of Airborne Contaminants Using Fresh Air Wristbands

Elizabeth Ziyang Lin and Krystal Pollitt, Yale University

Wearable passive samplers offer a cost-effective and non-invasive approach for personal exposure assessment across a broad range of environmental contaminants. These tools have recently been used to evaluate chemical exposures internationally across a diverse range of populations. The influence of ambient conditions (temperature, surface air velocity) on the chemical uptake has not been well characterized. Understanding the impact of these parameters is essential for expressing exposures as air concentrations. The sampling behavior of a wearable passive sampler (Fresh Air wristband) was evaluated in a controlled setting for 40 chemicals, including 16 polycyclic aromatic hydrocarbons (PAHs). Wristband sampling was characterized at three

temperatures (18, 21, 35 °C) and three surface air velocities (static, 0.5, 1.2 m/s) to simulate real-world exposures. The Fresh Air wristband contained four custom fabricated sorbent bars in a perforated sampling case. The impact of sheltering sorbents on sampling behavior was evaluated. Sampling conditions were further tested for two sorbent materials, polydimethylsiloxane (PDMS) and XAD impregnated PDMS (XAD-PDMS). The chemical uptake rate was positively related to the surface air velocity above both types of sorbent bars contaminated in the Fresh Air wristband. Sheltering the sorbent bars reduced the surface air velocity, which decreased the uptake rate (1.5 times). Ambient temperature was not found to influence the uptake rate of airborne chemicals. Across the evaluated parameters, a generic uptake rate of 0.3 m³ day⁻¹ was determined for PDMS sorbent bars, 1.3 m³ day⁻¹ for XAD-PDMS sorbent bars, in the Fresh Air wristband. Comparison of sampling behaviors of sorbent materials revealed enhanced uptake of PAHs (fourfold increase) by XAD-PDMS compared to PDMS under static conditions. Evaluation of the Fresh Air wristband under controlled conditions provided insight into the rate of chemical uptake by these wearable passive samplers. Understanding the influence of different sampling conditions on uptake supports the use of a sheltered sorbent in the wearable passive samplers. Furthermore, characterization of uptake rate enables chemical exposures to be reported as volumetric air concentrations (i.e., pg/m³), facilitating cross-study comparisons.

4.26.P-Mo-168 The Effects of Study Design Decisions on Fish Bioaccumulation Factors for Perfluoro-n-octanesulfonic Acid (PFOS)

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Protecting human health is a key driver in developing regulatory criteria for compounds of concern in surface waters. Criteria development approaches consider bioaccumulation in fish and other aquatic organisms as key exposure pathways, with bioaccumulation factors (BAFs) derived from field-collected data expressing the ratio of the contaminant in an organism relative to the concentration in the ambient environment. While robust bioaccumulation estimates are essential for development of regulatory decision making, field-derived BAFs can be highly variable within and across species due to chemical-, site-, and species-specific factors, and the degree of uncertainty is often predicated on the scientific approach of the studies used in their derivation. Available guidance for developing and conducting BAF field studies is necessarily general owing to the need for methods tailored to specific research objectives, ecosystems, chemicals, and species of interest, but a recent publication proposed that high-quality BAF studies for per- and polyfluoroalkyl substances (PFAS) were those in which BAFs were based on >3 water and tissue samples that were collected concurrently from collocated sites. To test these assertions and quantify how study design decisions regarding water and tissue samples (i.e., sampling quantity, spatial proximity, temporal coordination) affected BAF derivations, we used a publicly available dataset of perfluoro-n-octanesulfonic acid (PFOS) water and fish tissue concentrations spanning two states and >10 years. First, we calculated fish BAFs using the most temporally-proximate and spatially-linked water and fish tissue PFOS concentrations and applied random subsampling procedures to generate datasets that simulate a range of reductions in sampling effort with respect to the number of fish and the number of sites, respectively. Second, we focused on a subset of data from two waterbodies and linked fish tissue concentrations with water concentrations collected at different sites and times to evaluate the degree to which spatial and temporal proximity affected BAF patterns. This presentation describes how PFOS fish BAF patterns are affected by study design decisions, quantifies uncertainty associated with those decisions, and informs study design best practices and study quality evaluation criteria.

4.26.P-Mo-169 Monitoring Hydrophobic Organic Contaminant Concentration Changes Across Seasons in the Anacostia River Watershed

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Located within a major urban environment, the Anacostia River continues to receive inputs of legacy pollutants from its watershed, resulting in bioaccumulation in fish and the issuance of fish consumption advisories. A

detailed analysis of ongoing inputs of hydrophobic organic contaminants (HOC) from tributaries to the river was conducted to supplement the tidal Anacostia River Remedial Investigation/Feasibility Study. The study presented here focused on freely dissolved concentrations as it controls the HOC uptake in the food web. The objectives were to 1) measure several freely dissolved HOC concentrations in the tributaries of the Anacostia River 2) compare it with concentrations detected in the main stem to identify potential ongoing sources of contamination, and 3) determine if the freely dissolved concentrations vary across the seasons. This last measurement is essential to accurately estimate freely dissolved loadings from tributaries over a full year to understand seasonal inputs and its variability. Low density polyethylene (PE) passive samplers were deployed 3 months in the water column of the five main tributaries of the Anacostia River, four minor tributaries, as well as 5 locations within the river. Four deployment campaigns were successively performed to obtain time-integrated measurements of freely dissolved HOC for each season from Spring 2017 to Winter 2017/18. HOC measurements included 119 polychlorinated biphenyls (PCBs) congeners, 34 polyaromatic hydrocarbons (PAHs) and 10 organochlorine pesticides (OCPs). Results of the study identified one of the major tributaries, Lower Beaverdam Creek (LBC), as the main source of dissolved PCBs to the river with concentration up to 20 times higher than those detected in the Anacostia River. All the three LBC sites monitored showed a distinct PCB profile toward lower molecular weight congeners when compared to other tributaries. Most of the sites HOC concentrations measured two years apart did not show any difference in the patterns, however changes were prevalent between seasons. Notably, a two-fold decrease in concentrations was observed at most of the sites monitored between Summer and Fall 2017 for all HOC measured. Data from the seasonal changes in concentrations will be presented using measured parameters such as temperature, precipitation, and dissolved organic carbon concentration.

4.26.P-Mo-170 Using Refinements in Ion Chromatography to Shorten Run Times for the Determination of Perchlorate in Drinking Water

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Perchlorate is widely used as the oxidizing component in solid propellants for rockets, munitions, and fireworks. An unfortunate consequence of this production has been perchlorate contamination of soil, groundwater, and drinking water, leading to adverse health effects. In high doses perchlorate can disrupt thyroid function and hormone production, and therefore negatively impact the growth and development of fetuses and children and the healthy metabolism in adults. To minimize these adverse effects, several US states have established health-based goals or advisory levels for perchlorate in drinking water. A commonly used method for determination of perchlorate in drinking water is United States Environmental Protection Agency Method 314.0, which uses ion chromatography (IC) with suppressed conductivity detection. The work presented here will demonstrate the determination of perchlorate in drinking water at parts per billion levels using a high resolution and high capacity, small particle (4 μm) separation column that reduces analysis time per injection by three minutes, increasing throughput by 20%. Data was collected using an innovative new IC system that features enhancements that automate operation and increase component accessibility, decreasing analyst hands on time, while increasing reproducibility.

4.26.P-Mo-171 Assessment of Polychlorinated Biphenyl Emissions from the Portland Harbor Superfund Site into the Atmosphere

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The Portland Harbor Community Coalition (PHCC) is an organization concerned about the Portland Harbor Superfund Site. The Portland Harbor is contaminated with many hazardous substances, including polychlorinated biphenyls (PCBs), and the community asked us to evaluate the potential for PCB emissions from the site to their community because of dredging remediation. The Portland Harbor has historical and cultural resource significance making it one of the most complex Superfund sites designated by the United

States Environmental Protection Agency (EPA) because of its long history of shipping, industrial and commercial activity, and its key location on the Willamette River. We hypothesized there were elevated PCB concentrations in the atmosphere surrounding the Portland Harbor due to emissions and dispersion of PCBs from the contaminated water and that these emissions from the harbor contribute to inhalation risks to the surrounding community. To address this hypothesis, we conducted a study evaluating PCB emissions from the harbor, predicted dispersion of those emissions using EPA's regulatory model system AERMOD, and conducted measurements of airborne PCBs in the community to evaluate the predictive model. Working with the PHCC we identified monitoring sites along the harbor where we requested hosts who live in the area to allow placement of samplers on their properties. We used polyurethane foam passive air samplers (PUF-PAS), which were deployed for a 6-week period, to capture and measure long-term airborne PCBs. Samples were extracted and then analyzed using Tandem Mass Spectrometry GC-MS/MS (Agilent 7000) in multiple reaction monitoring (MRM) mode to quantify PCB congeners. PCB water concentrations were obtained from EPA water quality monitoring reports from 2018 to 2019 and we determined emissions using PCB concentrations and associated metadata (water velocities and sampling locations) collected and provided by the EPA. Our overall goal was to improve our collective understanding of the ambient air concentrations of PCBs and respond to and engage with the potentially impacted community of Portland Oregon.

4.26.P-Mo-172 Comprehensive Analysis of Phthalates and Phthalate Alternatives Using Gas Chromatography Mass Spectrometry with Real World Sample Demonstration

Kaley Adams, Caoilinn Haggerty, Ricky Scott, Steven O'Connell, Peter Hoffman and Kim Anderson, Oregon State University

Phthalates are anthropogenic chemicals having a wide array of commercial uses across the world, but resultant residues are frequently detected across a range of human exposures including foods and personal sampling devices. Countries around the world have added phthalates to environmental watch lists due to health effects that have been attributed to routine exposure, and interest in this chemical class has grown rapidly in the past decade. However, current analytical methods to measure phthalates are limited in the breadth of chemistry targeted, often targeting less than 15 compounds. We developed a selective ion monitoring (SIM) gas chromatography mass spectrometry (GCMS) method for quantitation of 27 phthalates and 3 phthalate alternatives covering boiling point ranges from 283-547 C°, and included several chemicals often understudied. Analytical parameters were optimized to improve resolution across the diversity of targeted chemicals, and the sensitivity of the method was compared to existing analyses. Instrument detection limits ranged from 17 to 231 ng/mL, with a median around 31 ng/mL. All phthalates were calibrated on a multi-level 6-point curve with r^2 values greater than 0.99. Finally, the application of the method was assessed with real world samples including a range of human exposure matrices such as olive oil, honey, and passive sampling silicone wristbands. The silicone wristbands were worn from a diverse pool of participants and potential exposures: roofers, children, pregnant women and farmworkers. Overall, replicates of real-world samples were found to have a relative percent differences less than 9%, representing a robust quantitative analysis.

4.26.P-Mo-173 Should We Assess the P&M Chemicals from a Perspective of the “Hazard” or “Exposure”?

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Persistent and mobile (P&M) chemicals are considered emerging threats to the environment and drinking water because they can be transported over long distances, penetrate natural and artificial barriers, and resist removal by traditional water treatment procedures. Current chemical regulatory practices define P&M chemicals based on the presence of chemicals in drinking water and the potential for human exposure. However, when screening the myriad of commercial chemicals for mobility, most existing efforts utilize bright-line criteria based on chemicals' intrinsic “hazard” properties, such as biodegradation half-lives for P and organic-carbon-water

partition coefficients for M. In this work, we compare and contrast the proposed intrinsic “hazard” criteria with chemicals’ concentration in drinking water predicted by a fate and transport model, based on the data of 112,000+ discrete organic chemicals registered in different countries’ chemical inventories. We evaluate the consistency of chemicals prioritized by “exposure” potential (chemicals with the highest concentrations in drinking water sources) and those by “hazard” assessment (chemicals with high P and M scores). We find that while chemicals with high P and M scores tend to possess a high potential to contaminate drinking water, P and M are not the only properties related to a chemical’s potential to contaminate drinking water. An example is the low potential of volatile chemicals to contaminate drinking water, even if they have high scores in P and M. Thus, screening chemicals based on the P and M hazard indicators alone may, unfortunately, lead to “false positives” and “false negatives”. It is therefore not appropriate to evaluate P&M chemicals by using hazard indicators as a proxy. This work addresses the academic and regulatory need for a better understanding of the processes and properties related to drinking water contamination and for developing and evaluating scientifically defensible methods and criteria to identify and potentially regulate chemicals of concern for the safe and sustainable use of chemicals in commerce.

4.26.P-Mo-174 Comparison of Methods for PCB Analyses for Biota Monitoring in a Regulatory Context

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Polychlorinated biphenyls (PCBs) remain an ongoing concern many years after phaseout due to their persistence, high concentrations at contaminated sites and increased reporting of “new” PCBs such as PCB-11 not arising from historical Aroclor use. Whereas many methods for PCBs have been developed and used over the years, most PCB data for regulatory compliance still rely on legacy Aroclor methods such as EPA 608 and EPA 8082. For characterizing watersheds still impacted by PCBs, measurements in fish and other aquatic/terrestrial animals have used high-resolution mass spectrometry (GC-HRMS) methods such as EPA 1668. GC-HRMS approaches have a significant advantage on sensitivity and specificity for PCBs, the ability to provide congener-specific and total PCB data, and toxicity equivalency (TEQ) data as well. However, comprehensive monitoring using 1668 is limited by relatively high cost and limited availability. Recently, the EPA released 1628 draft, a GC-MS method for PCBs that provides congener-specific, homolog, and total PCB data. Approaches like 1628 have great promise in expanding the comprehensiveness of monitoring due to lowered analysis costs, while providing the specificity of mass spectrometric data absent in Aroclor-based approaches. Since 2018, the U.S. Fish and Wildlife Service has examined PCB and organochlorine (OC) pesticide contamination in the Anacostia River watershed in Washington, DC and Maryland as part of a CERCLA-type investigation. Stationary forage fish, game fish, and caged freshwater mussels were sampled in the tidal river where there are sediment hot spots and in the non-tidal tributaries to detect continued loadings of bioavailable contaminants. We chose methods that were adequate for 1) making cleanup decisions, 2) before and after monitoring, 3) identifying ongoing sources, and 4) providing game fish data for updating consumption advisories. We met these objectives using Method 1628, enabling us to evaluate far more samples for individual and total congener concentrations, homolog distributions and OC pesticides within our budget than with a 1668 approach. We also report results of side by side analyses of a subset of the forage fish with both 1628 and 1668 to directly compare benefits and costs. Method selection should be based on consideration of project objectives and use of the data in a regulatory context; in many cases Method 1628 may be a wise choice.

4.26.P-Mo-175 Investigating Wildfire Smoke Composition and the Movement of Parent and Alkylated PAHs Between Air and Soil Before, During, and After a Wildfire to Understand Potential Human Exposure

Kelly O'Malley, Christine Ghetu, Diana Rohlman, Brian Smith, Ricky Scott, Kaley Adams, Peter Hoffman and Kim Anderson, Oregon State University

There is an increasing number of large wildfires (>1000 acres) in the Western United States (U.S.), resulting in a need for rapid responses to measure human exposure to wildfire smoke. This can be facilitated by working

with trained community members to deploy low-density polyethylene (LDPE) passive samplers before, during, and after wildfires to measure chemical contaminants. Wildfire smoke is a complex mixture and recent research has shown vapor-phase polycyclic aromatic hydrocarbons (PAHs) increased during a wildfire, concurrent with increased Air Quality Index (AQI), yet little is known about PAH movement, a valuable parameter for exposure risk assessment. Additionally, there are knowledge gaps of other vapor-phase organic contaminants present in wildfire smoke. Paired soil-poreair and air LDPE passive samplers were deployed by community members in the Western U.S. to determine chemical concentration using a gas chromatography mass spectrometry (GC-MS/MS) method, allowing calculation of diffusive flux (direction and magnitude) of PAHs and alkyl-PAHs between soil and air. We hypothesized that PAHs and alkyl-PAHs will deposit to soil during high (>200) AQI wildfires and volatilize after. The soil-poreair and air wildfire samples were also screened for 1500 chemicals using a GC-MS method to assess wildfire smoke composition. Generally for diffusive flux, we found 2 ringed PAHs diffused into air before, during, and after a wildfire, while 3 ringed PAHs deposited to soil, and 3-4 ringed alkyl-PAHs were in equilibrium or deposited at lower magnitudes compared to parent PAHs. During high AQI, there was a greater magnitude of 3-7 ringed PAHs and 3 ringed alkyl-PAHs depositing during a wildfire compared to pre-wildfire, and some post-wildfire locations showed volatilization of 4 ringed PAHs and 3 ringed alkyl-PAHs. For smoke composition, a total of 44 chemicals were detected either before, during, or after a wildfire, and 23 of these are potentially unique during a wildfire, most notably dibenzofuran. This study examines both the direction and magnitude of PAHs and alkyl-PAHs movement during all stages of a wildfire event, and fills in existing knowledge gaps of vapor-phase organic chemicals in wildfire smoke.

4.27.P-We Integrating Pesticide Exposure Models and Fate Data for Improved Risk Assessments

4.27.P-We-103 Does Pesticide Use Influence Phenology and Fitness of California Birds? A Study Using Citizen Science Data

Yuzhong Zhang and Andrea Bonisoli Alquati, California State Polytechnic University, Pomona

The widespread use of agricultural pesticides has proven a threat to non-target vertebrates. For example, farmland birds are in steeper decline than birds from other ecosystems, with insecticides as contributing factors. This study investigates whether agricultural insecticide use is associated with negative impacts on birds' fitness, as indicated by lower clutch size and fledging success, and delayed breeding dates. To this aim, we used breeding success and phenology data from the citizen science database NestWatch and related the observations to insecticide use data from the California Department of Pesticide Regulation. From 2008 to 2018, NestWatch collected 21,276 California nest records across 87 species. Seven species accounted for 91% of total records of clutch size, clutch initiation date, and fledging success. California agriculture used >1.2 billion lbs. of insecticides from 2008 to 2018. Of the 456 active chemicals, 50 accounted for >95% of usage and 88% of total application areas. We classified these 50 chemicals into 13 groups based on their chemical composition, mode of action, and risk level. High-risk groups such as Neonicotinoids, consisted of insecticides with acute toxicological effects or long environmental persistence. Low-risk groups such as oil and herbal extract, consisted of insecticides that were degradable, with no acute toxicity, or limited exposure to birds. Within group, we accounted for each chemical's toxicity by using relative potency factors. Insecticides with the lowest LD50 were used as index chemicals, while all others were converted into the toxic equivalents of the index chemical. Insecticide and citizen science data have been spatially joined in a GIS framework before assessing multi-insecticide effect using spatial autocorrelation analysis and regression analysis. We also test the correlation between crops and insecticide classes to account for the possible effect of specific crop types. Preliminary result show grapes having high correlation with fluorides and Neonicotinoids while almond with abamectin, phosphides, pyrethoids. Since habitat loss and climate change may also contribute to phenology changes and population declines, future investigations will incorporate descriptors of human disturbance, urbanization, and bioclimatic variables. This study tests the association between insecticide usage and birds' fitness, with associations potentially due to direct (toxicological) or indirect (ecological) pathways.

4.27.P-We-104 PROTEX: A Powerful Tool for Evaluating the Occurrence of and Exposure to Persistent and Mobile Pesticides and Agrochemicals

Zhizhen Zhang and Li Li, University of Nevada, Reno

Pesticides and other agrochemicals pose significant global public health concerns, necessitating the investigation of their environmental fate and resulting human and ecological exposure. Generic fate and exposure models, i.e., those built on mechanistic descriptions of physical, chemical, physiological, and behavioral processes and not specific to certain categories of chemicals, offer cost-effective and user-friendly tools for this purpose. One such example is the PROduction-To-EXposure (PROTEX) model, which supports tracking the transformation, transport, and accumulation of chemicals across the entire continuum from the production line to their presence in the human and organism bodies. However, existing generic fate and exposure models often fall short in assessing pesticides that resist degradation (“persistent”) and readily migrate in soil, sediment, and water (“mobile”), since they lack mechanistic characterization of (i) surface and sub-surface water mass balance and (ii) chemical sorption onto multiple geosorbents. In this presentation, we present our efforts to enhance and evaluate the PROTEX model, focusing on two key aspects: (i) incorporating regional surface and sub-surface water balance and including environmental compartments that are relevant for human exposure through drinking water, and (ii) considering the sorption of chemicals onto various soil and sediment components beyond natural organic matter. We will showcase the capabilities of the upgraded PROTEX model in predicting time-dependent pesticide concentrations, such as sulfamethoxazole, in surface water, riverbank filtrate, and deep groundwater following application, thereby estimating associated human exposure. This information is valuable for regulatory purposes, aiding in understanding the temporal accumulation and irreversible historical contamination of pesticides and agrochemicals. Overall, the PROTEX model offers a comprehensive computational approach to forecast the environmental fate and human and ecological exposure to pesticides and agrochemicals, thereby facilitating the assessment of potential risks associated with these substances.

4.27.P-We-105 A New Pesticides Data Visualization Tool for California

Ezra Miller, Tony Hale, Kelly Moran, Gemma Shusterman, Shira Bezalel and Cristina Grosso, San Francisco Estuary Institute

California has the most comprehensive pesticide application reporting system in the world, but using the data and combining it with relevant water quality monitoring, ecological effects, and human health data can be a challenge for even the most sophisticated analysts. Existing agency and scientific practices for data reporting and use can vary, as can existing reference values and methods for assessing the impacts of pesticides on ecological and human health. We are developing a new tool designed to make this valuable data more easily accessible to scientists, decision-makers, and others. It is an easy to use, web-based tool that enables interpretive visualization and download of high resolution geographic and temporal pesticide-related data. We envision the tool will be useful to a variety of users trying to answer a variety of scientific and management questions. For example, users can overlay maps of pesticide use, land use, and monitoring data at various watershed scales to identify patterns associated with the detection of pesticides in surface waters, or view a specific region to see where pesticide concentrations in their local area exceed aquatic toxicity thresholds and identify potential sources based on pesticide use data. This presentation will introduce the first iteration of the tool, which is focused on ecological health, along with our plans for future additions to the tool, including incorporation of human health-related data.

4.27.P-We-106 Evaluation of Pyrethroid Removal Efficacy in Agricultural Detention Basins

Berkley N. Anderson, Marie Vasi Stillway and Thomas Michael Young, University of California, Davis

Pyrethroid insecticides in agricultural runoff are known to be highly toxic to aquatic species, particularly benthic invertebrates, and can adversely affect ecosystems in receiving surface waters. The purpose of this study was to evaluate pyrethroid removal efficacy in field-based agricultural detention basins. Detention basins

can be used to detain runoff for extended periods of time (<48 hours) to control flow, allow particles to settle, and remove hydrophobic particle-bound contaminants such as pyrethroids. In this study, four agricultural sites in Salinas, California were sampled and analyzed to (1) determine site-specific organic carbon partition coefficients (K_{OC} and K_{DOC}) for target pyrethroid compounds, (2) measure particle settling and associated pyrethroid removal via small-scale settling column experiments, (3) evaluate pyrethroid toxicity removal via large-scale settling column experiments and toxicity tests using *Hyaella Azteca* (*H. Azteca*) and (4) develop a model for pyrethroid removal processes and simulate the performance of field-relevant detention basin designs in removing both pyrethroid compounds and associated toxicity. Significant particle removal (>80%) and reductions in toxicity to *H. Azteca* were observed in large-scale settling column experiments. Study findings reveal agricultural detention basins as a promising solution to remove pyrethroids and associated toxicity in agricultural runoff.

4.28.P-We Late Breaking Science: Chemistry and Exposure Assessment

4.28.P-We-196 Chemical Partitioning of Perfluorooctane Sulfonic Acid (PFOS) and Perfluorooctanoic Acid (PFOA) in Estuarine Mesocosm Exposures

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Per- and polyfluoroalkyl substances or PFAS are a class of compounds which, due their unique properties from partially or fully fluorinated carbon chains, are environmental contaminants which are both persistent and toxic to humans and wildlife. While there is an awareness of some of PFAS' environmental effects and interactions, there is a lack of knowledge about how they interact in unique environments, including estuaries. To expand knowledge in this area, mesocosm testing was performed to study the fate and effects of two PFAS in simulated estuarine environments, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). Mesocosm systems (n=24) were located in a greenhouse in Charleston, SC, USA where they were exposed to natural light and temperature conditions and experienced 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). In this 28 day experiment, there were six different treatments with four replicates per treatment. The treatments included 0.55 mg/L PFOS, 5.5 mg/L PFOS, 7 mg/L PFOA, 70 mg/L PFOA, 0.55 mg/L PFOS and 7 mg/L PFOA, and controls. To evaluate the compounds' interactions within the simulated environment, water samples and sediment samples were taken at nine different time points across the experiment. At the end of the experiment, organism tissue was collected for chemical analysis. Samples were extracted for PFOS and PFOA according to methods developed by the Environmental Protection Agency, National Institute of Standards and Technology, and the Food and Drug Administration, and analyzed using ultra high performance liquid chromatography tandem mass spectrometry. Data to be presented will examine the change in PFOS / PFOA concentration over the duration of the 28 day mesocosm experiment in the water, sediment and biological tissue samples, which will provide important information pertaining to how these compounds partition in estuarine environments. These compounds exist nearly everywhere and through examining how they interact in coastal habitats, priorities for methods of mitigation can be explored.

4.28.P-We-197 PFAS Analysis in Wastewater: A Comprehensive Workflow

Stephan Baumann, *Ruoji Luo*, *Matthew Giardina*, *Emily Parry*, *Linfeng Wu*, *Patrick Batoon* and *Tarun Anumol*, *Agilent Technologies, Inc.*

Per- and polyfluoroalkyl substances (PFAS) pose an increasing threat to the environment, animals, and human beings due to extreme chemical stability and bioaccumulation potential. Their detection at trace level is often interfered by the environmental matrices and background contaminants. A comprehensive workflow was

developed for the PFAS analysis in wastewater, based on the existing EPA draft Method 1633 with additional PFAS of varying size and functional group. This workflow contains off-line solid phase extraction (SPE), clean-up step, followed by LC-MS/MS analysis, and automatic reporting. The workflow demonstrates a reliable solution for the targeted analysis of PFAS in complex matrices with high robustness. In this study, a workflow solution was developed to achieve a highly robust detection of over 60 native PFAS in wastewater. This workflow contains a sample preparation, LC-MS/MS data acquisition and analysis, as well as automatic report generation. Briefly, 500 mL aqueous sample with spiked extracted internal standards (EIS) were concentrated using a conditioned weak anion exchange solid phase extraction (SPE) cartridge half-filled with silanized glass wool. Samples were eluted using 1% methanolic ammonium hydroxide. The eluate was acidified using acetic acid and cleaned up with carbon S. After centrifugation and filtration, samples were mix with non-extracted internal standards (NIS). Samples were stored at 4 °C prior to the LC-MS/MS analysis. After chromatographic separation, PFAS were analyzed using state-of-the-art LC/TQ instrument with optimized measurement conditions, which were accomplished by ion source optimizer and AI-based novel tuning utilizing particle swarm optimization. Performance criteria, such as method detection limit, recovery, precision, linearity, and reproducibility were investigated. The reproducibility was demonstrated by examining 300 continuous injections of real wastewater samples spiked with PFAS. Analytical results and required QC data were combined to a method specific reporting template that was automatically generated.

4.28.P-We-198 Assessment of Contamination Associated with Tobacco Product Waste within the Kendall-Frost Marsh Reserve

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Kendall-Frost Marsh Reserve is a wetland preserve in Mission Bay, San Diego, California. Due to urban runoff, stormwater pollution makes this area vulnerable to tobacco product waste (TPW) and their associated toxic chemicals. The most prevalent form of TPW found at beach cleanups are cigarette butts (CBs). It is important to assess contamination impacts from TPW, especially in natural reserve systems. In an urban reserve like Kendall-Frost Marsh Reserve, tidal marshes serve as important barriers for storm protection and erosion as well as productive filters of the watershed between estuaries and the ocean. In the reserve, water and sediment samples were collected from two sites on 13 separate occasions during wet and dry seasons. These samples were analyzed by a non-targeted analytical method based on comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS). As a result, 89 unique compounds were tentatively identified through this process. Of these, 57 were tobacco-related but most of these compounds have diverse uses such as in tire-wear particles, plasticizers, household products, manufacturing, and pesticides. Cotinine was the single compound that had tobacco-specific relation. There were 24 compounds ubiquitous in abundance or frequency of which 18 were tobacco-related, 14 were plastic use related, and 12 were associated with tire composition or combustion. Of these 24, according to EPA's CompTox database, pyrene, dibutyl phthalate, and benzene,1,1'-(1,3-propanediyl)bis- seem to be of most concern based on their persistence, bioaccumulation, and toxicity. Both pyrene and dibutyl phthalate have known tobacco associations and benzene,1,1'-(1,3-propanediyl)bis- is linked to uses in plastics, especially related to bisphenol-A. A physical survey of litter confirmed presence of TPW items in and outside of the reserve. In conclusion, the reserve's water channel and sediment was polluted with chemicals associated with tobacco products, possibly from tobacco use and disposal, and other anthropogenic products.

4.28.P-We-199 Simultaneous Determination of Fatty Acid Content of Flax Seed Oil (*Linum Usitatissimum* L.) in Corn Oil using Gas Chromatography-Flame Ionisation Detector /Mass Spectrometry

Purushottam Trivedi, Alpesh H Patel and Nadeem Ahmad Khan, Jai Research Foundation

Flaxseed (*Linum usitatissimum* L.) is a multi-purpose crop, and its consumption is beneficial to human health. Fatty acids are important components of the human body, having biological, structural, and functional roles.

Due to complex nature and presence of multiple components, flax seed oil analysis always imposes challenges for analytical chemists. In this study, a method is developed in corn oil as carrier. Method is validated to support pre-clinical toxicological study for the dose verification analysis. Linolenic acid, nonanoic acid, Tetra-decanoic Acid, Decanoic Acid, selected as analytical marker as a representative of complete flax seed oil. Linolenic acid, nonanoic acid, Tetra-decanoic Acid, Decanoic Acid has been derivatized with BF₃/MeOH reagent by using conventional heating process at 85 °C for 35 minutes. Samples prepared by spiking ethyl laurate as internal standard into matrix prior to sample extraction from dose career. Stabilwax (60 m x 0.32 mm I.D x 0.5 μm film thickness) stationary phase was used for quantitation methyl linolenate, nonanoic acid methyl ester, methyl tetradecanoate, decanoic acid methyl ester. The matrix-matched calibration curves with a correlation coefficient > 0.998, recovery range 82.23 – 109.64 % with relative standard deviation (RSD) 0.60 – 4.27 % and 24 h stable at 25 °C. Thus, the method is categorized effective and proved to be quick, robust, sensitive, and selective for routine analysis.

4.28.P-We-200 Nanoplastics Are Only a Small Fraction: Origin and Release of Submicron Particles During Washing of 12 Polyester Textiles

Tong Yang¹ and Bernd Nowack², (1)McGill University, QC, Canada, (2)Empa – Swiss Federal Laboratories for Material Science and Technology

Synthetic textiles are a significant source of microplastic fiber (MPF) pollution. While the MPF release mechanism during washing of synthetic textiles is well studied, little is known about the origin and release of nanoplastics during this process. First investigations about the nanoplastic fraction during washing and abrasion of polyester textiles have been published, however, questions were raised regarding the chemical composition of the observed submicron particles. In particular, the importance to distinguish between nanoplastics and water-insoluble oligomers was highlighted. This study aimed to establish a test protocol for extracting and analyzing submicron particles released during washing of polyester textiles and then applied it to a representative set of 12 different polyester (PET) textiles. A combination of analytical methods was used to characterize the particles, including nanoparticle tracking analysis (NTA), scanning electron microscopy (SEM), scanning transmissive electron microscope coupled with energy-dispersive X-ray spectroscopy (STEM-EDX) and Pyrolysis-GC-MS. The 12 textiles released $4.6 \times 10^{10} \sim 8.9 \times 10^{11}$ submicron particles during washing, with a mean size ranging from 122 to 191 nm. The number of released submicron particles was neither significantly influenced by the cutting method nor the textile structure, but was positively correlated (p-value < 0.01) with the number of submicron particles present on the fiber surface before washing estimated by SEM image analysis. This correlation, together with the fast decrease of particle release during repeated washes, suggests that washing is not a formation but a release mechanism for submicron particles from polyester textiles. 34-89% of the particles extracted from six selected fabrics are ethanol-soluble and they are most likely water insoluble PET oligomer particles, which is supported by the Pyrolysis-GC-MS results. Nanoplastics were therefore only a small fraction of all released submicron particles. Our results clearly show the urgent need to better understand the contribution of water insoluble oligomer particles to the pollution of the environment with anthropogenic submicron particles.

4.28.P-We-201 A Comprehensive Look at the Halogenated Organic Contaminants Impacting the Great Lakes Ecosystem

Saly Bolis Abadir Abskhroun, Clarkson University

Legacy organic contaminants have long posed a threat to the ecosystem of the Great Lakes due to their persistent, bioaccumulative, and toxic (PBT) properties. As a result, the United States Environmental Protection Agency has been monitoring the Great Lakes for PBTs since the 1970s. However, monitoring programs often focus on the compounds of interest. Therefore, non-targeted screening is required to reveal the true contaminant profile impacting the Great Lakes. The current research focuses on a novel analytical approach that enables one to see the complete volatile and semi-volatile contaminant profile in top predator fish collected

from the Great Lakes as part of the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP). In this case, a 2-dimensional gas chromatograph coupled to a high-resolution time-of-flight mass spectrometer (GCxGC-HR-ToF MS) was utilized for the non-targeted analysis of fish tissue. Samples were analyzed using electron ionization (EI) and negative chemical ionization (NCI) modes to better understand the chemical features detected. More halogenated features were observed by NCI which also yielded lower detection limits for highly chlorinated compounds. Therefore, the use of GCxGC-HRMS with NCI has further improved the ability to detect PBTs in the Great Lakes and enhanced our understanding of the nature and persistence of these compounds in the environment.

4.28.P-We-202 Unraveling Matrix Effects in the Quantification of Drugs of Abuse in Influent Wastewater Samples from Southern Ontario

Sandra Salic, Lily Warketin, Diana M. Cardenas-Soraca, Cheryl Chong, Paola A. Ortiz-Suarez, Rashne Vakharia, Leslie Bragg and Mark R. Servos, University of Waterloo

The consumption of drugs of abuse (DoA) has raised public concerns due to their impacts on human health, difficulties in assessing the extent of their use, and potential harm to aquatic organisms. However, challenges arise when quantifying these compounds in influent wastewater samples (WWs) due to co-extracts and interferences. Liquid chromatography-mass spectrometry is the common instrumental technique used to identify and quantify these substances in WWs. Nonetheless, the inherent soft ionization method in LC-MS/MS instruments leads to matrix effects (MEs), a phenomenon where target analyte ionization efficiency changes due to the presence of co-eluting compounds in the sample matrix. The research community has not yet established a standard strategy to mitigate MEs, but certain approaches can be applied to address these effects. These strategies include implementing a more selective extraction procedure, extensive clean-up, sample dilution, improving chromatography separation, and utilizing isotopically labelled internal standards. In this study, a solid phase extraction (SPE) method was optimized for 19 compounds to enhance sensitivity and minimize matrix effects during the extraction. The validation encompassed a linearity range from 50-1000 ng/L ($r^2 \geq 0.9912$), an absolute recovery (AR) $\geq 45\%$, and a reproducibility with RSD $\leq 20\%$ across three quality control levels. Finally, the method was applied to influent wastewater across seven different sites in Southern Ontario to calculate the MEs and examine how community contributions impact the quantitation of these compounds in real samples.

4.28.P-We-203 Are Biosolid-Impacted, Stocked Farm Ponds Understudied PFAS Hotspots?

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Because per and polyfluoroalkyl substances (PFAS) are ubiquitous in environmental media, it follows that wildlife are frequently exposed. Consumption of fish may be a major route of human exposure to PFAS and as a result, many states have begun PFAS monitoring programs focused on fish tissues and have begun to release consumption advisories. For example, in Indiana, the Department of Environmental Management (IDEM) has been monitoring PFAS in fish tissues from around the state since 2017 and collected 574 fish samples from 41 species, and have sampled diverse habitat types including streams, rivers, lakes, reservoirs, and even urban canals. However, despite that millions of small ponds have been constructed in the USA and that they are commonly stocked with fish for consumption, isolated farm ponds are not routinely sampled, largely because they are located on private lands. Stocked farm ponds may be PFAS hotspots, particularly if they are located near sites of agricultural biosolid applications, but data from these important habitats are sparse. In the present study, we summarize patterns of PFAS contamination in fish collected from various waterbodies in Indiana with respect to total loads and profiles, and we compare fish from the state's public waters (streams, rivers, lakes, and reservoirs) to fish from two farm ponds in Indiana: one that receives agricultural biosolids runoff and one that does not. We report on concentrations and profiles for recently sampled farm ponds alongside data from other waterbodies in the state and find that farm ponds may be PFAS hotspots, with high total loads compared

to other fish in the state and with different PFAS profiles. We discuss concentrations in comparison to available advisories and suggest that increased attention to farm ponds on private lands is necessary, as these sites may lead to dietary exposures for people consuming farm pond fish.

4.28.P-We-204 Toxic Tire Wear Compounds (6PPD-Q and 4-ADPA) Detected in Airborne Particulate Matter Along a Highway in Mississippi, USA

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Tire wear particles (TWPs) are a major category of microplastic pollution produced by friction between tires and road surfaces. This non-exhaust particulate matter (PM) containing leachable toxic compounds is released into the air, leading to inhalation concerns, and transported in stormwater runoff, leading to contamination of the aquatic environment. Airborne PM (~1-80 μm) were collected for ten consecutive days using Sigma-2 passive samplers at varying distances (5, 15 and 30 m) along a highway in Oxford, Mississippi, USA. Particles were subsequently extracted into solvent and the extracts were analyzed for selected oxidation biproducts of 6PPD by high resolution orbitrap mass spectrometry. This preliminary study focused solely on qualitative analyses to determine whether the selected TWP compounds were present in the extracts from airborne PM. The abundance of airborne TWPs increased with proximity to the road with deposition rates (TWPs/cm²/day) of 63, 47, and 23 at distances from the highway of 5 m, 15 m, and 30 m, respectively. Two 6PPD oxidation products, 6PPD-quinone and 4-ADPA were detected in all samples. Overall, this work indicates that airborne TWPs are a potential hazard to human health and the aquatic environment along busy roadways.

4.28.P-We-205 LC-MS/MS Analysis of Antimycin-A Using a Combined Response Approach

J. Nolan Steiner, U.S. Geological Survey

Antimycin-A (ANT-A) was the active ingredient in a historical pesticide formulation commonly used to control nuisance fish. ANT-A is a group of over twenty different structural analogs and these structural analogs share a dilactone ring and vary at two different functional group locations. ANT-A is produced by *Streptomyces* sp. and the analog compositions of ANT-A vary between fermented batches. ANT-A structural analogs A1, A2, A3, and A4 are the most abundant quantifiable compounds per batch and the percent composition of each varies among batches (e.g., 5 - 45%). This unique composition makes analytical verification of ANT-A difficult. The main challenge in ANT-A analytical verification and quantification is that the ANT-A reference standard structural analog percentage often differs from the experimental ANT-A. In 2013, Bernardy et al. established a method to determine ANT-A concentrations in water and used individual calculated concentrations from each of the analogs to determine the overall concentration. This presentation will describe the ANT-A quantification modifications since 2013 and a new combined ANT-A sum approach. The new ANT-A quantification uses the responses measured from the highest percent individual analogs (A1-A4), sums them together, and then creates a calibration curve from the combined responses as total ANT-A. This method can measure different manufacturing lots of ANT-A more accurately and measure pure individual analogs, which are currently being evaluated for pesticide formulation optimization. The versatility of the ANT-A sum quantification approach will be demonstrated by presenting ANT-A analytical verification results from multiple research applications. Overall, the combined sum approach is a robust method to measure complex mixtures of ANT-A analogs and will support a robust analytical method for U.S. Environmental Protection Agency pesticide registration and prospective nuisance fish management.

4.28.P-We-206 Screening for Elevated Blood Lead Levels Using Single Hair Strands: Accounting for External Contamination

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Hair has long been explored as a potential biomarker of lead exposure since lead is readily adsorbed into hair's keratinous matrix; however, the utility of hair as a biomarker for lead exposure is hampered by its susceptibility to external contamination. Specifically, lead particles can attach to the exposed hair surface, confounding estimates of endogenous concentrations causing overestimation of health risk. This study describes the development of a hair screening tool, in which the confounding influence of external contamination is mitigated by focusing on the unexposed hair root, to predict elevated blood lead levels (BLLs). This tool requires a single strand of scalp hair, which is analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Forty-four (44) workers with high potential for lead exposure, and 63 reference individuals (with no known lead exposure) volunteered for the study. Hair lead level (HLL) thresholds were developed using methods from clinical assessment to screen exceedances of BLL thresholds at 10 µg/dL and 20 µg/dL. Hair from both groups showed significantly lower lead concentrations in below-scalp portions compared to above the scalp (above-scalp was 11.2 times higher in workers, and 3.7 times higher in reference group). Hair lead concentrations below the scalp in workers significantly predicted BLLs. HLL thresholds for screening BLLs were 0.595 mg/kg and 0.85 mg/kg for BLLs of 10 µg/dL and 20 µg/dL, respectively. These HLL thresholds yielded high sensitivity (>85%), and slightly lower specificity (66.7% and 78.0%, for BLL thresholds of 10 µg/dL and 20 µg/dL, respectively). This study provides reference HLLs in non-contaminated portions of hair (<0.091 mg/kg), shows the significance of external contamination on exposed portions of hair even in a reference population, and assesses the effectiveness of below-scalp hair as a biomarker of BLL. This hair screening tool effectively predicted BLL exceedances and could be used as a non-invasive alternative to blood sampling.

4.28.P-We-207 Development and Validation of a New Sensitive LC-MS/MS Method for the Simultaneous Determination of 13 Pesticide Biomarkers, Including Dicamba, in Urine

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In order to assess the impact of exposure to various pesticides on health, several biomonitoring studies are carried out over the world. Among the pesticides of interest are organophosphate and synthetic pyrethroids insecticides and phenoxy herbicides. Since 2016, the release of seeds genetically modified to tolerate dicamba and/or 2,4-dichlorophenoxyacetic acid (2,4-D) has caused a significant increase in the use of these herbicides, raising concerns about their impact on reproductive health and cancer. To assess the degree of human exposure to dicamba, 2,4-D and multiple insecticides, a new analytical method using solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and validated under ISO/IEC 17025 guidance. This method detects simultaneously, in 250 µL of urine, 13 pesticide biomarkers, namely the herbicides dicamba, 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), organophosphate insecticides including malathion dicarboxylic acid (MDA), para-nitrophenol (PNP), 3,5,6-trichloro-2-pyridinol (TCPy), 2-diethylamino-6-methylpyrimidin-4-ol (DEAMPY), 2-isopropyl-6-methyl-4-pyrimidinol (IMPY), as well as cis-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DCCA), trans-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (trans-DCCA), 3-Phenoxybenzoic acid (3-PBA), 4-Fluoro-3-phenoxybenzoic acid (4-F-3-PBA) and cis-3-(2,2-Dibromovinyl)-2,2dimethylcyclopropane carboxylic acid (cis-DBCA), which are metabolites of synthetic pyrethroids insecticides. This method is suitable for human biomonitoring studies with detection limits (LOD) in urine samples ranging from 0.0038 µg/L to 0.10 µg/L, accuracy between 91.9 and 107.1% and inter-day precision between 1.8 and 7.8%, depending on the analyte. Samples from two External Quality Assessment Schemes, namely G-EQUAS and OSEQAS, were analyzed and

the measured values were within the defined tolerance range for these programs. Finally, preliminary results obtained following the analysis of 91 urine samples taken from pregnant women enrolled in the Heartland Study, a birth cohort study based in the United States, are also presented.

4.28.P-We-208 Health Care Delivery and Social Vulnerability Influence Pharmaceutical Discharges from Wastewater Treatment Plants and Water Quality Hazards

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It has become increasingly evident that human pharmaceuticals present important water quality risks. In arid and semiarid regions, such as the southwestern and south-central United States, these risks can be elevated when instream flows of lotic systems are dominated by or dependent on effluent discharges from wastewater treatment plants (WWTPs). Because pharmaceutical usage can vary throughout the year, and temporal studies of pharmaceutical discharges are relatively limited, we examined effluent discharges from two regional WWTPs in Texas over a 2-year period, using time-weighted 24-hour composited autosampling. We selected these two WWTPs because they serve similar population sizes; however, they service two areas that differ greatly in health care delivery and social vulnerability. Further, predicted environmental concentrations (PECs) from discharges inherently differ based on instream dilution characteristics of locations: site 1 discharges to a lotic system that is generally effluent dependent, while site 2 is effluent dominated during low-flow conditions. Following sample collection, pharmaceutical analysis was performed using isotope dilution mass spectrometry. We then examined temporal occurrence among seasons, and when sufficient data was available, employed probabilistic environmental hazard assessment to examine potential exceedances of water quality thresholds. We observed differences between sites that appear to be linked to health care delivery; for example, 20 of 23 pharmaceuticals analyzed were generally observed at higher values at site 1, which is located in a county with the 2nd highest county health ranking and low social vulnerability, compared to site 2, which has the 83rd (of 254) highest county health ranking and a medium to high level of social vulnerability. Further, select compounds, including sildenafil and haloperidol, were not detected at site 2, but were more commonly quantitated from site 1. However, 3 glucocorticoids were frequently observed at higher levels at site 2, including a higher probability of fluticasone propionate exceeding therapeutic hazard values. These findings indicate that health care delivery and social vulnerability status of local regions should be considered during water quality assessments, and challenge country scale environmental assessments by regulatory agencies for specific therapeutics that do not account for site specific usage and default instream dilution assumptions during PEC estimation.

4.28.P-We-209 Microbubble Extraction and Cleanup of SARS-CoV-2 in Raw Wastewater Samples

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Wastewater is an atypical matrix to process and analyze. Beginning with EPA method 1615, many wastewater testing labs have protocols that perform reasonably well but are very labor intensive and require large volumes of wastewater to achieve target sensitivity levels. Filtration based workflows challenges include throughput and requirement of time-consuming pasteurization and centrifugation processes for wastewater samples. The newly developed microbubbles developed for RNA extraction simultaneously improve ease-of-use, sensitivity, and method robustness starting with processing smaller volumes of wastewater using functionalized microbubbles to extract RNA for qPCR analysis. Two commonly practiced methods for wastewater concentration and viral processing were employed as a comparator to silica functionalized microbubbles, PEG/NaCl (PEG) precipitation and centrifugal ultrafiltration (UF). Both PEG and UF process 45 milliliters (mL) of wastewater sample and require the removal of suspended solids which in turn increase the amount of time spent on processing the sample. The microbubble protocol only requires 3 mL of raw wastewater (includes suspended solids) and provides equivalent or higher sensitivity with a quicker turnaround; thus being able to track a viral load within a community in a much more valuable time window. With over 100 measurements taken for sample

extraction and RNA isolation/cleanup, the microbubbles protocol performed 79% faster than PEG precipitation and 38% faster than UF. The use of 3 mL raw wastewater samples provided consistent results that were equivalent or in most cases proved to be more sensitive than PEG precipitation. The sensitivity captured with the silica functionalized microbubbles, can lead to identifying a spike in a virus earlier when analyzing trends within a population.

4.28.P-We-210 Combustion Ion Chromatography Technique with Inorganic Fluoride Reduction for Total Organic Fluorine Measurement in Aqueous Samples Containing Ultrashort Chain, Short-Chain, and Longer-Chain PFAS

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Efforts are underway to develop an analytical method to serve as a broad measure or indicator of perfluoroalkyl substances (PFAS) present in the environment. Combustion ion chromatography (CIC) is an approach that measures the fluoride generated by combustion of organofluorine compounds present in a sample. In environmental samples total fluoride (TF) may be comprised of inorganic fluoride (IF) and organic fluoride. Currently, total organic fluoride (TOF) is determined by subtracting measured IF from TF measured after combustion of the sample. CIC is a robust technique however the limit of quantification (LOQ) for the technique is typically higher than LOQs observed in speciated PFAS analysis utilizing Liquid Chromatography-Mass Spectrometry (LC/MS). Adsorbable organic fluorine (AOF) and extractable organic fluorine (EOF) methods attempt to remove IF and report an LOQ on the order of 0.300 µg/L in ultrapure water. EPA's draft AOF-based method 1621 reports removal of up to 8mg/L of IF in aqueous samples. TOF recoveries of 46-112% are reported for >C3 PFAS compounds using AOF. However, for ultrashort chain compounds (TFA, PFPA, PFPS) the TOF recoveries of 9.3% to 25.2% using AOF and 21.0 to 55.5% using EOF are reported in the literature. 3M EHS lab has developed a method to remove IF from the sample prior to measurement of TOF using CIC. The method involves filtering the sample through Alumina-N SPE cartridge followed by extraction with 1% dibutyl amine in methanol. The average recovery of a mixture of 18 PFAS comprised of TFA, 2233-TFPA, 2333-TFPA, PFPA, PFBA, PFPeA, PFOA, TFMS, PFES, PFBS, PFHxS, PFOS, TFSI, FBSA, N-MeFBSA, FBSE, FBSEdiol and HFPO-DA spiked in Milli-Q water was 102%±6.4%. Over 360 samples were analyzed using this method. The LOQ ranged from 83.6 to 125 µg/L. The average laboratory matrix spike recoveries spiked with this mixture were 116±7.0%. For two wastewater samples containing approximately 2 ppm of IF and detectable levels of TFA, PFPA, PFBA, TFMS, PFES, 2233-TFPA and 2333-TFPA, the TOF results from CIC were compared to results determined using the sum of targeted-LCMS analysis. The relative percent difference between the TOF results determined using CIC and LCMS was 17.2% for Sample 1 and for Sample 2 the TOF from CIC was <105 µg/L and 84.9 µg/L for LC/MS.

4.28.P-We-211 Canadian Ultraviolet Filters in Outdoor Swimming Pools, a Public Beach, and a Major WWTP on the Canadian Prairies

Alistair Kendrick Brown, Tori Maxwell, Jade Wish and Annemieke Farenhorst, University of Manitoba

UV Filters (UVFs) are found in a wide range of cosmetics, personal care products, and commercial stabilizers. UVFs are known to be toxic to aquatic organisms in both freshwater and marine environments, and are also known to be strong endocrine disruptors, thereby impacting human health. It is important to quantify human exposure to total UVFs in a recreational environment and to gauge the year-round input to the environment via wastewater. We quantified eight of the most popular and common UVFs approved for Canadian usage in three outdoor swimming pools in Winnipeg, Canada and a nearby freshwater beach over the 2022 summer season. UVFs were also quantified in wastewater weekly in 24 hour composite samples during July and August 2022 and then in February and March 2023 to elucidate seasonal trends. The chemicals were avobenzone, dioxybenzone, homosalate, octisalate, octinoxate, octocrylene, oxybenzone, and sulisobenzene. This was done using solid phase extraction coupled with polarity switching via ultra-high performance liquid chromatography-tandem mass spectrometry. For recreational waters seasonal, intraweek, and intraday trends of usage were

quantified. In pools there was a consistent trend of dioxybenzone, oxybenzone, and sulisobenzone mean levels of -1, avobenzone, homosalate, octisalate, and octinoxate mean levels of 0.009 to 0.25 $\mu\text{g L}^{-1}$, and mean octocrylene levels ranging from 1.52 to 2.49 $\mu\text{g L}^{-1}$. In the mesotrophic freshwater beach environment the range of mean concentrations were all between 0.017 $\mu\text{g L}^{-1}$ (sulisobenzone) and 0.16 $\mu\text{g L}^{-1}$ (oxybenzone). The differences between pools and beach are most likely due to much greater natural dilution of the lake and lack of chlorine ions to contribute to transformation. Also, the absence of soil, sediment, and suspended organic matter in the pools, in addition to the forced recycling of the water promoted their continuous suspension in the pool water. In wastewater, the greatest mean levels were sulisobenzone (0.3 $\mu\text{g L}^{-1}$) followed by salicylates (0.06 to 0.12 $\mu\text{g L}^{-1}$). Overall, there was no significant difference in mean concentrations from summer to winter. However, there was a noticeable increase in max octocrylene levels in winter, probably due to lesser dilution. Of important note, was that only dioxybenzone was removed during treatment. Moreover, sulisobenzone was not found in the surveyed sunscreens, indicating alternate personal care products or other industrial application sources.

4.28.P-We-212 Novel Extraction and Analysis Methods for the Measurement of 44 Diverse PFAS in Fish and Benthic Macroinvertebrate Tissues

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Measurements of per- and polyfluoroalkyl substances (PFAS) in water bodies and associated food webs are used to help better understand the fate and transport of PFAS in the environment. Monitoring PFAS also provides data to improve risk assessments and the development of water quality parameters. Such measurements require the continued development of analytical methods to measure PFAS in many types of environmental media. Current fish and macroinvertebrate tissue analytical methods include a limited number of PFAS compounds. An additional analytical method requiring three different liquid chromatography-mass spectrometry (LC-MS) methods was developed for the analysis of 44 different PFAS compounds in fish and macroinvertebrate tissues. This group of compounds represented a diverse array of PFAS compounds having a wide range of physical-chemical properties, including ultrashort chain and longer chain precursor compounds. This method was applied to the analysis of PFAS in samples collected as part of a large food web study conducted over 42 nautical river miles of the Mississippi River between St Paul, MN and Red Wing, MN. As part of this study, over 790 fish and benthic macroinvertebrates specimens were collected in summer 2021 and extracted and analyzed for PFAS over the following two years. The fish samples were comprised of 10 unique species with both whole-body and fillet samples. Of note, a non-PFAS interferent was present in the fish tissue samples resulting in a significant positive bias in the measured concentration of perfluorobutanoate (PFBA) in the collected fish tissues. The interferent was evaluated using high resolution mass spectrometry and improvements in the chromatographic conditions of the analysis were done to resolve the interferent from the PFBA. The method uncertainty for each of the 44 analytes ranged between 15% and 41%, and the species-dependence of the uncertainty was also evaluated. The lower limit of quantitation (LLOQ) ranged from 0.0232 to 73.2 ng/g, where ultrashort chain analytes such as 2,2,3,3-tetrafluoropropionic acid had the highest LLOQs, and the C4-C13 carboxylic acids ranged from 0.0250 to 5.00 ng/g. Method performance varied across fish species and between whole-body and fillet samples of the same fish type. The method has limited extraction and clean-up steps, allowing faster data production.

4.28.P-We-213 Occurrence of Dissolved Organic Nitrogen (DON) in Low-Relief Streams on the Eastern Shore of Virginia, USA

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The agricultural use of nitrogenous fertilizer in watersheds along the Atlantic Coast, USA, has fueled concerns and investigations into the upland-derived nitrate (NO_3^-) discharging to coastal waters. Past studies of low-

relief, gaining streams in small watersheds on the Eastern Shore of Virginia, USA, have quantified the NO_3^- -N flux to seaside lagoons and the Atlantic Ocean. The contribution of dissolved organic nitrogen (DON) to the total nitrogen loading to coastal waters had not previously been evaluated. This study quantified concentrations of DON, NO_3^- , and total dissolved nitrogen (TDN) under baseflow conditions in 15 streams varying in watershed size and cropland use on the Eastern Shore of Virginia across a one-year period. Mean concentrations of DON in streams ranged from 0.328 to 2.14 mg N L⁻¹ and represented 12 to 70% of the TDN pool. In 14 of the 15 streams, NO_3^- was the principal form of nitrogen ranging in mean concentrations from 0.094 to 6.06 mg N L⁻¹. Instream DON concentrations were independent of NO_3^- concentrations, watershed area, and cropland use. Unlike NO_3^- , DON varied seasonally with highest DON concentrations observed in spring. DON ranged from 6 to 41% of the TDN in shallow groundwater with concentrations from 0.776 to 2.12 mg N L⁻¹. These concentrations were lower than the respective concentrations determined in overlying surface-water samples (0.001 to 0.773 mg N L⁻¹) collected concurrently. In a laboratory experiment, DON of 1.02 mg N L⁻¹ was eluted in the effluent from an intact streambed sediment core using artificial groundwater influent containing NO_3^- only and represented nearly 60% of the TDN in the core effluent. The results of this study establish DON as an important and dynamic constituent of the TDN pool in freshwater streams discharging from the Eastern Shore of Virginia, USA, to the coastal waters of the Atlantic Ocean.

4.28.P-We-214 Hair Screening Tool Feasibility Study: Detection of Elevated Exposure to Copper

Jennie Christensen and Geriene LaBine, TrichAnalytics Laboratory

In this study, single human hairs were examined as a potential biomarker of elevated copper exposure. Hair has long been used as a tool to assess copper exposure; however, the high likelihood of external contamination of the hair strand upon scalp emergence precludes hair from being fully accepted, as it is difficult to distinguish between endogenous and exogenous copper sources. Here, we use laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) on hair strands from 50 individuals above and below the scalp. The main objectives were: 1) to determine if a double ablation technique (i.e., ablating the hair twice) removes copper contamination on the exposed hair strand; 2) to calculate a normal reference range of copper levels in the cuticle and cortex layers; and 3) to determine if a single hair can represent multiple hairs from the same individual. Copper concentrations were, on average, 4.5 times higher in hair above the scalp than below the scalp, indicating significant external contamination in most individuals. Double ablation was successful at burning off the contaminated cuticle layer in all cases, leaving only endogenous copper concentrations within the inner cortex layer. The resulting (non-contaminated) reference range (10th to 90th percentile) for copper was narrow at 7.3 to 15.2 mg/kg. A subset of the 50 individuals were used to determine if a single hair can represent any hair strand from an individual. Multiple hairs were taken from 6 people and the copper concentrations were compared. The relative percent difference was low ranging from 5.3% to 15%, suggesting high replicability among hairs. The findings of this preliminary feasibility study indicate that the use of single hairs to monitor for copper exposure are promising, as long as the portion of hair analyzed is 1) below the scalp (where it is protected from exogenous copper sources); or 2) if above the scalp, the contaminated cuticle is first burned off, and only the inner cortex results are interpreted. This is the first publication highlighting the extent of copper contamination on hair chemistry results and with a proven approach of double ablation to mitigate those confounding influences.

4.28.P-We-215 Forest to Field: Comparing Pollutant Contributions of Forest and Agricultural Land Along River Transects

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Continuing pollutant loads are a major concern for watersheds such as those flowing into the Chesapeake Bay, despite ongoing progress in reducing some contaminant input sources. The upper reaches of the Shenandoah River represent a unique watershed system where there are significant headwater areas in the mountains that are

heavily forested and protected, transitioning abruptly to intensively farmed areas in the valley that lack many water quality best management practices. While intense agricultural activity, such as that found in the Shenandoah Valley, are understood as key sources of nutrients, sediments and bacteria, the role of the heavily forested headwater regions has not been quantified. The contribution of pollutants by historic and ongoing forest activities has not been established. Nitrate, phosphate, sediment, conductivity and bacteria were monitored over a two year period along three river transects that originate in heavily forested mountains, and transition into intense valley farmland. Forestland had lower levels of all pollutant parameters on average, but levels vary significantly with specific location and time. For instance, geometric mean nitrate concentrations were 1.13 and 0.51 ppm in agricultural valley and mountain forest samples, respectively. Nitrate levels exceeded impairment levels (0.75 ppm) in 69% of samples in agricultural valley and 23% of samples in mountain forest stretches. Fecal coliforms were present in 41% and 11% of agricultural valley and mountain forest samples, respectively. Pollutants were generally lower in waterways for the first 10 kilometers after entering agricultural valley lands, suggesting that slow accumulation from multiple inputs is most important for causing higher pollutant levels downstream. Results indicate a major forest area that should be understood as having lower, but significant, levels of the major pollutants of concern that are inputs to the overall watershed. Historic and current activities may be responsible for some inputs, in addition to normal forest processes.

4.28.P-We-217 Compositional Changes in a Vertically Sectioned Oil Residue

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Oil residues can remain on rocks and permanent structures for years after a spill. This is particularly true for spills of crude and heavy fuel oils. Once deposited in the coastal environment, these oil residues experience differential weathering; residue surface is most exposed to weathering while the oil beneath the surface is sheltered from these processes. Visual inspection shows the residue surface to have distinct color and textural changes compared to the bulk material underneath which appears shiny and unweathered. These differences in physical appearance suggest that there may be distinct chemical changes between the layers. To explore such differences, oil samples were artificially weathered using a full spectrum lamp, vertically sectioned, and analyzed via gas chromatography with mass spectrometry detection. Here we present detailed polycyclic aromatic hydrocarbon and total petroleum hydrocarbon data for these vertical sections of laboratory-weathered oil samples. Our analyses demonstrate the physical protection of bulk oil by surface skin formation. The differential composition of the surface and deeper layers of bulk oil has implications for accurate assessment of the bioavailability of toxic petroleum compounds following a spill.

4.28.P-We-219 Monitoring of Transboundary Rivers in Alaska and Washington for Mining Impacts

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British Columbia is home to some of the largest operating and proposed precious metal mines in the world. Mineral exploration projects and proposals for mines in the mineral rich “Golden Triangle” of northwestern BC has resulted in a high density of active mining claims in a few watersheds. These areas also form the headwaters of rivers that drain to the Pacific Ocean and provide critical habitat for Pacific salmon; many who are listed as “endangered” or “at-risk”. Baseline information about water quality conditions in these USA-Canada transboundary rivers is being collected by the US Geological Survey at 5 river locations in southeast Alaska (Asek, Taku, Stikine, Unuk, and Salmon rivers) and 2 rivers in northern Washington state (the upper Skagit and Similkameen). All 7 rivers have continuous discharge and quality sensor data available online at the USGS NWIS website; search by river name. Equal discharge or equal width increment sampling of river water for major ions, nutrients, metals and carbon occurs approximately 6 times a year per site. To date, US EPA water quality criteria exceedances for dissolved metals are rare, with concentrations of copper in the Unuk River and copper and cobalt in the Salmon River appearing more likely than others to approach criteria. Mine density per watershed area and mine density per mean daily discharge was weakly related in rank order to

dissolved metal concentrations for only copper, cadmium, and selenium. Preliminary dissolved metal loads have been calculated. Metal chemistry on suspended and bed sediments as well as fish tissue and moss are measured annually per site and summarized.

4.28.P-We-221 Effects of Conservation and Conventional Tillage on Constituent Loading into Surface Waters in the Dryland Agricultural Region of the Inland Pacific Northwest – A Paired Watershed Study

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The productive silt loam soils found in the Palouse dryland cropping region of the Pacific Northwest are ideal for growing wheat and legume crops, but the steep topography, winter precipitation, and soil physical properties generate some of the highest erosion rates nationwide. To address this issue, a regional conservation partnership group used a voluntary incentive-based conservation program to convert or keep over 15,000 hectares of farmland as conservation tilled for five years. A paired-watershed study was conducted to identify if change from conventional tillage to conservation tillage practices in this region reduced suspended sediment, nutrients, and pesticides loading into surface waters. Roughly 80% of the treatment watershed, Kamiache Creek, was managed under conservation tillage during the study period, while the control watershed, Thorn Creek, was managed under roughly 80% conventional tillage. Grab samples were collected from fall 2016 to summer 2023. The US Geological Survey's Load Estimator was used to approximate the constituent loading into each watershed and was normalized by watershed area. Initial findings indicate greater nutrient loading in both watersheds during the winter months with the highest nitrate loads per acre from the control watershed. Tillage practices explain some differences in constituent loading, but more accurate estimates could be developed by incorporating precipitation, crop rotation, and stream bank erosion analyses. This study highlights the importance of having crop residues on farmland during the months of high precipitation when erosion is most prominent to decrease constituent loading into surface waters.

4.28.P-We-222 Simulations of Field-Realistic Pesticide Residues Relevant for Pollinator Exposures

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Modeling field-realistic pesticide residues across spatially and temporally explicit dimensions is an important step when estimating risks to non-target species. Residues in air, soil, nectar, and pollen are particularly relevant for pollinator species, which may be exposed during foraging and nesting in or near agricultural locations. Here, we describe a methodology for adapting available data and models to simulate exposure landscapes relevant to pollinators. We use a suite of environmental fate models to simulate daily residues from foliar, seed, and soil applications in relevant exposure media on and off-field over time and compare outputs with available measured residue values. Models used include the U.S. EPA's AgDRIFT model for foliar drift, the Pesticide in Water Calculator, and additional models for measuring residues in pollen and nectar. We also test several novel methods for estimating residues in compartments from pesticide seed treatments. Estimates were conservative when compared against available residue measurements and provides a basis for comparing relative exposures between different application methods and exposure pathways for pollinators. This work addresses the need for a comprehensive set of exposure routines that directly link spatially explicit application rates from common application methods to measurable residues in media over time.

4.28.P-We-224 Persistent Organic Pollutant Accumulation in Pacific Abyssal Plain Sediments and Biota: Implications on Sources, Transport, and Deep-Sea Mining

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Despite its vast size, ecological, and economic importance, the deep sea is one of the least understood

ecosystems on Earth. While much remains to be discovered, researchers have established that the deep sea is being exposed to numerous anthropogenic stressors, one of which is chemical pollution. Persistent organic pollutants (POPs), defined by their persistence in the environment, bioaccumulation, and high toxicity, are continually discharged and transported into the deep sea despite protections under global and regional conventions. Few studies have provided insight into POP transport processes and accumulation in the deep sea and none to our knowledge have examined POPs at the seafloor of the Pacific abyssal plain; an area currently being targeted for deep-sea mining and previously assumed to be too far removed to be affected by POPs. Here, we provide baseline data on POPs in deep-sea fish from a region of the deep abyssal plain targeted for deep-sea mining. All congeners of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and multiple per- and polyfluoroalkyl substances (PFAS) were measured in sediment, fish muscle, and liver tissue of a deep-ocean predator and scavenger, *Coryphaenoides sp.*, to provide information on sources, transport, accumulation, and contributions of legacy versus emerging (e.g., unintentional PCBs) POPs. Here, we provide the first evidence of PFAS, PCBs, and PBDEs in sediment and biota in this region of the Pacific abyssal plain. Preliminary results show that PCBs were more evenly distributed over the sampling region, while PBDEs and PFAS had patchy distributions and concentrations. Unintentional PCBs (e.g., PCB 11, a component of pigments) significantly contributed to sediment and muscle tissue concentrations, suggesting unregulated PCB releases are accumulating in the deep Pacific Ocean. PFAS was detected in one sediment sample and all biota at levels as high as those found in freshwater ecosystems. Results suggest that the flux of carrion, organic matter particles and vertically migrating species may present an important transport process for POPs that result in patchy contaminant distributions and differences seen in scavenging biota versus sediment. However, for PCBs, with a longer production duration, sinking particle transport may be a more important driver, with raining particles (e.g., POM and plastics) laden with adsorbed PCBs, resulting in a more uniform distribution across this region.

4.28.P-We-225 Bioaccumulation Kinetics of Per- and Polyfluoroalkyl Substances in the Aquatic Model Organism, *Pimphales 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 uptake of 19 PFAS, which were selected at low levels not eliciting standardized adverse outcomes, by fathead minnows (*Pimphales promelas*) over 7 days. 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. Uptake kinetics were estimated for each PFAS with non-linear regressions for both tissue and plasma. Increasing PFAS tissue concentrations and uptake rates were observed with increasing chain length in tissue and plasma for both PFCAs and PFSAs. By the end of the uptake period, PFOA concentrations had accumulated up to 209 µg/kg in tissue and 888 ng/mL in plasma. Additionally, kinetic and ratio based bioaccumulation factors (BAF) and blood:water partitioning coefficients (P_{BW}) were also estimated. For example, the ratio and kinetic based BAFs for PFOA were 16.4 and 27.3 L/kg, respectively, while the ratio and kinetic P_{BWs} were 35.4 and 44.1, respectively, showing general agreement. When applicable, the apparent volume of distribution (V_D) was estimated to examine the distribution of PFAS. V_D estimates were all below 1 L/kg, indicating PFAS were more distributed in the plasma than the tissue. For both PFCAs and PFSAs, V_D estimates generally decreased with increasing chain length. Data from this experiment provides BAFs, P_{BWs} and V_D s for PFAS of differing chain lengths and

functional groups for a common fish model and supports ongoing uptake and elimination studies. Further, this work will contribute to the development of predictive models for PFAS bioaccumulation, especially in PFAS contaminated sites, where these chemicals exist as mixtures.

4.28.P-We-226 Field-Deployable LC-MS Platform for On-Site Screening of Per-, and Poly- Fluoroalkyl Substances (PFAS) in Environmental Samples

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Per- and poly-fluoroalkyl substances (PFAS) are ubiquitous in the environment. Their ingress into the global food chain has led to measurable levels in nearly the entire human population in developed countries, with real and potential health effects reported worldwide. For example, due to their widespread uses as components in fire-fighting foams, increased levels of PFAS have been detected at sites where fire-fighting activities have been conducted. There are a few screening methods that offer the capability to detect total PFAS, but none of them provide the required selectivity and sensitivity to measure individual PFAS at concentrations as low as ng/L for environmental water samples, or sub- $\mu\text{g}/\text{kg}$ for soils. Liquid chromatography coupled with triple quadrupole mass spectrometry (LC-QqQ-MS) is the most effective laboratory-based technique for determination of PFAS in various types of samples ^[1]; however, sample transport to the central laboratory has created significant latency in sample analysis. With minimal sample preparation on site, a proof-of-concept field-deployable LC-single quadrupole-MS (LC-Q-MS) platform was demonstrated for addressing on-site environmental monitoring requirements ^[2]. Results achieved in an enhanced field screening workflow based on the proof-of-concept platform have been compared with those obtained from a LC-QqQ-MS method performed in a centralized laboratory.

4.28.P-Th Late Breaking Science: Chemistry and Exposure Assessment

4.28.P-Th-100 Application of Proposed EPA PFAS Methods for Wastewater

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The Environmental Protection Agency (EPA) has published multiple drafts of Method 1633 for analysis of Per- and Polyfluorinated Substances (PFAS) in aqueous matrices, including wastewater. Both Method 1633 and laboratory method 537M (modified) by isotope dilution were included and are currently used for the analysis of PFAS analytes in wastewater discharged under National Pollution Discharge Elimination System (NPDES) permits. Non-targeted methods EPA Method 1621 and Total Oxidizable Precursors (TOP) assay were also investigated, these methods are comprehensive in their analysis and currently outside the scope of NPDES permits. We contracted the analysis of replicate pulp and paper industry-associated waters (influent, effluent, synthetic wastewater, river water, and blanks) at a total of eight EPA-accredited laboratories with the various PFAS methodologies to (1) enhance our understanding of the applicability and relevance of Method 1633 compared to existing targeted and emerging non-targeted PFAS methods and (2) evaluate the capabilities of EPA-accredited contract laboratories and consistency of reported results. We reviewed Level IV reports to evaluate adherence to test methods (i.e., sample preparation protocols and QA/QC measures), and identify reporting biases, false positives, and QA/QC intricacies. Upon reviewing EPA Method 1633 reported data, inconsistencies in the initial sample handling protocols among certified laboratories were associated with disparities in reported concentrations of PFAS analytes in replicate samples. The inconsistency occurred when total suspended solids (TSS) data was greater than 50 mg/L causing some laboratories to subsample from the sampling bottle or dilute the sample, with both actions not advised according to Method 1633. Among the 13 commonly detected PFAS compounds in the same influent sample measuring 1500 mg/L TSS, concentrations

ranged from non-detect to 110 ng/L, with labs having to dilute the sample or extract to deal with the interferant to achieve passing QC typically reporting non-detects. Comparative PFAS concentrations across methods are reported for each sample type. These findings have significant implications for stakeholders and emphasize the need for refinement of sample preparation protocols among certified laboratories. Such refinement is particularly important when analyzing complex matrices such as pulp and paper industry wastewaters, and instrumental in achieving water quality objectives.

4.28.P-Th-125 Detection and Isomer Differentiation of PFAS using MALDI-TOF Mass Spectrometry with Trapped Ion Mobility

Aidan Reynolds, Abby Smith, Irfath Jasmin Reza and Tian Autumn Qiu, Michigan State University

Poly- and perfluoroalkyl substances (PFAS) are a class of organic compounds that have attracted global attention for their persistence in the environment, exposure to biological organisms, and their adverse health effects. There is an urgent need to develop analytical methodologies for quantification and characterization of PFAS in various sample matrices. Current efforts in PFAS characterization research have centered around chromatography-coupled tandem mass spectrometry (MS). While chromatography-based methods have many advantages, they usually require extensive sample preparation, costs longer time in analysis, and prone to contaminations during processing. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a chromatography-free MS method that performs laser-based ionization and in situ analysis on samples. Herein we present PFAS analysis by MALDI-TOF MS with trapped ion mobility (TIMS), which provides an additional dimension of gas phase separation based on the size-to-charge ratios. MALDI matrix composition and key instrument parameters were optimized to produce different ranges of calibration curves. We achieved limits of detection of perfluorosulfonic acids (PFSAs) at parts per trillion concentrations and perfluorocarboxylic acids (PFCAs) at parts per billion concentrations. Using TIMS, we successfully separated three perfluorooctanesulfonic acid (PFOS) structural isomers in the gas phase. Finally, we showed the application of this MALDI-TIMS-TOF analysis for PFAS detection in bacterial biomasses. Our results demonstrated the new development of utilizing MALDI-TIMS-TOF MS for fast, quantitative and sensitive analysis of PFAS, paving ways to future high-throughput and in situ analysis of PFAS such as MS imaging applications.

4.28.V Late Breaking Science: Chemistry and Exposure Assessment

4.28.V-022 Using Machine Learning to Predict Response Factors of ESI-LC-MS/MS Data in Standardized Solvent Mixtures for Non-Targeted Analysis

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Non-targeted analysis (NTA) allows us to gain a rich understanding of a sample's chemical makeup by embracing a wide spectrum of compounds, opening exciting possibilities for discovery. One challenge with current workflows and instrumentation used in NTA is producing quantitative information. This is mainly due to large differences in ionization efficiency of different chemicals influencing the detector response. In some cases, peaks with areas the same size can differ in concentration by up to three orders of magnitude. This has forced NTA to become a screening tool for the discovery of new or suspect chemicals that are then used to develop targeted analysis methods to more reliably quantify the contaminants identified. The process of transitioning between one analysis type to another not only takes time and significant expertise but requires specialized chemicals and analytical standards to accurately report and quantify a compound. It has been estimated that only 2% of chemicals listed by the EPA's DSSTox list are even available as pure analytical standards. Advancing NTA to a point where it becomes quantitative could save time and resources while improving overall outcomes. In this project, a set of 8 chemical mixtures was analyzed with ESI-LC-QTOF/MS

with the data being used to develop, train, and evaluate a series of machine learning algorithms. The algorithms used different physicochemical properties and chemical parameters as inputs to predict the relative response factors (RRF) of the chemicals and from there estimate the concentrations of the of analytes in the mixture. Predicted values were plotted against true values to evaluate the linear relationship between the two and the predictive accuracy of the algorithms. Results showed that a model using Mordred descriptors had the best prediction of RRF, while a combination of parts from multiple descriptor models did not appear to improve the model accuracy. This preliminary work shows significant promise, especially when considering different sets of physicochemical descriptors. Success for comparison with true values will require more tailored models rather than maximizing the number of descriptors and parameters used when applying machine learning. Future goals of the project will be to build upon these standard-based results to reliably predict true values of compounds using NTA in real-life environmental analysis and clinical settings.

Track 5: Environmental Risk Assessment

5.01.P-Th Advancing Wastewater Surveillance to Complement Community and Environmental Health Measures

5.01.P-Th-132 Drugs Discharged at Rest Areas and Truck Servicing Facilities During Federal Holidays in the United States

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The National Highway Traffic Safety Administration reported a higher motor vehicle traffic crashes during holiday than non-holiday periods from 1982-2019. Drug-involved traffic fatalities during six major holidays (New Year's Day, Memorial Day, July 4th, Labor Day, Thanksgiving, and Christmas) were significantly higher than a typical day in 2019. In this study, the amount of several discharged drug residues including stimulants, opioids, antidepressants, and hallucinogens were measured in raw wastewater collected from five interstate highway rest areas and two truck servicing facilities in Kentucky on major federal holidays over a 12-month period (2021-2022). Three stimulants (cocaine, methamphetamine, and amphetamine), two opioids (hydrocodone and tramadol), tetrahydrocannabinol metabolite, and four antidepressants (venlafaxine, citalopram, fluoxetine, and sertraline) were detected in all wastewater samples. Population normalized mass loads of amphetamine, methamphetamine, cocaine, and tramadol were higher on Christmas and July 4th compared to the weekday and weekend periods in a typical week. However, the average mass load on holiday periods (3-5 days) was not consistently different from the corresponding non-holiday periods. Despite higher drug-involved traffic incidents along the interstate highways during holidays, this study represents the first comprehensive and quantitative report of drugs discharged during holidays and non-holidays areas along interstate rest areas and truck servicing facilities.

5.01.P-Th-133 Low Level Quantification of Sucralose and Acesulfame Sweeteners in Wastewater

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Wastewater surveillance (WS) is a useful public health tool for assessing community disease prevalence complementing clinical diagnostic tests. During the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), many organizations such as the U.S Centers for Disease Control and Prevention and the Public Health Agency of Canada adopted WS for national surveillance programs. Because up to 89% of infected individuals shed viral particles into their feces, public health organizations can monitor the prevalence of SARS-CoV-2 in wastewater. However, composition of wastewater is constantly changing due to temperature, flow rate, dilution by other sources of water, and population dynamics. These factors and variability in viral shedding between people can cause large variations in the viral loads detected in wastewater

samples. This means that WS may not reflect the actual prevalence in the community. Therefore, population normalization is critical for accurate interpretation of WS data. There is no consensus on which indicator should be used to normalize viral loads. Recently, the artificial sweetener acesulfame (ACE) was shown to outperform other biomarkers during holiday periods when there is increased population movement. The artificial sweeteners, ACE and sucralose (SUC), are common chemical markers for municipal wastewater because ACE and SUC are not naturally occurring, widely consumed by humans, stable in wastewater, and greater than 99% of consumed ACE is excreted into urine and 80% of consumed SUC is excreted into feces. Samples were collected from two wastewater treatment plants and underwent a 24 hour composite of raw sewage after physical filtration. The resulting wastewater samples were filtered further before analysis with 0.25 µm syringe filter and further diluted with water before analysis on the SCIEX QTRAP 7500 system. The mean concentration of acesulfame in 141 wastewater samples was found to be 22 µg/L while sucralose was found to be 41 µg/L showing that a sensitive and robust assay can be achieved on the SCIEX QTRAP 7500 system using minimal sample clean up techniques allowing the screening of artificial sweeteners as biomarker of population size.

5.01.P-Th-135 Moving Beyond COVID-19: Monitoring Wastewater for Influenza and Respiratory Syncytial Virus, a Pilot Study

Rebecca B Fahney, Adelaide J Roguet and Dagmara Sieprawska Antkiewicz, University of Wisconsin

Wastewater-based epidemiology (WBE) has recently gained recognition as a complementary and independent epidemiological tool for monitoring community levels of SARS-CoV-2, the agent causing COVID-19.

Wastewater surveillance can provide an early warning of both COVID-19 transmission and variant spread in communities. It often foreshadows clinical surveillance, picking up signal in asymptomatic or mild cases that may not be detected by a healthcare system. In addition, WBE provides better equity in public health surveillance as all communities served by a sewer system are equally tested, while traditional health surveillance can overlook populations that lack access to health services despite being at the highest risk for poor health outcomes. Leveraging wastewater surveillance, public health officials can better understand disease burden in these vulnerable communities. In fall of 2020, the Wisconsin State Lab of Hygiene (WSLH) in collaboration with partners at the University of Wisconsin-Milwaukee and the Wisconsin Department of Health Services (DHS), built and implemented a statewide wastewater surveillance network for SARS-CoV-2. In efforts to expand the wastewater monitoring program to include other pathogens of concern, a pilot study was conducted to monitor influenza and respiratory syncytial virus (RSV) in Wisconsin communities. Using techniques similar to routine COVID-19 wastewater monitoring methods, influenza and RSV viral loads were measured in wastewater from multiple communities across the state during the influenza season. By comparing clinical cases, levels detected in wastewater were found to reflect syndromic clinical levels, showing that WBE for these two pathogens can be used as a complementary tool alongside traditional epidemiological methods.

5.01.P-Th-136 Variation in Locational Response to Omicron Measured Using Wastewater-Based Surveillance at Wastewater Treatment Plants, Correctional Facilities and Assisted Living Facilities in Texas

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In late 2021, the variant named Omicron was identified due to an S gene drop out during regular sample checking in South Africa. This variant spread extraordinary rapidly with the initial wave of BA.1 from December to February 2022, followed by the emergence of another subvariant named BA.2. Regular monitoring of wastewater treatment plants under an ongoing wastewater-based surveillance study with the state of Texas identified Omicron two weeks prior in Denton before its detection in Waco. Hereafter, it was detected in numerous other facilities. Sampling consisted of (a) once weekly sampling and analysis of wildtype (N1, N2)

and omicron specific indicator (P13L), (2) normalization to fecal indicator (PMMoV) and (c) comparison with active cases/hospitalizations where possible. The time profiles of SARS-CoV-2 in wastewater broadly matched the waves of active cases, but with two notable differences between assisted living facilities (n = 5) and correctional facilities (n = 6). For these two locations, differences were detected in the frequency of positive detects for targets, with assisted living facilities having less than 50 % agreement between targets compared to > 90 % agreement between targets for the correctional facilities (> 100 % agreement up to April). Sequencing of the wastewater treatment plants agreed with the trends already observed using PCR. However, as omicron has evolved, unique sequences attributable to one variant are becoming more limited, which is where a combined sequencing and PCR approach will become increasingly necessary. Following the successful application of this approach to sequencing at the wastewater treatment plants, samples from the correctional and assisted living facilities have now been sent for sequencing to determine if the assisted living facilities represent a potential source for the emergence of mutations/cryptic lineages given the suggested frequency of drop offs in nucleotides suggested by the results. These results will assist in improving our understanding of the spread and mutations associated with this ongoing infectious disease.

5.01.T Advancing Wastewater Surveillance to Complement Community and Environmental Health Measures

5.01.T-01 Advancing a Wastewater-Based Framework for Monitoring Drug Use in Communities

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Drug-involved overdoses took the lives of more than 100,000 people last year in the United States and are largely attributable to synthetic opioids, namely illicitly manufactured fentanyl. Treatment and prevention are critical to addressing this national crisis which rely on knowledge of specific types, quantities, and consumption patterns of specific substances. Wastewater-based epidemiology (WBE) is an innovative yet mature approach that can identify drug use trends rapidly on a community scale as they change and shift in near real-time. Community-level drug trends have been successfully generated from wastewater for targets of current concern (e.g., fentanyl); however, implementation of WBE as a holistic framework that contributes reliable, consistent, and trusted data to stakeholders is still being developed. Our team, called GatorWATCH™, in partnership with the NIH National Drug Early Warning System (NDEWS) Coordinating Center generated 9-month fentanyl consumption trends from wastewater collected from treatment plants at four US cities from October 2021 – July 2022. We sampled from three wastewater reclamation facilities which allowed us to quantify fentanyl and its primary metabolite, norfentanyl, in sub-city level sewer sheds. To accomplish this, 24 hour composite influent wastewater samples were collected one day per week and were filtered and processed using solid phase extraction followed by analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Concentrations of fentanyl and norfentanyl were quantified and norfentanyl was used to calculate fentanyl consumption rate (CR, mg/day/1,000 people). Using this data, we evaluated the association of fentanyl CR with fentanyl-related overdose deaths. A log-unit change in fentanyl consumption rate was associated with 55% higher incidence of fentanyl overdose deaths. At the highest quartile of fentanyl CR, we observed 155% increase in overdose deaths when compared with the lowest quartile of fentanyl consumption rates. Data generated from this study support that wastewater sampling at the sub-city level produced rapid trends of community consumption of fentanyl that are associated with time-matched fentanyl-related overdose

deaths. Furthermore, our approach of advancing drug measurement methodology in tandem with sophisticated data harmonization will provide a blueprint for expansion and use of WBE as an integral part of public health prevention and interventions.

5.01.T-02 Wastewater as a Tool to Evaluate the Efficacy of a Statewide Drug Takeback Program

Daniel Burgard, Diana Tran, Myah Stauffer and Katie Gray, University of Puget Sound

In 2018, Washington State passed the Safe Medication Return program, the nation's first unified, state-wide, medication return program. This program is managed by the WA Department of Health (WADOH), operated by a third party, and in November 2020 began accepting unwanted medication with both take-back kiosks and mail back packaging. The goals of the WA State program are three-fold: 1) reduce prescription opioid abuse, 2) reduce accidental poisonings, and 3) reduce environmental contamination. WADOH will evaluate the program's effectiveness by weighing the returned goods from each kiosk and mailer, however, there will not be an inventory as to what is being weighed. Since generic mass does not necessarily equate to the goals of the program, there is a gap in the understanding of the program's impact. Hydrocodone is the most distributed opioid in WA. In single takeback events, hydrocodone has been shown to be the ninth most returned compound and to have an average unused rate of 62 percent. The U.S. FDA currently maintains a list of 15 active ingredients, 10 of which are opioids (including hydrocodone and oxycodone), that should be flushed when they are no longer needed. Thus a wastewater-based approach has the potential to evaluate the effectiveness of the program by monitoring a decrease in flushed drugs, presumably removed by the program. This paper will present the initial findings of a three year monitoring program of wastewater from seven wastewater treatment plants in WA State. Levels of hydrocodone and its unique major urinary metabolite norhydrocodone, as well as oxycodone and its metabolite noroxycodone will be quantified in raw influent wastewater samples. From 2020 to 2022, 24-hour composite samples were collected daily in the months of April and August. Samples are extracted with solid phase extraction and quantified with liquid chromatography tandem mass spectrometry. This sampling period provides two months of baseline, pre-takeback program data to be compared to levels post implementation of the program. Results will be presented in terms of parent drug to metabolite ratios, where a decrease would indicate lower levels of flushing and a positive impact of the takeback program.

5.01.T-03 Wastewater Surveillance Signals for Xylazine in Kentucky

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(1)University of Kentucky, (2)Murray State University

Wastewater surveillance of potent novel psychoactive substances is a growing public health strategy. Fatal overdoses involving the veterinary sedative, xylazine, have exponentially increased in the United States from 2018 to 2021. In Kentucky, xylazine was detected in raw wastewater from two interstate rest areas and one truck servicing facility. From September to December 2021, 54% (61 of 112) samples tested positive for xylazine; decreasing to 38% (January to June 2022) before doubling to 78% (184 of 237 samples) from July to December 2022. The doubling of the detection rate in wastewater corresponded with a similar increase in xylazine in Kentucky law enforcement samples (1.6 to 2.9 per 1,000 submissions) submitted to the National Forensic Laboratory Information System. Further, samples from four wastewater treatment facilities in Eastern Kentucky had a 28% xylazine detection rate (16 of 58 samples, February to August 2022). In the first few months of 2023, interstate wastewater detection increased yet again to 83% (49 of 59 samples) with >80% and >65% detection for rest areas and truck facilities, respectively. Though xylazine was commonly detected in wastewater samples, xylazine-involved fatal overdoses (< 5 deaths) and positivity from publicly-reported healthcare samples (e.g., urine drug screens) remained relatively rare over most of this period in Kentucky. Population use rates calculated from wastewater samples are preferred to qualitative detection rates but are challenging because human excretion studies of xylazine are lacking. Though xylazine is present in populations

using interstate roadways and community wastewater samples throughout the state, the lack of xylazine involvement in fatal and observed nonfatal overdoses may indicate that xylazine in the drug supply may not be leading to severe health outcomes.

5.01.T-04 Application of Wastewater-Based Epidemiology to Monitor Substance Use Trends in Eastern Kentucky Communities

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Introduction: Wastewater analysis is a tool to identify and quantify substances in communal wastewater samples to provide near real-time, non-invasive, and inexpensive substance use epidemiology. Applications include identifying a) geographic and time trends of substance use, b) emerging substances, and c) changes in substance use after community intervention. In this work we examine wastewater samples for multiple substances in vulnerable communities in Eastern Kentucky. **Methods:** Convenience samples from four wastewater treatment facilities (WWTFs) in Eastern Kentucky were collected weekly for three to six months. Concentrations of 29 licit and illicit substances were measured using high-performance liquid chromatography (HPLC) in split samples at the Analytical Chemistry Lab at Murray State University and UK's Equine Analytical Chemistry Laboratory. Then, each substance's mass loads (human consumption in mg/day/1000 people) were estimated for the WWTF service areas. Mass load temporal trends were generated and compared to select prescription rates for WWTF service areas. **Results:** Sixty-six wastewater samples were analyzed (mass loads range: 0.2 to 372.5mg/day/1000 people). Substance positivity rates in wastewater were: antidepressants (%82), stimulants (%75), opioids (%63), sedatives (%44), and hallucinogens (%34). We identified xylazine (the veterinary sedative recently found in the illicit drug supply) and naloxone (overdose reversal medication distributed by mitigation programs such as those associated with the HEALing Communities Study in the region) in %25 and %44 of the samples respectively. **Conclusion:** This work highlights the potential of a wastewater-based substance use monitoring system that enables in-depth public health, epidemiological, and policy studies on substance use in Eastern Kentucky.

RETRACTED

5.01.T-05 Wastewater -Based Epidemiology Exposome: Mapping Increased Places of Concern for Metals Exposure

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Community wastewater surveillance is an established means to measure health threats across geographic scales from buildings and neighborhoods to entire cities. However, research into using wastewater to detect areas of increased exposure to environmental toxicants which may cause a human health risk has been limited. Increased metal body levels are associated with several chronic diseases, including metabolic syndrome, cardiovascular diseases, and cancers. This research aims to use Jefferson County, Kentucky, as an exposome case study to analyze the metal levels in the wastewater as a gradient of community toxicant exposure, and to test the feasibility of using wastewater to identify community areas of elevated metals exposure. Twenty-six grab samples were collected in December 2022 from influent wastewater. Levels of 25 metals were determined in the wastewater samples using Inductively Coupled Plasma Mass Spectrometry. Results indicate seventeen of the sites had one or more metal results greater than one standard deviation above the mean. Commonly (9/26 studied sites), more than one metal was elevated. Where there are Maximum Contaminant Level (MCL)s, the results (12/25 studied metals) are below enforceable standards for drinking water. Examination of the spatial patterns of metal concentrations using Getis-Ord G_i^* indicates hot spots of high values for metals (e.g., As, Cr, Mn, Pb) were observed in neighborhoods that are disproportionately resided by minority and lower-income households. Furthermore, the distribution of toxic release inventory (TRI) facilities in the study area is spatially

associated with elevated metal concentrations, indicating potential exposure to toxicants released from those industrial facilities. The findings of this study contribute to the existing literature on environmental injustice and shed light on potential linkages between metal exposures measured in wastewater and adverse health outcomes after accounting for other social and environmental confounding factors.

5.01.T-06 Bioinformatics Based Screening of Wastewater Samples May Provide Information for Selecting Targeted Wastewater Surveillance of Potentially Emerging Viral Disease

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Monitoring of potentially pathogenic human viruses in wastewater is of crucial importance to understand disease trends in the community, predict potential outbreaks, and boost preparedness and response by public health departments. Considering the overall disease burden caused by diverse pathogenic viruses in wastewater, surveillance tools that are both practical to apply and able to identify diverse viral communities accurately are urgently needed. High throughput metagenomic sequencing circumvents the limitations and opens an opportunity and expands the capabilities of wastewater surveillance. However, there are major bottlenecks in the metagenomic enabled wastewater surveillance, which involve complexities in selecting appropriate sampling and concentration methods, random amplification methods, and bioinformatic analysis of complex samples with low human virus concentrations. Untreated wastewater samples were collected from the Great Lakes Water Authority (GLWA) Wastewater Treatment Facility in Detroit and virus diversity in both centralized interceptor sites and the localized neighborhood sewersheds was investigated during the COVID-19 pandemic. Bacteriophage affiliated with the families *Siphoviridae*, *Myoviridae* and *Podoviridae* were found to be abundant in all the wastewater samples. The proportion ranges of viral-related contigs from bacteriophage families *Siphoviridae*, *Myoviridae* and *Podoviridae* population were 22.13% ~ 32.76%, 30.95% ~ 40.14% and 7.22% ~ 14.81%, respectively. Comparison against a custom Swiss-Prot human virus database indicated the potential prevalence of pathogenic viruses including *Orthopoxvirus*, *Rhadinovirus*, *Parapoxvirus*, *Varicellovirus*, *Hepatovirus*, *Simplexvirus*, *Bocaparvovirus*, *Molluscipoxvirus*, *Parechovirus*, *Roseolovirus*, *Lymphocryptovirus*, *Alphavirus*, *Spumavirus*, *Lentivirus*, *Deltaretrovirus*, *Enterovirus*, *Betacoronavirus*, *Kobuvirus*, *Gammaretrovirus*, *Cardiovirus*, *Erythroparvovirus*, *Salivirus*, *Rubivirus*, *Orthohepevirus*, *Cytomegalovirus*, *Norovirus* and *Mamastrovirus* in the wastewater samples. The quality of the sequences was assessed and phylogenetic analysis of nearly complete viral genomes was explored further to reveal virus identification in a finer resolution. This study shows a demonstrative application of metagenome sequencing in identifying human viruses of potential concern and can be used to inform further testing for identification of human viruses of concern in the species or genotype level.

5.01.V Advancing Wastewater Surveillance to Complement Community and Environmental Health Measures

5.01.V-014 Comparison of Different Nucleic Acid Extraction Methods: An Investigation on Abu Dhabi Wastewater

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Recently wastewater-based epidemiological surveillance has shown a growing interest among researchers. Evaluation of wastewater for pathogens requires rapid, reproducible, and consistently precise analysis. Unlike clinical samples, community wastewater is diluted by several thousand folds, processing such a diluted sample requires a highly sensitive detection method. Therefore, molecular detection methods (PCR) are more suited than classical culture methods. However, the extraction of nucleic acid from the wastewater is challenging due to the low concentration and diversity of the collected sample. The current study compares different Nucleic

acid concentrations and extraction methods using commercial extraction kits and further compares the merits and demerits of different extraction strategies. We investigated different regimes for sample processing, i.e., A) silica spin column-based extraction, B) extraction using paramagnetic beads, C) Filtration followed by bead beating and silica spin column extraction D) Pre-concentration and extraction using paramagnetic beads. The quality and the quantity of extracted nucleic are compared as it is crucial in downstream processing such as variant analysis and whole genome sequencing. The current study will throw some light on choosing the right method of nucleic acid extraction from wastewater.

5.01.V-015 Profiling Microbial Community and Potential Pathogens through Wastewater Surveillance in UAE using MALDI-TOF Mass Spectrometry

Surabhi Singh, Giovanni Odivilas, Helio Santos, Degan Abdissalam, Sumayya Almansoori, Maryam AlQaydi, Bhuvanesh Kumar Shanmugam, Amina Ismail Ahmed, Monika Shukla, Ashraf Adlan, Shaikha Alameri and Wael Elamin, G42 Healthcare

Microbial community profiling for wastewater has been less explored despite containing a diverse array of microbes that may help to gain insights about community health. A wide variety of infectious disease-causing microbes can be identified via monitoring of wastewater samples, which allows us to detect, contain and provide an early warning about outbreaks. Isolating microbes from the wastewater matrices is difficult as the wastewater is saturated with environmental microflora (*E. coli* and *Aeromonas*). In this study, an attempt has been made to untangle the complex microbial community using a structured protocol that involves pre-enrichment and selective isolation of bacterial species from wastewater. The isolates were identified by Matrix-assisted laser desorption/ionization- Time of Flight (MALDI-TOF MS). The potential pathogenic bacterial members identified from the samples included *Bacillus spp.*, *Klebsiella spp.*, *Pseudomonas spp.*, *Salmonella spp.*, *Shigella spp.*, *Staphylococcus spp.*, and *Vibrio spp.* The fungi identified were *Aspergillus spp.*, *Trichophyton spp.*, *Trichosporon spp.*, and *Candida spp.* The study shows that the wastewater microbiome provides useful public health information, and monitoring wastewater through high throughput techniques may aid in proactively detecting and characterizing pathogenic agents circulating in a community.

5.02.P-Mo Bayesian Networks in Environmental Risk Assessment and Management

5.02.P-Mo-176 Bayesian Network Models of Socio-Ecological River Systems to Determine Sustainability Requirements and Manage Multiple Stressors

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Many of southern Africa's socio-ecologically important water resources are over utilized, threatened by multiple stressors and do not provide the ecosystem services required by venerable African human communities. Regional Integrated Water Resource Management (IWRM) has been established to manage water resources holistically, but being dominated by the South African Water Act (NWA). The NWA proposes to achieve a sustainable balance between the use and protection of significant water resources through use management to minimize use, thereby assuming adequate protection. While the NWA considers the management of water quality, quantity and habitat stressors, it does not consider river connectivity, water borne diseases and disturbance to wildlife stressors. As a result, management plans do not adequately represent resources, and or how they respond to multiple stressors, simply because these plans do not consider all relevant stressors. Advances in IWRM include probability modelling approaches that can address these shortcomings. One new approach is Regional Scale Ecological Risk Assessments (ERA) incorporating Bayesian Networks (BN). This ERA-BN approach has tested in the Limpopo and Incomati trans-boundary basins, to characterise multiple stressors impacting a system and evaluate the risk that stressors have on selected social and ecological endpoints. These case studies have identified excessive water abstraction, pollution, climate change, alien

invasive species, over harvesting of natural resources and existing and planned river connectivity disruptions as key drivers of the lack of sustainability of these systems. Many of these rivers that were perennial are now seasonal and seasonal rivers are episodic resulting in deteriorated river conditions and reduced ecosystem services. Environmental flow requirements range between 38 and 50% of the mean annual runoff of water in the rivers, most of this is available as freshet and flood flows but storage limitations affect annual supply. Cumulative impacts of water quality stressors from mines, industries, urban centres and agriculture pollute rivers have been identified and we proposed water use licence amendments. Emerging climate stressors, alien invasions, overfishing and water disease management recommendations have been provided This new approach is being tested to contribute to the implementation of IWRM in the region.

5.02.P-Mo-177 A Modular Approach to Risk Assessment with Structural Models

John F. Carriger, U.S. Environmental Protection Agency

Structural models based on Bayesian networks are powerful knowledge representation tools that can capture diverse problem analysis structures and provide a basis for quantitative analysis and weight of evidence. They have only recently been developed and applied in fields ranging from law to medicine to product safety. They have also been applied to environmental risk assessment and their uptake is increasing. Conceptual models in risk assessment are frequently used to capture the causal information in a risk problem. Bayesian networks provide an additional means for including uncertainty in the relationships of the variables in the conceptual models through conditional probabilities. Their development with a conceptual model also allows additional variables that can influence the fate and transport of chemicals to be included, including intervention decisions, and modifying factors. This presentation will overview past applications of Bayesian network and probabilistic model fragments for organizational risk management and demonstrate how these tools can allow advanced modeling and more useful assessment and management models. An approach using structural causal models will be emphasized for building a library of risk model structures for re-use and adaptation for individual risk problems in an organizational context. Various model types will be discussed but causality will be emphasized for capturing key understanding and components of the risk problem. EPA disclaimer: 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.

5.02.P-Mo-178 Bayesian Networks as Flexible and Comprehensive Tools for Use in the Natural Resource Damage Assessment Process

April D Reed and Wayne G. Landis, Western Washington University

Ecological risk assessments (EcoRAs) are often used to inform remediation decisions for the clean-up of contaminated environments. The Bayesian Network – Relative Risk Model (BN-RRM) is a progressive tool that was developed for EcoRA applications. The BN-RRM can quantitatively and probabilistically evaluate ecosystems at a regional scale because of its capacity for incorporating multiple stressors and multiple endpoints into a single model. This tool has not yet been developed for, or applied to, the federal Natural Resource Damage Assessment and Restoration (NRDAR) process. The NRDAR program was developed to restore natural resources and ecosystem services that have been lost or impacted due to the anthropogenic release of contaminants into the environment. This study will adapt the BN-RRM for use as a tool that will facilitate and enhance the NRDAR process. As a case study, this research will adapt and apply BN-RRM to a regional-scale natural resource damage assessment for the Little Mississinewa River (LMR) Superfund site near Union City, Indiana. The PCB contamination at the LMR site was investigated and cleaned up in the early 2000s. At the time, it was decided that NRDAR was not warranted for the site. We will be conducting an assessment now to demonstrate the effective adaptation of the BN-RRM for the NRDAR process. The addition of utility and decision nodes into our Bayesian network models will add the functionality needed for the valuation of impacted resources and evaluation of restoration alternatives. This research is funded by a grant from the United States Department of the Interior which oversees the NRDAR program.

5.02.P-Mo-179 Ecological Risk Assessment Using a Bayesian Network Relative Risk Model (BN-RRM) for Microplastics in the Upper San Francisco Bay Region

Cynthia C. Kuhn, Emma Sharpe, Wayne G. Landis, Skyler Elmstrom and Erika Whitney, Western Washington University

This project is conducting an ecological risk assessment for micro- plastics 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 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. Also included is mercury toxicity data, and toxicity data from studies on the pesticide Bifenthrin, 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. This project is relying on plastic particle toxicity data generated by Oregon State 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 is funded by the National Science Foundation Growing Convergence Research Grant (1935018) program.

5.02.P-Mo-180 Assessing Pollinator Decline Associated Risks to Endangered Plant Populations using Bayesian Networks

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Endangered plant populations experience a wide array of stressors such as habitat loss, competition from invasive species, weather events, fire management practices and other human activities. Insect pollinators play essential or facultative roles in the vast majority of plant species' reproduction. Studies have shown that concurrent with the pollinator loss, comes a reduction in plant reproduction dependent upon lost pollination services. Endangered plant species already experiencing low densities can be particularly vulnerable to reductions on pollination services. Estimating the effect of pollination loss on plants is challenging because pollination is a function of numerous direct and indirect interactions between ecological factors and management practices. This difficulty is compounded when there is little data describing these drivers and their interactions. Bayesian Networks provide options for understanding emergent risk from multifaceted processes with limited data. Coupling Bayesian inference with network science, Bayesian Networks are specifically optimized to provide evidence-based inference across complex causal chains, even with limited data. This is supported by: 1) the flexible nature of Bayesian Networks which can make use of relationships between both quantitative and categorical data and 2) the network structure of conditional probability which propagates direct and indirect inference across different types of data. Furthermore, Bayesian Networks are readily updated as better data becomes available to produce inference reflecting the best available data. Here we show how Bayesian Networks can be used to evaluate the risk faced by endangered plant species experiencing potential losses in insect pollination due to insecticide exposure. Using the Bayesian network development software Netica, we created and visualized a Bayesian network describing risk facing endangered plants in southern Florida. We then present sensitivity analysis across network outcomes using the RNetica software package in R to visualize how risk changes as a function of focal species and application practices.

5.02.T Bayesian Networks in Environmental Risk Assessment and Management

5.02.T-01 Probabilistic Risk of Chemical Mixtures: Relative and Cumulative Risk of Pesticides Within Freshwater Biological Communities

Jannicke Moe¹, Nikiforos A Alygizakis², Mirco Bundschuh³, Anders Madsen⁴, Sophie Mentzel¹, Andreu Rico⁵, Francisco Sylvester⁶, Samuel Welch¹ and Paul van den Brink⁷, (1)Norwegian Institute for Water Research (NIVA), (2)Environmental Institute, (3)University of Kaiserslautern-Landau (RPTU), (4)Hugin Expert, (5)University of Valencia, (6)Goethe University Frankfurt, (7)Wageningen University & Research

We have developed a hierarchical probabilistic model, an object-oriented Bayesian network (BN), for exploring relative and cumulative risk of pesticides mixtures to non-target biological endpoints in freshwater ecosystems. Our study is based on monitoring data for 10 stream sites in Germany, with three pesticides per site, in total 13 different pesticides. Traditional environmental risk assessment (ERA) approaches are largely based on single-substance and single-species testing. Two major challenges for more realistic ERA are therefore chemical mixtures and community-level responses. Modelling methods developed for handling these challenges often aim to reduce the complexity of the chemical stressors (e.g., a Mixture Assessment Factor) and of the community response. Our BN model, in contrast, incorporates an empirical dose-response relationship for each pesticide-to-endpoint pathway. For each pesticide, we used the existing model PERPEST (Predicts the Ecological Risks of PESTicides) to predict probabilities of three alternative effect classes (no, weak, clear) for up to 10 biological endpoints. PERPEST uses a database of mesocosm studies and case-based reasoning for this purpose. We then used the BN to aggregate the predicted effect probabilities from single pesticides to mixtures by joint probability (OR) expressions. Furthermore, the BN aggregated the probability of effect classes from the level of individual endpoints (e.g., algae, rotifers, insects), to endpoint groups (e.g., plants, zooplankton, macroinvertebrates), and further to the community level. This BN enables the exploring of risk from different angles by joint probability calculation in combination with alternative assessment rules. For example, we can address questions such as following, for a given site. (1) What is the probability that at least one pesticide will have a clear effect on at least one biological endpoint? (2) What is the probability that none of the pesticides will have an effect on any of the endpoints? (3) Which endpoint groups are most likely to be affected by the observed pesticide mixtures, and to single pesticides? This work results from the workshop "Improving the use of (semi-)field data for the risk assessment of chemicals" (WG2 Bioassays), organized in November 2022 by the Expert Group on "Ecosystem level effects of chemicals of emerging concern on aquatic ecosystems" within the NORMAN Network of reference laboratories, research centers and related organizations.

5.02.T-02 The Relative Contributions of Contaminants to Environmental Risk in the Upper San Francisco Estuary

Emma Sharpe¹, Eric J. Lawrence¹, Steven Eikenbary¹, Ethan Brown², Mikayla Bowers¹, April Markiewicz¹, and Wayne G. Landis¹, (1)Western Washington University, (2)University of Notre Dame

The objective of this ecological risk assessment was to determine which contaminants in the Upper San Francisco Estuary contribute to the overall risk for pelagic fish and macroinvertebrate communities. The Upper San Francisco Estuary, also known as the Sacramento-San Joaquin River Delta, is one of the largest estuaries in North America. Many organisms rely on this area for migration and nursery. It is surrounded by urban, industrial, and agricultural land use and has historical contamination from a variety of sources. This has resulted in a complex suite of contaminants that requires a comprehensive and dynamic risk assessment method. The method used to conduct this risk assessment was the Bayesian network relative risk model (BN-RRM). The BN-RRM is ideal for multiple stressor regional-scale ecological risk assessment because it uses Bayesian networks to model complex relationships between variables with probability distributions. Chinook salmon and

Delta smelt were used for fish endpoints. Macroinvertebrate community structure was determined using multivariate analysis and comparing variable such as contaminant concentration and water quality. Environmental concentration data, used to estimated exposure, was downloaded from the California Environmental Data Exchange Network (CEDEN) and California Surface Water (SURF) databases and combined into a single dataset. Dose-response models connecting acetylcholinesterase inhibition to mortality in multiple fish species for a mixture of malathion and diazinon were derived from Laetz et al. (2009). Dose-response models for a mixture of chlorpyrifos and bifenthrin for silverside mortality were estimated using a mixture additive model to estimate toxicity from single chemical toxicity data for bifenthrin and chlorpyrifos from Hutton et al. (2021). Dose-response models for mercury for fathead minnow, brook trout, and rainbow trout mortality were derived from Dillon et al. 2010. Initial results show that the diazinon and malathion mixture have the most impact on fish mortality in the modeled scenario. This is likely due to synergism between these two contaminants. This model can continue to be extended as sufficient dose-response data becomes available.

5.02.T-03 Bayesian Network Model of Mercury Exposure to Aquatic Ecosystems of the Mackenzie Watershed

Una Jermilova, Trent University

A Bayesian Network Relative Risk Model (BN-RRM) was developed to assess the recent (2005- 2020) state of mercury (Hg) in the freshwater ecosystems of Great Slave Lake and the Mackenzie River, in the Canadian Northwest Territories. Data from environmental models, Hg monitoring projects, and community reports were organized into a single causal model which considered six Hg input pathways. Sensitivity analysis was used to predict the sources influencing Hg concentrations in freshwater and fish tissue across eight study regions. The output of the BN-RRMs differed significantly throughout the study area (800,000 m²), with atmospheric Hg deposition and soil erosion Hg release consistently flagged as important explanatory variables. However, the low sensitivity values imply that only a fraction of the observed Hg concentrations can be attributed to the input pathways. Analysis of the endpoint uncertainties revealed gaps in knowledge and in Hg datasets, which should be the focus of study for future monitoring programs.

5.02.T-04 Regional Scale Ecological Risk Assessment Incorporating Bayesian Networks to Characterise the Risk of Multiple Stressors to Social and Ecological Endpoints of the Limpopo River Basin, Southern Africa

Gordon Craig O'Brien¹, Chris Dickens², Melissa Wade¹ and Victor Wepener³, (1)University of Mpumalanga, (2)International Water Management Institute, (3) North-West University

The trans-boundary water resources of the Limpopo River Basin sustains high, unique biodiversity, and >10 million vulnerable Africans. While important the perennial nature of the rivers of the basin have been transformed into seasonal rivers and the seasonal rivers into episodic systems resulting significant losses in ecosystem services. Knowledge of this shared socio-ecological system is limited, and while being “everybody’s” in the region, it is also “nobody’s”, and as such poorly managed. We carried out a regional scale ecological risk assessment of multiple stressors affecting the ecosystem services of the Limpopo Basin, incorporating Bayesian Network probability modelling methods. We divided the basin up into 25 risk regions or sub-hydrological basins according to socio-ecological scenarios and regional and international interests. We developed Bayesian Network probability models to represent the socio-ecological systems for each risk region and aligned/linked models to represent upstream (migration) and downstream (drift) relationships. We used the risk assessment to characterise environmental flows for the basin and then considered the implications of different source-stressor vs. protection management scenarios. The risk of multiple stressors to the ecosystem services of the basin is currently high, demonstrating that a significant change in the state of the resource and

availability of services has occurred. Major stressors including water quantity and quality alterations, habitat removal and river connectivity disruptions, disturbance to wildlife and climate change. While the total volume of water required to achieve environmental flows for the basin, towards sustainability, is available, water storage is limited and base flows are currently used excessively resulting in significant loss of ecosystem services in particular. Of the cultural, supporting, regulating and provisioning services considered in the study, the supporting services that represent the wellbeing of the ecosystem, with its biodiversity and associated ecosystem processes, was determined to be most impacted. Future climate change scenarios identified extremely high risk for the services of the basin. Without urgent stressor mitigation and sustainable management the Limpopo Basin its ecosystems, and the people who depend on them are at great risk of service losses, services they are all dependent on. This study has supported regional water resources management of the basin.

5.02.T-05 Applications of Object Oriented Bayesian Networks for Assessing Pharmaceutical Pollution

Samuel Welch¹, Merete Grung¹, Anders Madsen² and Jannicke Moe¹, (1)Norwegian Institute for Water Research (NIVA), (2)Hugin Expert

Understanding pharmaceuticals' contributions to future environmental risk requires consideration of how the environment, population, and climate will evolve in the future. Forecasts of these factors are uncertain, and will become more so further into the future – making probabilistic environmental risk assessment, which retains uncertainty, a valuable tool for exploring future risk. We present an Object-Oriented Bayesian Network (OOBN) for the prediction and prioritisation of environmental risk posed by six Active Pharmaceutical Ingredients (APIs) in Norway, across two time points (2020 and 2050), eleven counties, three population (low, main, high), and four wastewater treatment (WWT) scenarios (current, upgrade of all to at least secondary, upgrade to tertiary, best possible removal). Sales weights were predicted by year and population from historic sales, and used to predict environmental concentrations in WWT influent, effluent, and surface waters. Surface water Risk Quotients (RQ) per API were predicted under each scenario, and a Sum of RQ and joint probability of RQ threshold exceedance calculated across the 6 APIs. We found that in 2020, predicted SumRQ was highest in urban counties, with higher population density and better wastewater treatment, and lower in rural counties. By 2050, risk was consistent across urban and rural counties, but lower in peri-urban counties. Comparing Sum of RQ across counties in 2050 (default population scenario), risk is expected to increase if WWT is upgraded to secondary or tertiary due to lower removal of risk-driving APIs under these treatments. Risk in urban counties is mostly unaffected due to the efficient technologies already used in WWT. However, both in rural counties and across the country as a whole, switching to the best available treatment technology is expected to lower overall risk. Under low, main, and high population scenarios captured by the OOBN Norway's population could range from 4.5 to 7.5 million, but little effect was seen on risk. Perhaps as population growth drives both modelled sales and dilution, Sum RQ across the whole country was predicted to drop in the low growth scenario and be unaffected by high growth. This work represents a useful case study for the use of a spatial and temporally discretised OOBN for prediction of environmental risk and identification of APIs driving risk distributions with the full consideration and transparent presentation of uncertainty.

5.02.T-06 Integration of Climate Change into the Multiple Stressor Risk Assessment for the Yakima River, Washington, USA

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A key question in understanding the implications of climate change is how to integrate ecological risk assessments that focus on contaminants with the environmental alterations from climate predictions. This report summarizes the results of integrating climate change with the use of agricultural pesticides in the Yakima River

basin of Washington State. The original study (Mitchell et al. 2021) used the Bayesian network relative risk model to integrate risk. The Bayesian network incorporated an adverse outcome-based pathway to describe the effects of pesticide mixtures on fish survivorship and reproduction. Other pathways described the effects of water quality. The endpoint was no net loss to the Chinook population. Inputs to the model were all based on direct measurements from the site. In our study, a number of additional pathways were integrated to capture possible effects of a changing climate. Different levels of pesticide application were incorporated to capture different strategies to control pests in the future. Projected changes in water quality parameters were based on Ficklin et al. (2013), who used a suite of climate change models to generate probability distributions describing climatic conditions in the years 2050 and 2080. Our findings indicate that the largest driver of risk is the direct impacts climate change on temperature and dissolved oxygen content, rather than changes in pesticide exposures. The results suggest the Yakima metapopulation will not meet the goal of no net loss. It appears that climate change will have a major influence on the population dynamics and therefore risk to the Chinook population. If preservation of Chinook salmon is the goal, risk mitigation options should be planned and implemented by state, federal regulators and stakeholders in the near term. This research is a product of the SETAC Pellston conference “Integrating Global Climate Change in Ecological Risk Assessment” held June 2022 at the Oscarborg Fortress Conference Center, Oslo, Norway.

5.03.T Mechanistic Effect Modeling to Support Ecological Risk Assessment: Moving Between Ecological Scales with Limited Datasets

5.03.T-01 An Evolving View of Quantitative Adverse Outcome Pathways and their Application

Daniel L. Villeneuve, U.S. Environmental Protection Agency

Quantitative adverse outcome pathways (qAOPs) have been broadly defined as adverse outcome pathways (AOPs) for which the quantitative understanding of the key event relationships is sufficient to allow for estimation of the probability or severity of the adverse outcome occurring based on measured or modeled changes in an early key event in the pathway. This presentation reports on testing and evaluation of an early example of a qAOP based on a system of interconnected, mechanistically-based, computational models. Simulations generally underestimated impacts on early key events but yielded EC50s that were within a factor of 10 of experimentally determined lowest observed effect concentrations. Recognizing that the pace of similar qAOP development has not been sufficient to support the growing interest in application of new approach methodologies in chemical safety decision-making, alternative approaches for developing fit-for-purpose qAOPs are considered. For example, anchoring AOP development to prototypical stressors for which there is a strong database of concentration-response data across key events may provide an alternative approach. This approach has been applied in conjunction with effect-based monitoring to identify mechanistically-based groups of chemicals that or high or low concern for potential ecological effects at a given site. However, this strategy several key assumptions that should be evaluated to determine whether relationships developed for the prototypical stressor and domain of empirical evidence can be extended to the plausible domain of applicability for the AOP. *The contents of this abstract neither constitute, nor necessarily reflect, US EPA policy.*

5.03.T-02 Mixtures with Limited Experimental Data – Filling Data Gaps using Read-Across or Estimations

Chrissy Coley, Savannah Sierco and Laura Morlacci, SRC, Inc.

In chemical hazard assessment, data gaps are often filled using predicted values. Predictions are generated using methods such as model estimations or read-across to analogous chemicals to help inform a chemical’s hazard evaluation. Predicting values becomes more challenging for mixtures because the complexity of the substance being evaluated increases and the prediction methods become less straightforward. Mixtures may be well-defined with known components and composition (identity and ratio of components); however, some mixtures are not well characterized and have variable components and composition based on production methods and

processing. Some mixtures are relatively homogeneous (e.g., fatty acid methyl esters) while some are very complex and have many different types of components (e.g., an essential oil). The aim of this work is to compare read-across and model estimation approaches for predicting hazard data of mixtures with incomplete datasets; predictions for environmental fate, ecotoxicity, and human health endpoints are included. Model estimations will be performed using EPI Suite™, ECOSAR, and OECD QSAR Toolbox. Model estimations will be dependent upon the best representative structures for the mixture. Representative structures may be chosen based on least or most toxic/persistent component(s), composition, or major component(s) of the mixture. Analogues for read-across will be identified using AIM, Comptox Chemicals Dashboard, PubChem, and professional judgment. Factors considered for read-across include evaluation of the analogue selection process, through a component based or composition focused approach. The quality and availability of analogue data is also considered. Results from this high-level demonstration show that a one-size-fits all approach is not recommended. Identifying limitations of each strategy and selecting the method that best represents the mixture is necessary to optimize the predicted values used to inform a chemical's hazard evaluation.

5.03.T-03 What is a Toxicity Translator?

Matthew Etterson, Nathan Pollesch, Jill Awkerman, David Miller, Tom Purucker and Sandy Raimondo, U.S. Environmental Protection Agency

Assessing the risks of contaminant exposure to invertebrates, fish, and wildlife based on toxicity test results is a major challenge in ecotoxicology. This has always been true with toxicity tests for apical outcomes with well-known endpoints (LD50, ECx, NOEC, etc.). The challenge has increased as the field has evolved to leverage advanced cellular and molecular techniques. These provide valuable information about the early key events of Adverse Outcome Pathways, but we do not always fully understand the subsequent key events leading to adverse effects on vital rates, fitness, and population health nor can we confidently quantify associated model parameters. USEPA's Toxicity Translator program was conceived to help EPA's Office of Pesticide Programs (OPP) interpret standard toxicity test results submitted by registrants under the Federal Insecticide, Fungicide, and Rodenticide Act. Model development began in 2005 with the Markov Chain Nest Productivity Model (MCnest), which is now routinely used by OPP during pesticide registration to help understand pesticide risks to breeding birds. As the field has evolved, so has the Toxicity Translator program. In this presentation we will define toxicity translators and briefly review the various translator models under development at USEPA. We will describe the current strengths and weaknesses of the models and the current development priorities. We will also describe the development timelines and availability of the models for testing and implementation. Finally, we will describe some of the major challenges we face in implementation of the toxicity translator approaches. 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.

5.03.T-04 Developing an Interactive Tool to Support Ecological Risk Assessment for Fish Populations

Nathan Pollesch and Matthew Etterson, U.S. Environmental Protection Agency

Adoption of mathematical and statistical models to support ecological risk assessment is critical to synthesize complex data streams, extrapolate across species and context, and to project forward in time to explore potential future scenarios. Successful incorporation into risk assessment workflows depends on multiple factors. These include sound modeling approaches, feasibility of parameterization, meaningful model outputs, and overall user experience. Ecotoxicological modeling tools are developed in a variety of programming languages by model developers, and an important step in development is how the model will ultimately be deployed to users and stakeholders. In this talk, I will discuss the development of computational workflows and the current state of a graphical user interface with USEPA's Fish Toxicity Translator model as a case study. I will discuss the advantages and challenges of deployment of a graphical user interface using the R Shiny programming

language and give a demonstration of its features. The goal of this talk will be to provide insight into the deployment aspect of model development, and to help create a dialogue between model developers and risk assessors surrounding this critical step in ecological risk assessment tool development and utilization.

5.03.T-05 Using Traits-Based Approaches to Inform Population Modeling of Threatened and Endangered Species

Valery Forbes¹, Maxime Vaugeois², Richard Brain² and Nika Galic², (1)Florida Atlantic University (FAU), (2)Syngenta Crop Protection

Mechanistic effect models, such as population models, are powerful tools for quantitatively linking responses of stressors in individuals to potential impacts on populations. Their use in ecological risk assessment is increasing as a result of improved guidance and a growing number of case studies demonstrating their value and increasing regulatory confidence. Developing effective population models requires data on species life-history traits and ecology, which can be particularly challenging for species listed as threatened or endangered under the US Endangered Species Act for which such data are often lacking. Using terrestrial plants and freshwater mussels as examples, we have been developing approaches to identify relatively data-rich non-listed species for population modeling that can represent groups of data-poor listed species. The goal is to use such models to assess the potential risks of pesticides and other stressors to listed species and to inform effective management and recovery planning. Using life-history databases in combination with statistical and demographic analyses, we found no consistent differences in life-history traits between listed and non-listed species for either taxonomic group assessed. For terrestrial plants, listed species are widely distributed across plant phylogeny and life-history clusters, suggesting that listed species do not share a common evolution or life-history characteristics that would make them uniquely vulnerable. For freshwater mussels, listed species tended to have equilibrium or periodic life-history traits, and none were found to have opportunistic life-history strategies. Since life-history traits drive the relationship between individual responses to stress and population-level impacts, traits-based approaches to categorize species can help to identify suitable representatives for population modeling to inform the risk assessment and management of listed species.

5.03.T-06 Use of a Plant Community Model to Simulate Ecosystem Services of Importance for Indirect Effects on Endangered Species

Ismael M Rodea-Palomares, Bayer AG - Crop Science Division

Endangered Species Assessment (ESA) is of critical importance to ensure pesticides are used in such a way that ensures protection of non-listed and endangered species. Besides direct effects of pesticides on endangered species, effects on biotic elements on which endangered species rely on for e.g., food and shelter are critically important for a successful ESA. Among those biotic elements, the plant community that provides both food and shelter to the rest of the ecosystem is probably the most evident biotic element that, if affected by pesticide use, can result in detrimental indirect effects on endangered animals (such as terrestrial arthropods, mammals, birds, etc.). In this work we explore a framework for model plant communities in field margins and some of their ecosystem services through mechanistic plant community modelling. For this, we use an Agent-Based plant community model that simulates a grassland plant community through the definition of plant functional types. As the modelling is based on plant functional types rather than specific plant species, it can be considered to represent plant attributes that are generic to any plant species if the species belong to a similar functional type (e.g., small rosette vs tall plants, etc.). The model can represent various plant functional types and their interaction through competition for resources above and below ground, and the effect of agronomic practices (such as annual plowing, mowing, etc.) in the plant interactions. A module to represent effects of pesticides based on standard plant toxicity studies is also incorporated in the model. In the presentation we will explore

a framework to use this model in the context of indirect effects on endangered species. We will explore how endpoints for effects on some of the ecosystem services linked to indirect effects on endangered species (such as shelter and food provision) could be derived and used in the context of ESA assessment of pesticides.

5.03.V Mechanistic Effect Modeling to Support Ecological Risk Assessment: Moving Between Ecological Scales with Limited Datasets

5.03.V-017 A Multi-Tiered Risk Assessment of Exposure of Aquatic Invertebrates to Thiamethoxam Using Data-Driven Tools

Maxime Vaugeois¹, Silke Claßen², Natalie Carmen Dallmann², Jana Gerhard², Daniel Gerth², Josef Koch², Tido Strauss² and Nika Galic³, (1)Syngenta Crop Protection, (2)gaiac - Research Institute for Ecosystem Analysis and Assessment,(3)Syngenta Crop Protection

Thiamethoxam (TMX) is a systemic neonicotinoid insecticide that is used to protect crops against pests. In a recent ecological risk assessment, the US EPA identified potential risk from chronic TMX exposure to freshwater aquatic invertebrates. Chironomids were identified as one of the most sensitive taxa. To better quantify the chronic toxicity of TMX in chironomids, we developed a multi-scale, multi-tiered risk assessment approach to characterize the risks at the individual and population levels under realistic environmental conditions and exposure to TMX. We first developed a quantitative mechanistic effect model for *Chironomus riparius*, to simulate the species' life history under dynamic temperatures and exposure concentrations. The model design was based on the state-of-the-art mechanistic approach of combining the Dynamic Energy Budget (DEB) theory with toxicokinetic-toxicodynamic (TKTD) modeling. The model was calibrated on data from tailor-made experiments. Furthermore, the calibrated model delivered good comparisons to independent data, which had been specifically collected for validation purposes. We then used the DEB-TKTD model to estimate the potential risk associated with realistic exposure profiles and variable temperatures using a moving-time-window (MTW) approach. This approach allows for the determination of the most adverse exposure window, resulting from a critical combination of life-stage sensitivity and the timing and magnitude of chemical exposure. We estimated exposure multiplication factors (EMFs) associated with different levels of effects on mortality (EP10 to EP50) for two US watersheds most vulnerable to runoff. Finally, we parametrized an Agent-Based Model (ABM), which integrates the previously developed DEB-TKTD model, to estimate risk at the population level. This model was parameterized and validated using literature data as well as tailor-made 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, the risk estimation is based on the combination of data generation and the best data-driven tools available, which were developed following the most up-to-date guidelines and recommendations, notably regarding model testing and transparent documentation.

5.04.P-We Methods and Data for Cumulative Impact Assessment in the Context of Environmental Justice

5.04.P-We-108 Understanding ECEJ Census Data for a Large Group of Manufacturing Sites with a Customized Dashboard

Tom Fewless and Tamara House-Knight, GHD

The US government has made census information available to encourage analysis and consideration of impacted communities with a focus on Environmental, Climate, and Economic Justice (ECEJ). This is a complex issue with a large and complex dataset that can be applied in many situations. Because of the complexity of the data, understanding and developing actionable outcomes can be difficult. For ECEJ initiatives to be successful stakeholders need to derive insights from these data to inform their (better) decisions. This tool provides analysis and recommendations to enable understanding of and interaction with the census data through

an interactive dashboard. The objective of this tool/dashboard is to assist with proactively engaging marginalized communities and understanding the ECEJ metrics. This tool analyzes the ECEJ information of communities within a user-defined radius/area to provide a clear picture of the ECEJ landscape within a community. This tool was developed to assist with visualizing this information so it can be used as a decision-making tool by facilitating identification of priority locations. By providing actionable recommendations based on an improved understanding of the information, users can align their policies with the interests of surrounding marginalized communities. This dashboard compiles the data, allowing for examination of statistics, trends, and other key metrics. By using these results, users can evaluate the impact of implemented community efforts. Businesses can access these datasets, view their community impact relative to their geographic footprint, and allow individual users and teams to understand specific parameters of interest. This presentation is intended to provide an overview of the dashboarding tool, how it analyzes the data, its use, and the results for a large manufacturing business with property in the United States.

5.04.P-We-109 Utilizing Existing Samples to Characterize Chemical Components of PM_{2.5} in Jackson, Mississippi

Amelia Craze Smith and Courtney Roper, University of Mississippi

In Jackson, Mississippi (MS), over 80% of the population identifies as Black or African American. Strained healthcare infrastructure and environmental injustices threaten the health of this population. Mississippians have higher rates of morbidities such as cancer, cardiovascular disease, chronic obstructive pulmonary disease (COPD), and long-term health impacts from COVID-19 than the national average. Poor air quality can exacerbate these conditions and lead to premature mortality. There are few studies on air quality in the American South, with even less in MS. Interest in fine particulate matter (PM_{2.5}), a complex mixture of air pollution, has grown due to associations with systemic health effects. An underlying mechanism for these effects is the ability for PM_{2.5} to induce oxidative stress. Federal regulations are in place to monitor PM_{2.5} concentrations throughout the U.S. with routine filter-based sampling. However, after concentrations are determined, the filters are not commonly used for additional analysis; such as, measuring health relevant constituents including polycyclic aromatic hydrocarbons (PAHs). In the interest of sustainable science, this project utilizes filters previously collected by the Mississippi Department of Environmental Quality (MDEQ) to determine the chemical and toxicological profile of PM_{2.5}. The objective of this study was to characterize the chemical components of PM_{2.5} across seasons. For this project, we used filters collected in Jackson, MS at two sites over an entire year. Filters were extracted and pooled based on sampling week prior to subsequent analyses. The Dithiothreitol (DTT) Assay will be used to measure oxidative potential of samples. Chemical analysis will be performed by GC-MS to quantify 16 parent PAHs commonly bound to PM_{2.5}. Initial analysis of PM_{2.5} concentrations explored seasonal differences, with summer having the highest average PM_{2.5} concentrations (14.8 mg/m³ ± 8.49) with variation between sampling locations (ex. Summer 16.06mg/m³ ± 9.29 vs. 11.30 mg/m³ ± 3.91). Of note, these values are above the recommended annual and daily PM_{2.5} concentrations several regulatory agencies. We anticipate that variation in season and meteorological conditions will impact our results which will be compared to other sampling locations in rural areas of MS. This work will contribute to identifying chemical stressors to support a cumulative impact assessment in a region experiencing multiple environmental issues.

5.04.P-We-110 Evaluating Biometrics in National Health and Nutrition Survey (NHANES) Data to Estimate Allostatic Load in Cumulative Impact Assessments

Alexander East, William D Klaren, Cara Frankenfeld and William Rish, ToxStrategies, LLC

Characterization of non-chemical stress is an evolving field in cumulative impact assessments. Multiple publications have taken the approach of estimating allostatic load; a metric which represents chronic basal stress via aggregation of selected biometrics. Quantification of chronic stress with tools like allostatic load can be useful in determining chemical susceptibility within communities and adequately addressing it within

assessments. While these tools hold promise, there is uncertainty about which biomarkers are better suited at representing chronic stress, the correlated nature of the commonly used biometrics, and if any should be given additional weight. To this end, by using National Health and Nutrition Survey (NHANES) data, we evaluated 14 potential biomarkers across multiple organ systems (Systemic - Body Weight, Waist Circumference, Body Mass Index; Cardiovascular - Diastolic Blood Pressure, Systolic Blood Pressure; Serum lipids - High Density Lipoprotein Cholesterol, Total Cholesterol, Serum Triglycerides; Immunological - C-Reactive Protein; Hepatic - Serum Albumin, Lactate Dehydrogenase; Renal - Serum Creatinine, Creatine Phosphokinase; and Endocrine - Glycohemoglobin) for their contributions to allostatic load scoring. NHANES participants were divided into subpopulations based on sociodemographic factors (Income, Healthcare Access, Educational Attainment, and Food Insecurity) to investigate allostatic load differences among the groups as well as contributions of underlying biometrics to those differences. Biometric values varied among the selected subpopulations, suggesting the need for careful review when integrating results into a cumulative impact assessment that includes diverse subpopulations. Observed differences suggest that diastolic blood pressure, high density lipoprotein, and body mass index are the most frequent indicators of significant differences in stress between potentially vulnerable populations and remaining populations. Additionally, the largest gradients were observed in C-reactive protein, serum triglycerides, high density lipoprotein cholesterol, and creatine phosphokinase when comparing means between subpopulations and remaining populations. Further evaluation of these biometrics is required to develop models and tools that will effectively capture non-chemical stress and chemical susceptibility.

5.04.T Methods and Data for Cumulative Impact Assessment in the Context of Environmental Justice

5.04.T-01 An Integrated Approach to Cumulative Impact Assessment in Support of Projects and Actions within Delineated Environmental Justice Areas

Charlie Menzie, Ann Morrison, Melissa Kleven, Andrew Deines and Qiuyan Yu, Exponent Inc.

Approaches to conducting cumulative impact assessment (CIA) in the context of actions within EJ communities are being considered at the national and state level. This paper draws upon our experience with assessing the risks/impacts of multiple stressors and involvement with complex projects involving multiple stakeholder groups. We view the scope of CIA as situation specific and involving addressing questions raised in concert with a proposed or existing project, permit, or set of conditions identified as problematic within the community. Because of varied multiple stressors and disparate nature of information, the foundational approach is necessarily generic and requires a means of integrating quantitative and qualitative information. The center of the approach incorporates a communication program and a suite of goals that will be stakeholder specific and a need to bring these together. The overall goal of the process involving CIA is informing decisions and thus the envisioned CIA approach is designed to compare alternative solutions that consider the stakeholders and project objectives and benefits along with project-related and cumulative impacts. The approach is place-based and stressor-based which means that the CIA must have a focus germane to the questions. We present a set of twelve principles that can lead to well-constructed and focused CIAs. We describe how tools such as the the Relative Risk Model (RRM), influence diagrams, Bayesian analyses, artificial intelligence, and urban landscape models might be employed to represent combinations (e.g., additivity and/or interactions) of stressors and situations; we discuss the strengths and limitations of these methods. We view environmental conditions as part of human well being, and illustrate how ecosystem services are incorporated along with other stressors that impact directly or indirectly on human health. We diagram how communication and impact assessment fit into a process that is informed by structured communications among all parties to arrive at a solution or potential alternative solutions for addressing identified impacts from a project-specific and EJ perspective. Given the diverse nature of stakeholders, results need to be presented in an understandable manner. We utilize a variety of visual techniques for this purpose. Finally, we provide an example to illustrate the approach.

5.04.T-02 Exploring a Community Adjustment Factor: Epidemiological Data for Cumulative Impact and Risk Assessment

Mary Fox, Johns Hopkins University

Within established human health risk assessment methods there are steps where protective adjustments are made. For example, in developing Reference Doses or Reference Concentrations “uncertainty” factors are applied and when developing slope factors or unit risks for mutagenic carcinogens age-dependent adjustment factors are applied. These adjustments are made to account for important sources of variability and data gaps or uncertainties. There are other sources of variability and uncertainty that influence human health that have not yet been accounted for in risk assessment, namely the social determinants of health. It is through a better understanding of these determinants that we may begin to identify and respond to social and environmental injustices. This work will explore the concept of a community adjustment factor by: 1) describing the data and justifications for current adjustment factors to outline a roadmap for development of new adjustments; and 2) applying an effects-based approach to identify case examples of quantitative adjustments for community-level social determinants drawn from epidemiological literature. Environmental injustice will not be resolved through risk assessment procedures but building real population-level variability into the process acknowledges complex non-chemical exposures to improve risk estimates and inform stronger public health protections.

5.04.T-03 Linkage Between Allostatic Load from Non-Chemical Stressors and Susceptibility to Environmental Chemical Exposure: State of the Science Review

William D Klaren, William Rish and Alexander East, ToxStrategies, LLC

Cumulative impact assessment includes consideration of non-chemical stressors in (1) screening tools for identification of the location of subpopulations that have the potential for increased susceptibility to health impacts from environmental chemical exposure, and (2) assessing the extent to which exposure to non-chemical stressors may exacerbate these health impacts. The hypothesis is that increased susceptibility and exacerbation of adverse health effects from exposure to non-chemical stressors is linked to allostatic load. This hypothesis is predominantly supported by numerous studies of the statistical association between selected measures of allostatic load, non-chemical stressors, and specific health effects. The authors will present findings from a comprehensive review of scientific literature from 1994 to 2023 on the relationship of allostatic load to health effects from chemical exposure. A summary of the statistical association evidence and its relationship to non-chemical stressors being used in screening tools and cumulative impacts assessment frameworks will be presented. In addition, research into the causality of increased susceptibility to chemical exposure health effects from exposure to non-chemical stressors will be summarized, along with key uncertainties and future research needs.

5.04.T-04 EPA’s Ecological Research to Advance the Science of Cumulative Impacts

Chris Frey, U.S. Environmental Protection Agency

In response to a series of executive orders under the Biden Administration, the U.S. Environmental Protection Agency (EPA) is developing and adopting relevant scientific approaches to inform decisions that advance environmental justice for overburdened communities. These approaches include cumulative impact assessment, which seeks to understand the combined effects of multiple stressors on human health and the environment with a goal of informing decisions aimed at improving health, well-being, and quality of life for overburdened communities. In 2022, the EPA Office of Research and Development published a report that describes five areas where research is needed to advance the science underlying cumulative impact assessments. These five areas are: (1) establish the decision context and partner engagement; (2) address scientific considerations for meeting partner needs; (3) empower local decisions and actions through science; (4) support science translation and delivery; and (5) provide research management support for cumulative impact assessment. Altogether, this strategic approach has structured ORD’s cumulative impacts research portfolio to be responsive to partner needs and provides a blueprint for other research institutions to advance their own research on cumulative impacts.

This talk will focus on how ecological research influences health, well-being, and quality of life outcomes for communities and the importance of ecological research in improving human health as it relates to cumulative impact assessments.

5.04.T-05 National Aquatic Resource Surveys (NARS) Data for Addressing Impacts and Risk

Amanda M. Nahlik¹, Michael Dumelle¹, Alan T. Herlihy², Donald J. Benkendorf¹, Kerry L. Kuntz¹ and Susan Holdsworth¹, (1)U.S. Environmental Protection Agency, (2)Oregon State University

The National Aquatic Resource Survey (NARS) program, a collaboration among United States (US) Environmental Protection Agency Office of Water, Office of Research and Development, and states and tribes, assesses progress towards the objective of the Clean Water Act through field-based assessments conducted annually on a five-year cycle in lakes, rivers, streams, coasts, and wetlands. NARS is the only national program that produces publicly available, long-term, probabilistic, field-based data for the nation's aquatic resources. Since its inception in 2007, NARS has amassed physical, chemical, and biological data from over 16,000 sites. The probabilistic design enables the use of data collected in the field from individual sites to make estimates with confidence to regional and national populations of any given aquatic resource. In addition to reporting on aquatic system condition across the conterminous US and the change in condition over time, NARS data supports the calculation of relative and attributable risk of different stressors, such as nutrients (e.g., total nitrogen, total phosphorus), acidification (acid neutralizing capacity), and sediment contaminants, on the condition of aquatic systems at national and regional scales. In this talk, we demonstrate the power of NARS data and analysis methods to answer research questions, such as whether environmental degradation of flowing waters due to human activities are distributed inequitably in society. This study uses NARS rivers and streams data to assess the condition of flowing waters in predominantly urban areas of the conterminous US and its relationship to measures of environmental justice (EJ). The analysis showed that urban flowing waters are more degraded than their non-urban counterparts and that the proportion of urban waters in poor condition was often nearly twice as high as for the nation's flowing waters. However, relative and attributable risk of EJ measures (percent low income and percent minority race) were not associated with increased degradation of flowing waters. Several factors may obscure relationships between flowing water condition and environmental justice indicators including transformation and dilution of analytes, cumulative environmental stress, and scale of available data. More research is needed to assess the potential inequitable access to healthy rivers and streams.

5.04.T-06 Improving Cumulative Impact Assessment in Fenceline Communities: A Case Study in Southeastern Pennsylvania

Andrea Chiger¹, Echo Alford², Eve S Miari², Kearni N Warren³, Lora Snyder², Thom Nixon², Mike Ewall⁴, Ryan D Kennedy¹, Carolyn Gigot¹, Ellis Robinson¹, Mina Tehrani¹, Mary Fox¹, Kirsten Koehler¹, Ana Rule¹, Thomas Burke¹, Sara N Lupolt¹, Peter DeCarlo¹ and Keeve Nachman¹, (1)Johns Hopkins University, (2)Marcus Hook Area Neighbors for Public Health, (3)Delco Environmental Justice, (4)Delco Environmental Justice

Cumulative impact assessment is key to characterizing health risks in fenceline communities facing environmental pollution and challenging socioeconomic conditions. We used a mixed-methods approach to better characterize the burden from chemical and non-chemical stressors on physical and mental health in communities on the fenceline of industrial facilities in southeastern PA. Our first objective was to improve quantitative methods for evaluating health risks by both moving beyond inclusion of only the most sensitive health endpoint for each chemical and considering co-exposures to psychosocial stress. We demonstrate our innovative approach using data from our Hazardous Air Pollutant Monitoring and Assessment Project (HAP-MAP), which employed state-of-the-art mobile measurements to estimate inhalation exposures to over 30 volatile organic compounds in southeastern PA. We compiled hazard information from ATSDR Toxicological

Profiles and EPA's CompTox Chemicals Dashboard for all non-cancer health endpoints associated with each measured chemical and calculated hazard indices. Our approach found neurological, reproductive, and respiratory risks (hazard index >1) in HAP-MAP fenceline communities, whereas no risks were identified using standard risk assessment approaches. We also reviewed literature on co-exposures to chemicals and psychosocial stress, which indicated that stress may increase susceptibility to chemicals. We explored risk management approaches to account for relationships between chemical and non-chemical stressors. Our second objective was to improve understanding of non-chemical stressors in fenceline communities in southeastern PA and to examine how these stressors can act independently and in conjunction with chemical exposures to affect physical and mental health. Using a community-based participatory research (CBPR) approach, we developed a community health survey that included questions on pollution and chemical exposures, financial stressors, healthcare, health conditions, and priorities for policymakers. We will present our preliminary results using quantitative (e.g., descriptive statistics and ANOVA to test for potential differences by demographic factors) and qualitative (e.g., inductive and deductive analytical approaches to coding of open-ended responses) methods. Our work highlights the need for transdisciplinary collaborations, innovative approaches, and CBPR to improve methods for cumulative impact assessment.

5.05.A.T Pharmaceuticals in the Environment - A One Health Perspective - Part A

5.05.A.T-01 Incorporating Farm Management Practices to Support Simplified Environmental Assessment of Veterinary Drugs

Margaret Fleming and Jane Staveley, Exponent Inc.

Under the National Environmental Policy Act of 1969, the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM) must evaluate the environmental impacts of any major actions, resulting in an environmental assessment (EA), an environmental impact statement (EIS), or a categorical exclusion. A categorical exclusion applies to actions that have been determined not to have a significant effect on the environment. When applicable, a categorical exclusion allows a drug sponsor to avoid the expense and effort of completing a full EA for a proposed action. This presentation will provide an overview of actions eligible to receive a categorical exclusion and illustrate the categorical exclusion justification process using a case study. This presentation will also review "exceptional circumstances" that are considered prior to the determination that an action does not have a significant effect on the environment. The case study justification was prepared on behalf of a drug sponsor for a supplemental New Animal Drug Application with the purpose of removing a precaution statement that precluded the use of an approved anti-microbial in lactating or pregnant sows. CVM guidance provides a procedure for calculation of the predicted environmental concentration (PEC) of applied veterinary pharmaceuticals in soil. If the PEC_{soil} is less than 100 µg/kg, it can be concluded that risks to the environment are not anticipated. The PEC_{soil} is calculated based on the concentration of the drug in the manure (PEC_{manure}) and the application of this manure to agricultural land. To develop the PEC_{manure} it is appropriate to consider farm practices, the prescribed treatment, and the sow cycle. Through the integration of information describing pharmaceutical metabolism and typical manure management practices that impact the concentration of the excreted pharmaceutical in land-applied manure, Exponent calculated a PEC_{soil} below the 100 µg/kg threshold. This outcome, in addition to the absence of extraordinary circumstances, resulted in the determination by CVM that no environmental risks are anticipated and that the preparation of an EA was not needed.

5.05.A.T-03 Psychoactive Drugs in Non-Target Species – Neurotoxicity of Venlafaxine in Zebrafish (*Danio rerio*) Embryos

Katharina Brotzmann¹, Maria Fischer², Raoul Wolf³ and Thomas Braunbeck¹, (1)University of Heidelberg, (2)Centre for Organismal Studies, (3)Norwegian Institute for Water Research (NIVA)

The continuous increase in the consumption of pharmaceuticals in our society results in elevated concentrations in open water bodies. Designed to be stable and act already at low doses, antidepressants are of special concern. Interfering with neurotransmitter homeostasis within synaptic clefts and the brain, antidepressants may cause developmental disorders and manipulate neurobehavioral patterns. Given the high conservation of binding sites across vertebrate groups, fish are likely to also be affected by unintentional exposure. Especially in early life-stages, the nervous system is vulnerable to external stressors, which bears the risk of irreversible adverse effects in later developmental stages. Thus, there is a need for sensitive and robust testing systems in early life-stages in non-target species to determine potential risks of neurotoxic compounds. As a non-protected vertebrate model, zebrafish embryos have received increasing attention for testing behavioral and morphological effects within developmental neurotoxicity research. In this study, effects of venlafaxine on the coiling activity as the earliest detectable motor behavior and on the lateral line organ were investigated. Movements were recorded from 21 to 47 hours postfertilization (hpf) and analyzed with respect to coil duration and frequency. A generalized additive model was applied to distinguish between nontreatment-related effects (random effects) resulting from natural variation within groups and replicates and fixed treatment effects of the chemical concentrations. For venlafaxine, an altered coiling behavior was observed with a LOEC of 1 mg/L. For the lateral line organ, the neuromast assay assesses the ototoxicity of chemicals at 96 hpf by staining with DAPI and DASPEI. Comparisons with negative controls indicated no significant deviation at concentrations of 0.3 - 1 mg/L. As a conclusion, the methods chosen indicate (1) that venlafaxine induces behavioral changes within the first 48 hpf and (2) that the mode of action of venlafaxine in fish does not involve neuromast changes. As a model, zebrafish embryos represent a sensitive system for the identification of potential risks by neurotoxic compounds in early life-stages of non-target species. Supported by BMBF (724-40003-03F0906A) within the PharmaSea consortium, AquaticPollutants Joint Transnational Call by Water JPI.

5.05.A.T-04 Effect of Soil Amendments on Mobility and Plant Uptake of PPCPs in Sandy Soil Irrigated with Wastewater

Ali Mawof, Stéphane Bayen, Shiv Prasher and Lan Liu, McGill University

Wastewater can supplement freshwater in agriculture; however, it can contain emerging organic contaminants (*e.g.*, pharmaceuticals and personal care products (PPCPs)) that can pose major risks to human health and the environment. We investigated the effects of soil amendment with barley straw biochar and green and table waste compost, both alone and mixed, on PPCPs (*e.g.*, caffeine, carbamazepine, DEET, diclofenac, and triclosan) mobility in soil and uptake by potato (*Solanum tuberosum* L.) irrigated with synthetic wastewater for two years. In each year of the field lysimeter study (2017, 2018) conducted under wastewater (WW) irrigation, a thrice-replicated completely randomized design (CRD) tested the effect of a factorial combination of three levels of barley (*Hordeum vulgare* L.) straw biochar amendment (none, 1% and 3%) and two levels of mixed green and table waste compost amendment (none, 7.5%). Assessing the soil mobility of PPCPs in the presence of different amendments provides a wider understanding of the relationship between PPCPs' properties (*e.g.*, water solubility, octanol-water partition coefficient, acid dissociation constant) and their behaviour in soil. 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. We conclude that amending soil with barley straw biochar and/or compost produced from city green table waste can improve the safety of wastewater irrigated potatoes, depending on the biochar application rate and the PPCP compound.

5.05.A.T-05 New Approach Methodologies for the Ecological Assessment of Pharmaceuticals: An Overview of Recent Developments

Gerald T. Ankley and Carlie LaLone, U.S. Environmental Protection Agency

Toxicity data to assess the ecological risks of many human and veterinary pharmaceuticals are usually limited.

While this is not uncommon for environmental contaminants, there often is some level of understanding of potential molecular and biochemical activities of pharmaceuticals which can provide a foundation for understanding mode(s) of action and predicting potential adverse effects. Over the past few years substantial progress has been made in the development of new approach methodologies (NAMs) to efficiently quantify biological activity of a variety of chemicals, including pharmaceuticals, using computational models and pathway-based in vitro and in vivo assays. The availability of open-access knowledgebases to curate biological response (including NAM) data, and bioinformatics tools to help interpret the information also has significantly increased. Importantly, advances in development and implementation of the adverse outcome pathway framework provide the underpinnings needed to translate NAM data into predictions of ecologically relevant outcomes required by risk assessors. This talk will provide an overview of the evolution and integration of these various data sources, tools, and concepts as a basis for efficiently assessing the ecological risks of pharmaceuticals. *The contents of this presentation neither constitute, nor necessarily reflect, official US EPA policy.*

5.05.A.T-06 Risk Assessment and Biological Effects of Pharmaceuticals Discharged Via a Wastewater Treatment Plant in the Marine Environment

Daniela Maria Pampanin¹, Jason Magnuson¹, Magne O. Sydnes¹ and Daniel Schlenk², (1)University of Stavanger, (2)University of California, Riverside

Marine sewage outfalls are major contributors of complex mixtures of emerging and legacy contaminants, which together represent important ecological challenges in aquatic environments. Most traditional wastewater treatment plants (WWTPs) are not designed to remove pharmaceuticals. To face such challenge the One Health approach has been chosen to combine the impact of discharged pharmaceuticals on human, organisms, and environmental health, by focussing on the interaction between disciplines and effects on the total environment. The case scenario of the wastewater discharge of Stavanger, the 4th largest city in Norway, is presented as an example of evaluation of the presence, effects and risk of pharmaceuticals in the marine environment. The risk related to thirty commonly used human pharmaceuticals was assessed using DREAM (Dose related Risk and Effect Assessment Model), a numerical model that is utilised to simulate continuous discharges of complex mixtures of chemicals. Chemical analyses allowed the quantification of the selected compounds in the inlet and outlet water samples, as well as environmental samples of sediment and biota. However, most compounds were below detection limits in the seawater. The highest risk was related to compounds such as: ciprofloxacin, metoprolol, amitriptyline and carbamazepine. Caffeine, a central nervous system stimulant, commonly present in drinking coffee and many energy drinks, was used as a tracer of human contamination. The possibility of transfer of pharmaceuticals from the sediment to biota was tested using amitriptyline, the most prescribed antidepressant in Norway. Amitriptyline was detected in inlet and outlet samples from the WWTP and sediment and was accumulated in polychaetes exposed to field-collected sediments, suggesting that it has the potential for trophic transfer in marine systems. In addition, sublethal biological effects were observed in the brains of Atlantic cod caged for 4 weeks at the discharge point, including estrogenic responses in males and neurodegeneration and inflammation in females. Hence, exposure to WWTP discharge in caged animals can have multiple targets within the brain that can impair multiple functions in native fish species. WWTP discharge poses a risk to the ecosystem and the organisms living in it, potentially influencing human health. The ecological assessment of the risk of compounds discharged from WWTPs needs further investigation at the ecosystem level.

5.05.B.T Pharmaceuticals in the Environment - A One Health Perspective - Part B

5.05.B.T-01 Combatting Antimicrobial Resistance in Europe: Strategies and Regulatory Actions for the Environment

Kathi Westphal-Settele, Patrick Schröder, Jens Schönfeld, Daniela Gildemeister and Silke Hickmann, German Environment Agency (UBA)

Antimicrobial resistance (AMR) is a global public health concern. Consensus exists that the challenge of AMR can only be met with the One Health approach. The widespread global occurrence of antibiotics in the environment, potentially leading to AMR, is e.g. shown by the UBA data base on pharmaceuticals in the environment (IPChem Portal (europa.eu)IPChem Portal (europa.eu)). From a regulatory perspective, the environmental dimension of AMR has received comparatively less attention in the past than the human and veterinary sectors. However, this has changed. At the European level, the challenge of AMR has led to the implementation of the One Health concept in EU policy in recent years. The Strategic Approach to PiE (Pharmaceuticals in the Environment) includes initial requirements regarding the public health impact of active pharmaceutical ingredients via the environment. The Pharmaceutical Strategy for Europe contains measures and targets aiming at addressing AMR across all sectors. Furthermore, environmental aspects were considered in the EU's Green Deal, in particular under the Zero Pollution Ambition and the Farm to Fork strategy. The new EU's veterinary legislation (Regulation (EU) 2019/6) applying since January 2022, intends to strengthen action to tackle AMR by ensuring prudent and responsible use of antimicrobials in animals. Resistance in the environment shall be addressed in the authorisation procedure of antimicrobials and assessment concepts are being developed. In April 2023, a new proposal for the EU pharmaceutical legislation on medicinal products for human use and a proposal for a Council Recommendation on antimicrobial resistance have been published. Both plan to set specific objectives and measures to strengthen Member States' actions to combat AMR according to One Health. In particular, it is proposed that the impact of emissions from the production on the development of antimicrobial resistances in the environment inside and outside Europe should be considered as part of the authorization process. We explicitly support EU strategies and welcome the promotion of research and integrated surveillance to better understand the important role of the environment as a reservoir and vector of AMR. This is critical to curbing the spread of AMR in the spirit of One Health. The presentation gives an overview on current EU regulatory actions and measures tackling AMR in human and veterinary medicine in Europe from an environmental perspective.

5.05.B.T-02 Developing a National Scale Monitoring Program for Antimicrobial Resistance in Surface Waters

Alison Franklin¹, Jay Garland¹, Patrick McDermott² and Kim Cook³, (1)U.S. Environmental Protection Agency, (2)U.S. Food and Drug Administration, (3)U.S. Department of Agriculture

Antimicrobial resistance (AMR) is a world-wide public health threat projected to lead to 10 million deaths annually by 2050. Recognition of this threat has led to action plans to combat AMR, including improved antimicrobial stewardship, development of new antimicrobials, and advanced surveillance. The National Antimicrobial Resistance Monitoring System (NARMS) (Food and Drug Administration, Centers for Disease Control, and U.S. Department of Agriculture) monitors antimicrobial resistant bacteria in food animals, retail meats, and humans. Because human and animal health are linked to environmental health, NARMS is exploring an integrated One Health surveillance model. The Environmental Protection Agency (EPA) is leading an interagency NARMS effort to develop and implement an environmental AMR monitoring program at watershed and national scales. The East Fork Watershed (SE Ohio) was chosen to evaluate selected methods for the national monitoring program since it is impacted by anthropogenic and agricultural inputs (e.g., wastewater effluent and runoff from farms) and has been a long-term EPA study area. Watershed samples were collected from 35 surface water sites (August 2022 - May 2023) with most sites sampled every 3 weeks and 4 sites

sampled weekly. Samples were analyzed for culture targets (*Escherichia coli*, extended spectrum beta-lactamase producing *E. coli* (ESBL *E. coli*), *Enterococcus*, vancomycin-resistant *Enterococcus* (VRE), and *Salmonella*) and will be analyzed for molecular targets (metagenomics and targeted ARG analysis). Counts of total *E. coli* and *Enterococcus* (CFU/100 mL) ranged from no detection to 30,000 and 3 to 7,450, respectively. About 25% of the sites had *E. coli* counts above recreational water quality criteria limits, while about 50% of the sites had *Enterococcus* counts above these limits. Percent of total *E. coli* and *Enterococcus* that were ESBL *E. coli* and VRE ranged from 0 to 25% and 0 to 5%, respectively. Quantifiable ESBL *E. coli* and VRE were detected in 34% and 23% of the samples, respectively, while *Salmonella* was detected in 37%. This data will help develop robust surface water monitoring programs with the goal of assessing risks associated with AMR pathogens in surface water, provide a fuller picture of how resistant strains are related spatially and temporally within a watershed, and help assess how anthropogenic drivers and intervention strategies impact the transmission of AMR within human, animal, and environmental systems.

5.05.B.T-03 Analysis of the Prevalence of Antibiotic Resistant (AR) Bacteria, AR Genes, and Antibiotic Residues in a Mixed-Use Watershed

Sohyun Cho¹, Lari M. Hiott¹, Quentin D. Read¹, Julian Damashek², Jason Westrich³, Martinique Edwards³, Roland F Seim⁴, Donna A. Glinsk⁴, Jacob M. Bateman McDonald⁵, Elizabeth A. Ottesen³, Erin K. Lipp³, W. Matthew Henderson⁴, Charlene R. Jackson¹ and **Jonathan G. Frye¹**, (1)U.S. Department of Agriculture, (2)Utica University, (3)University of Georgia, (4)U.S. Environmental Protection Agency, (5)University of North Georgia

The environment is a potential driver of antibiotic resistance (AR) and is an important part of the One Health approach to combat AR. To provide data on AR in the environment, a survey of antibiotic resistant bacteria (ARB), antibiotic resistance genes (ARG), and antibiotic residues was conducted in a mixed-use watershed. A culture-based approach was used to determine prevalence and diversity of ARB in surface water. Low levels of AR *Salmonella* (9.6%; 29/303) and *Escherichia coli* (6.5%; 11/170) were detected while all *Enterococcus* isolates were resistant to at least one antibiotic tested. Extended spectrum β -lactamase (ESBL)-producing Enterobacteriaceae were isolated from 17.3% of water samples and carbapenem-resistant Enterobacteriaceae (CRE) were isolated from samples at 7.7%. Six ARGs were assayed with quantitative PCR and the erythromycin-resistance gene, *ermB*, was detected most often. Twenty-six antibiotics were measured, with almost all water samples (98.7%; 149/151) having detectable levels of at least one antibiotic. Correlations between presence of ARB or ARGs and the antibiotics detected in the same sample were not significant. This could be due to sampling flowing water where interactions between bacteria and antibiotics would be transient. Analysis of wastewater samples from three wastewater treatment plants (WWTPs) within the watershed showed that WWTPs could reduce AR-associated contaminants but did not eliminate them. WWTP effluent discharges still contained high levels of ARGs and antibiotics, confirming previous reports that WWTPs are a source of these AR-associated contaminants in receiving water. However, there was no significant difference in ARGs and antibiotics between the upstream and downstream water. This combined with detection of ARB, ARGs, and antibiotics throughout the watershed suggests that there are other sources of AR contamination that warrant further investigations. The widespread detection of antibiotics, ARB, and genes associated with resistance to these antibiotics, demonstrates that surface water may be a medium for the collection of AR-associated contaminants, their mixing, and their interaction with downstream environments, as well as animals and humans who are exposed to contaminated surface water.

5.05.B.T-04 Reducing Health Risks Posed by Tetracycline-Resistant *Aeromonas hydrophila* Under Oxytetracycline Stress in a One Health Framework

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Aeromonas hydrophila has ability to spread tetracycline resistance under stresses of oxytetracycline (OTC), one of the most important antibiotics in aquaculture industry. Even though the environmental reservoir of *Aeromonas* allows it to be at the interface of all the One Health components, there is a lack of quantitative models to properly assess health risk. Therefore, the main objective of this study is to assess aquaculture species-associated infection risk and human-associated resistance gene transfer risk posed by *A. hydrophila* and to propose the control measure models on managing antibiotic resistance-related risks from the One Health perspective. To this end, dose–response models are constructed for the effects on mortality for aquaculture species and transfer of resistance genes for human. Furthermore, aquaculture species-associated *A. hydrophila* infection risk and human-associated tetracycline resistance genes transfer risk are assessed in various scenarios by integrating the fugacity, dose–response, and population dynamic models. Finally, the control measure models are developed to evaluate the effect of control strategies. Results showed that in One Health-based study regions, it is likely that farmed shrimp exposed to tetracycline-resistant *A. hydrophila* and OTC would have higher risk being infected with 95%-tile relative risk (RR) estimates of 1.25–1.34. On the other hand, tetracycline resistance genes transfer risks for human working in shrimp ponds (5.48×10^{-5} – 2.81×10^{-4}) and swimming in coastal areas (1.94×10^{-6} – 6.41×10^{-6}) during autumn exceeded acceptable risk 10^{-6} per year, indicating that health risk posed by tetracycline-resistant *A. hydrophila* via these exposure routes was alarming. To protect health of environment, farmed shrimp, and human, reductions in OTC emission rate together with concentration of *A. hydrophila* (70–99%) were recommended. In conclusion, the integrated One Health-based risk assessment framework is developed to systematically assess tetracycline resistance-related health risks to provide risk-based control strategies for addressing health risks in an aquaculture species–human–environmental system. The quantitative methodology can inform public health decision-making and antibiotic stewardship in aquaculture.

5.05.B.T-06 Profiles of Selected Antibiotic Residues and Resistomes in Urban Aquatic Systems Of Lusaka, Zambia Using High-Throughput Quantitative PCR Analysis

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The effects due to presence of cocktail antibiotic residues to environmental microorganisms is not clearly known. Risk of antibiotic resistance selection in aquatic ecosystems presents a global health issue of concern. This study aimed to determine the measured environmental concentration (MEC) of selected antibiotic residues, resistome profiles of numerous antibiotic resistance genes (ARGs), mobile genetic elements (MGEs) and other genes in urban water systems in Lusaka, Zambia. Samples collected included wastewater influent, effluent, sediments and sludge, surface water and ground water. Analysis of surface water samples for 144 genes was done using high-throughput quantitative PCR arrays. Multiresidue liquid chromatography tandem mass spectrometric method was used for analysis of antibiotic residues (trimethoprim, tetracycline, oxytetracycline ciprofloxacin, sulfamethoxazole, sulfadoxine, sulfamethoxypyrazine, erythromycin, amoxicillin, ofloxacin and rifampicin). There was 100% detection frequency for the selected antibiotics with MEC ranging from below quantification limit (LOQ) to 4415 ngL^{-1} . A total of 134 genes were detected in at least one of the samples, including 117 ARGs conferring resistance to 11 classes of antibiotics, 2 integrase MGEs and 15 other genes. Enhanced relative ARG abundance was observed in the wastewater, sludge and sediments and the river water. ARGs against vancomycin, tetracycline, β -lactams, and sulfonamides showed the highest average relative gene

abundance. In Chunga river ARGs were detected with higher relative gene abundance in downstream samples of the wastewater treatment plant compared to upstream samples. 14 ARGs were also detected in underground water sample including 5 multidrug resistant (MDR) genes and 3 β -lactams. This highlighted potential risk posed by the consumption of untreated water from these sources. Other notable ARGs detected included *vanA*, *vanB_1*, *vanC_2*, *vanC1*, *vanXB*, *intI*, *sulI*, *tetX* and *dfrA1*. This study provides the first quantitative assessment of aquatic environmental resistomes in Lusaka Zambia, underscoring the ubiquitous pollution by antibiotic residues and resistomes in urban waters systems. Efforts to improve sanitation in the peri-urban areas of Lusaka were noted with increased activity on decentralized sanitation systems that emphasize on use of dry toilets, source separation and treatment principles.

5.05.P-Tu Pharmaceuticals in the Environment - A One Health Perspective - Part A

5.05.P-Tu-192 Activities of the Federal Interagency Workgroup on Pharmaceuticals in Water

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In 2021, a four-Party Memorandum of Understanding (MOU) among the US Environmental Protection Agency Office of Water, the US Department of Agriculture (USDA), Agricultural Research Service, the US Department of Health and Human Services Food and Drug Administration, and the US Department of the Interior US Geological Survey, was signed with the goal of improving and sustaining federal coordination and collaboration on ecological and human health issues related to pharmaceuticals in drinking water. This MOU supports a Pharmaceuticals in Water (PIW) Workgroup consisting of the four signatory agencies of the MOU as well as the Centers for Disease Control and Prevention, the National Institutes of Environmental Health Sciences National Toxicology Program, the National Oceanic and Atmospheric Administration, the Army Office of the Surgeon General, and the Defense Centers for Public Health-Aberdeen. The PIW facilitates a forum for the exchange of information, coordination of joint studies, and interagency consultation on research findings and implications of detecting pharmaceuticals in the water resources and in drinking water. The Workgroup has created an internal database describing known federal research activities related to pharmaceuticals in water. Potential data gaps in research needs were identified and group based on the Boxall et al. (2012) "Big Questions" article. Ongoing activities to address data gaps include developing human health benchmarks for pharmaceuticals as an approach to prioritize potential human health risks of pharmaceuticals in water; developing water quality benchmarks for veterinary pharmaceuticals, prioritizing pharmaceutical classes for environmental assessment under National Environmental Policy Act regulations; studying the effects of pharmaceutical mixtures; and monitoring pharmaceuticals and other contaminants of emerging concern in water. The workgroup has resulted in a better understanding of federal research activities related to pharmaceuticals in water across agencies and provided a forum to solicit feedback on these activities.

Disclaimer: The views expressed in this abstract are those of the authors and should not be construed to represent the views or policies of the US EPA, US FDA, DOD, USDA, NIH, or OTSG.

5.05.P-Tu-193 Reexamining US FDA's Environmental Screening Levels for Human Pharmaceuticals: Trends and Emerging Issues

Xiaoqin Wu and *James Laurenson*, U.S. Food and Drug Administration

Under the National Environmental Policy Act of 1969 (NEPA), the Food and Drug Administration (FDA) is required, as a part of its regulatory process, to consider the environmental impacts of its actions, such as #SETACLouisville

approving drug applications. Thus, all drug applications must be accompanied by either an environmental assessment (EA) or a claim of categorical exclusion from an EA. Per FDA's regulation 21 CFR 25.31(b) and the EA Guidance (1998), one of the categorical exclusions is when the expected introduction concentration (EIC), i.e., the estimated concentration of the substance at the point of entry into the aquatic environment, is below the screening level of 1 part per billion (< 1 ppb, or $\mu\text{g/L}$). Since the promulgation of this categorical exclusion, there have been numerous changes in drug development, production, and use, as well as significant advances in the scientific understanding of pharmaceutical exposure issues in aquatic environments, which bring a need for FDA to review and update, if needed, the categorical exclusion screening level of 1 ppb to ensure the threshold to be ecologically relevant. In response in part to a 2013 citizen petition (FDA-2010-P-0377), FDA published a guidance in 2016 for drugs with estrogenic, androgenic, or thyroid activity, and is now considering the appropriateness of a lower screening level for these and possibly other classes of drugs during a possible broader future update to the 21 CFR 25 regulations. FDA currently is analyzing data collected for EAs, supported categorical exclusions, data on national pharmaceutical sales, and other environmental monitoring and ecotoxicity data. We have identified several emerging issues that FDA environmental scientists are interested in for this effort, including concerns over hormones and hormone antagonists, antimicrobials that could contribute to antimicrobial resistance, cumulative risks, short-term usage of drugs such as antivirals during pandemics, and agricultural application of treated wastewater and biosolids. Future efforts also are needed in developing comprehensive exposure and toxicity models for predicting the impact of FDA-approved human pharmaceuticals in wastewater, biosolids, and surface water.

5.05.P-Tu-194 Limitations of Using Quantitative Structure-Activity Relationship (QSAR) Models to Predict Organic Carbon Water Partition Coefficient (Koc) for Complex Active Pharmaceutical Ingredients (APIs)

Wasfia Hoque, Jeff Rominger and Tim Verslycke, Gradient

Active pharmaceutical ingredients (APIs) can enter the environment during production, use, and disposal of pharmaceutical products, resulting in their presence in a variety of environmental media, including biosolids, wastewater, sediment, soil, groundwater, and surface water. One important environmental fate process for APIs is the partitioning between solid (soil/sludge/sediment) and aqueous (groundwater, surface water) phases. One of the most prevalent methods to study this partitioning process is by measuring the organic carbon water partition coefficient (Koc). In the absence of empirical Koc data, quantitative structure-activity relationship (QSAR) models have been used to predict the Koc of APIs. While QSARs are relatively reliable for small/mono-constituent substances, their performance can be less reliable for complex chemicals with large molecular weights, ionizable charges, and varying functional groups. Given the growing shift in drug development away from small molecules to the development of biologics and new chemical modalities (e.g., small interfering RNA (siRNA) drugs, antisense oligonucleotides, antibody drug conjugates, pegylated peptides), it is important to understand the reliability of QSAR models for key environmental fate parameters, such as the Koc. This study examines the performance of different QSAR models for predicting the Koc of multiple APIs with complex structures. We applied QSARs based on structurally derived parameters, such as molecular connectivity indices (MCIs), as well as models based on correlations with physical and chemical properties, such as n-octanol/water partition coefficient (Kow). Understanding the limitations of QSARs can help identify the need for more QSAR development or empirical data to improve environmental exposure and risk assessments of complex APIs.

5.05.P-Tu-195 MSC OK? – Minimum Selective Concentrations (MSCs) for the Assessment of AMR in the Environment

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This project aims to close the methodological gap in assessing the risk of antimicrobial resistance (AMR) spreading in the environment within the scope of the authorisation of medicinal products. Currently, various approaches are being discussed for the computational derivation of minimum selective concentration (MSC) values as a basis for assessment. In these approaches, MSC values are calculated from publicly available MIC (Minimum Inhibitory Concentration) values. However, to date, it has not been experimentally determined which of the approaches is most realistic. Therefore, the goal of this project is to verify the approaches and then determine the most appropriate computational approach for risk assessment by comparing calculated and experimentally generated MSC values. To this end, MSC values will be experimentally determined for thirty antibiotics in combination with five environmental bacterial species. Preliminary data on these comparisons are presented. Isogenic strains differing exclusively in their fluorescence profile (encoding mScarlet or YFP) were designed e.g., from an environmental *Sphingomonas* sp. isolate. For this strain, the MIC for more than twenty antibiotics was first determined using a broth microdilution assay. Subsequently, the mScarlet-encoding strain was allowed to acquire naturally occurring resistance genes for each of the antibiotics through horizontal gene transfer in liquid mating assays with environmental microbial communities from soils or wastewater in- and effluents. Then, the resulting resistant strain was grown in competition with the susceptible isogenic strain encoding YFP across a gradient of the corresponding antibiotic. Growth rates of the resistant strain relative to its susceptible counterpart were determined by recording both fluorescence signals. The MSC corresponds to the tested antibiotic concentration at which the growth rates of the two strains are identical. The experimentally and computationally determined MSC values are then compared for each antibiotic to determine if existing computational approaches could correctly predict MSC values for environmentally acquired resistances. Finally, the most appropriate computational approach should serve as the basis for developing methodological guidance on how to assess the risk of AMR development and spread in the environment within the authorisation procedure of medicinal products.

5.05.P-Tu-196 Using Fish Toxicokinetics and Mammalian Toxicity Data to Evaluate the Risk for Fish Reproductive Toxicity by a Pharmaceutical

Michael Lee and Alison Nimrod Perkins, Lilly

Pirtobrutinib is a highly selective, non-covalent (reversible) BTKi approved in January 2023 by the FDA for relapsed/refractory mantle cell lymphoma. Standard studies (physical chemical, fate, aquatic and sediment ecotoxicity) were used to conduct an environmental risk assessment (ERA). The dataset included a fish early life-stage toxicity test with fathead minnow (OECD 210). The dataset also included a non-standard fish toxicokinetic study that quantified pirtobrutinib concentrations in plasma and tissue of adult fathead minnows exposed via water. Although the standard ERA concluded that there is no risk for aquatic or sediment species (e.g., PEC/PNEC < 1), teratogenicity is a known class effect for BTK inhibitors. A read-across approach using the fish toxicokinetic and mammalian toxicity data was used to evaluate the potential for teratogenic or reproductive toxicity in fish. At the highest concentration tested in the OECD 210 study, also the no-observed effect concentration, fish embryos were exposed directly to concentrations up to one million times greater than the PEC_{surfacewater}. The expected pirtobrutinib plasma concentrations in fish at the PEC_{surfacewater} are at least 400,000 times lower than plasma concentrations in maternal rats at the no-observed effect dose level in the embryo-fetal development study. The measured concentrations of pirtobrutinib in fish tissues are not

sufficiently high enough to support a maternal-embryo transfer of sufficient magnitude to result in embryo toxicity at the $PEC_{\text{surfacewater}}$. These considerations are the basis for the conclusion that pirtobrutinib is not expected to result in teratogenicity or reproductive toxicity in fish at the $PEC_{\text{surfacewater}}$.

5.05.P-Tu-197 Use Pharmaceutical PNECs with Caution

Neil Parke, Alison Nimrod Perkins and Michael Lee, Lilly

In recent years, substantial effort has been made to measure concentrations of pharmaceuticals in the aquatic environment. By comparing measured environmental concentrations (MECs) to predicted no effect concentrations (PNECs), locales at risk from pharmaceutical micropollutants can be identified. However, not all PNECs are created equal. There are several published or presented assessments where poorly-derived PNECs were used to identify risks from MECs. PNECs considered poorly-derived include those based on predicted toxicity when empirical data is available or those based on unreliable ecotoxicity data. Examples are presented and discussed, including some reported PNECs that are orders of magnitude different from PNECs based on high quality data. Researchers should critically evaluate PNECs before using them to determine whether an MEC is safe or a concern. Elements of that critical evaluation are discussed. PNECs for pharmaceuticals should be derived using standard methodology (e.g., EPA Water Quality Standards Methodology or EU Water Framework Directive) using the best available, empirical, most recent, and preferably chronic ecotoxicity data. It is recognized that there is currently not a comprehensive database with ecotoxicity data on pharmaceuticals and not all pharmaceuticals have ecotoxicity data. Available sources for data to develop PNECs will be presented. Finally, when PNECs are used to identify risks, their origin and basis should be transparent. Only high quality PNECs should be used to make decisions by risk assessors and policy makers.

5.05.P-Tu-223 Environmental Safety Assessment of Data-Poor Pharmaceuticals Using Read-Across

Ifeoluwa Bamgbose, Janet Vo and Tim Verslycke, Gradient

Environmental safety assessments are required for market approval of new active pharmaceutical ingredients (APIs) in the US and the EU. However, ecotoxicological information to support such assessments is lacking for many drugs, particularly for drugs that were approved prior to the implementation of environmental assessment regulations and for new drugs that qualify for a categorical exclusion (e.g., expected introduction concentration below US FDA's screening level of 1 $\mu\text{g/L}$). To evaluate environmental risks of drugs lacking ecotoxicity data typically requires the use of quantitative structure-activity modeling (QSAR) or read-across approaches. In 2012, we proposed a read-across approach based on drug therapeutic class (Verslycke et al., 2012). Specifically, existing aquatic toxicity data from 184 drugs belonging to 14 therapeutic classes were used to derive aquatic toxicity thresholds (i.e., predicted no-effect concentrations [PNECs]) for each therapeutic class. Since 2012, a substantial amount of aquatic toxicity data has been generated as part of new drug approvals and this data was used to re-evaluate our prior approach. We compiled aquatic toxicity data and PNECs for 306 APIs across the 14 therapeutic classes that are reported into the Swedish National Formulary (FASS.se) database. These data were used to derive PNEC ranges for each therapeutic class and to identify the most sensitive trophic level (algae, invertebrates, or fish) within each class. With a few exceptions (e.g., estrogen actives, iron chelators, anti-infectives), the proposed PNEC ranges provide an appropriate basis for using read-across within each therapeutic class or subclass. Using a case study, we will illustrate how the proposed approach has been successfully applied to support a categorical exclusion request for a new drug in the US.

5.06.P-Tu Surrogacy in Endangered Species Pesticide Risk Assessment: Strategies for Testing and Conceptual Applications

5.06.P-Tu-198 The Importance of Accurate Field Metabolic Rate Estimates in the Endangered Species Assessment Process

Colleen Priest^{1,2} and *Dwayne R.J. Moore*², (1)*Corteva Agriscience*, (2)*Intrinsic Ltd.*

Field metabolic rate (FMR) is the amount of energy expended by an animal while performing daily activities (e.g., foraging, hunting, nesting, mating, sleeping) under natural conditions. Knowledge of daily energy expenditure is required to estimate dietary consumption of a pesticide by endangered and threatened (listed) species. The United States Environmental Protection Agency (EPA) uses the Terrestrial Residue Exposure (T-REX) model to estimate pesticide exposure to both listed and non-listed species. Food intake rates for birds and mammals are estimated in T-REX with allometric equations compiled over two decades ago from data in the literature. However, FMR has been measured for numerous species since that time and the current data include a wider range of habitats and diets. Therefore, more species-specific data are available and may be more applicable to the unique species listed as endangered or threatened in the United States. An updated literature search was conducted to compile available FMR data for birds, mammals, and reptiles. Preference was given to data collected from free-ranging (i.e., not captive), seasonally active, adults. Allometric relationships were determined using power regression analyses. Data were divided by species type (bird, mammal, reptile), taxonomic order, habitat (e.g., desert, marine, mountain), and dietary preference. Overall, taxonomic order was the best predictor of FMR, except for species found in extreme environments where lower FMRs are common. This relationship was particularly evident for desert bird species, which had significantly lower FMRs than bird species from other habitat types. In practical applications, knowledge of the habitat and dietary preferences of listed species may help to further refine predicted dietary requirements and associated exposure estimates, thereby providing more accurate risk conclusions.

5.06.P-Tu-199 Establishing Protective Neonicotinoid Sediment Toxicity Thresholds For Aquatic Insects Through a Combination of Field and Laboratory Studies

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Neonicotinoid application as agricultural pesticides has increased rapidly since the early 2000s when treated seeds and foliar sprays for major crops became commercially available. However, in recent years, widespread and prophylactic neonicotinoid use as seed treatments has caused controversy due to lethal and sublethal effects on non-target species. High leachability and water solubility contribute to the mobility of neonicotinoids from agroecosystems into non-target aquatic ecosystems. Aquatic invertebrates that inhabit freshwater systems and provide important ecosystem services may be exposed to neonicotinoids through water run-off or accumulation in sediments over multiple years of seed application. Lethal and sublethal effects such as decreased growth and delayed time to emergence have been observed in laboratory aquatic insect bioassays. Additionally, changes in aquatic invertebrate community structures and abundance associated with neonicotinoid concentrations have been reported in both field and mesocosm studies. Recent research reported neonicotinoid concentrations in Missouri wetland sediments were an order of magnitude greater than aqueous concentrations observed in the same wetlands. Although neonicotinoid benchmark concentrations for aquatic insects have been determined in water, sediment toxicity thresholds have not yet been established and thus, the implications of contaminated sediment for aquatic insects in freshwater systems are largely unknown. To address this knowledge gap, we will present data detailing the persistence and bioavailability of neonicotinoid-associated sediments using Missouri wetland sediments of differing organic carbon content. Next, to quantify conservative toxicity thresholds for sediment, we will conduct chronic sediment toxicity tests using the established model species, *Chironomus dilutus* as well as the more sensitive mayfly species, *Neocloeon triangulifer*. In addition, we are collecting

aquatic invertebrate samples from Missouri streams and wetlands that have invertebrate community data collected prior to the widespread use of neonicotinoids to evaluate changes in not only invertebrate community structure but presence/absence of at-risk species over time. Combined results from our field and laboratory studies will help establish sediment toxicity thresholds that are protective of sensitive and threatened aquatic invertebrate species.

5.06.P-Tu-200 Hazards of Anticoagulant Rodenticides to Early Life Stages of Pacific Salmon

Melissa Driessnack¹, Lillian Pavord¹ and Jenifer McIntyre², (1)Washington State University Puyallup, (2)Washington State University

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 AR-containing pellets to support the conservation of native species. In the Aleutian Islands, invasive rats are being targeted due to their negative impacts on nesting seabird populations. Prior to aerial dispersion, surveys are conducted to evaluate the potential for non-target organism mortalities, including impacts on aquatic organisms. In addition to seabirds, the Aleutian Islands are also home to Pacific salmon, including coho (*Oncorhynchus tshawytscha*) and pink (*O. gorbuscha*). The standard timing for rat eradication efforts, late summer to early fall, is of importance on this island, as it overlaps with the spawning and developmental timeline for coho salmon embryos and alevin. Coho salmon spawn in early fall in gravel redds, where the embryos then develop over the winter. The hatched alevin then spend approximately one month in the gravel before emerging to feed as fry and reside in streams for one year before migrating to the ocean. Eradication efforts targeted for late summer mean that pellets entering streams will be present while salmon embryos are developing, and possibly into the alevin and fry stages. However, few studies exist that have assessed the impacts of AR in aquatic habitats. To begin estimating effects for early life stages, we raised coho embryos from fertilization through hatching in a flow-through system in the presence of an environmentally relevant exposure to AR-containing bait pellets. Treatments consisted of a control (no pellets), control pellets (no AR added), pellets containing brodifacoum (BROD), and pellets containing diphacinone (DIPH). Six replicates (n = 6) were run for all treatments, with all pellet-containing treatments receiving two pellets per replicate. Additionally, pellets were replaced after 28 days to simulate a second pellet drop that occurs during eradication efforts. BROD had the most noticeable effect on embryo survival and development, including a strong tendency towards pronounced cranial hemorrhage. Effects of DIPH exposure were less pronounced but still significant relative to controls. The results of this work will be discussed in the context it will be used to inform rat eradication efforts on the Aleutian Islands.

5.06.P-Tu-201 Are There Opportunities to Reduce In Vivo Avian Toxicity Tests Using Species Surrogacy? A Case Study Using Multiple Lines of Evidence for Cross-Species Extrapolations

Audrey Bone and Katherine K. Coady, Bayer AG - Crop Science Division

Toxicity testing in avian species is required for registering pesticide products in various jurisdictions across the globe. Required avian tests include an acute oral test (OECD TG 223; OCSPP 850.2100) with two species, a sub-chronic dietary test (OECD TG 205; OCSPP 850-2200) with two species, and a chronic reproduction test (OECD 206; OCSPP 850.2300) also with two species. Collectively, an avian toxicity data package for a pesticide active ingredient can use > 5,000 birds. The data collected from these studies are used to classify pesticide active ingredients into hazard categories and to perform ecological risk assessments with the aim to define acceptable use patterns for pesticide products that are protective of avian wildlife, including endangered species. One way to approach reducing animal use in ecotoxicity testing is to consider the value that data from other species or taxa may hold for informing the overall risk assessment. Toxicity testing to support human health risk assessment is a rich source of terrestrial vertebrate data which may be applicable to an avian risk assessment in certain use cases. In this case study we investigate the utility of different lines of evidence to refine, reduce, or replace avian toxicity studies via cross-species extrapolation including toxicokinetic tools,

molecular homology, interspecies correlations using existing toxicity data, and ecological/life-history trait-based comparisons. As a case study, we apply these concepts to one class of pesticides to illustrate the potential utility of this type of approach. Using multiple lines of evidence for cross-species extrapolations can improve the understanding of how species surrogacy can improve hazard and risk approaches for the protection of avian wildlife while minimizing animal use.

5.06.T Surrogacy in Endangered Species Pesticide Risk Assessment: Strategies for Testing and Conceptual Applications

5.06.T-01 Addressing Surrogacy Challenges using Bioinformatics with Special Focus on Endangered Species

Carlie LaLone, U.S. Environmental Protection Agency

The process used for the evaluation of whether a chemical may affect an endangered species involves a number of factors including understanding the extent to which the chemical use may overlap with the species range, the magnitude of direct and indirect effects, and the overall vulnerability of the species. The evaluation aims to determine whether these factors represent a likelihood of jeopardy to the species' population. Through this process, toxicity results from model organisms are used as surrogates to predict impacts to untested species and taxa. There is an ongoing challenge in understanding whether the surrogate species are predictive for untested species, such as listed endangered species, or if they have marked differences in their biology. Bioinformatics approaches, such as the US Environmental Protection Agency's Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) tool, are poised to address this challenge, particularly as it pertains to threatened and endangered species. The SeqAPASS tool compares protein sequence and structural information based on a known chemical-protein interaction in a species or test system (model organism) and predicts chemical susceptibility based on conservation of that protein target in other species. This method for species extrapolation can be rapidly applied to understand whether there are lines of evidence to support extrapolation from surrogate species to endangered species. Case studies have been created to demonstrate this application of the tool in the context of pesticides, demonstrating its utility in providing scientific evidence for contextualizing the utility of model organisms as surrogates to represent untested listed species. This abstract neither constitutes nor necessarily reflects USEPA policy.

5.06.T-02 Methodologies to Identify Surrogate Species Representative of Threatened and Endangered Species

Tamara Dawn Lunsman, Bridget O'Neill, Kristine LeRoy and John Marton, Corteva Agriscience

Understanding crop protection product toxicity to species that are listed as threatened and/or endangered (T&E) under the US Endangered Species Act is paramount for the United States Environmental Protection Agency (US EPA) to conduct an accurate pesticide Effects Determination. Characterizing the way crop protection products interact and affect T&E species' survival, growth, reproduction, habitat needs, food abundance, and life cycle are important aspects of the assessment. Because toxicity data for listed species cannot be generated, this presentation explores a methodology to phylogenetically identify the most appropriate species to use as surrogates. A case study using a representative insecticide will compare using data from traditional laboratory species versus a more closely related surrogate in a risk assessment. We will also discuss future testing plans to address points of concern where higher tiered testing could provide a more accurate assessment.

5.06.T-03 Endangered Species Evaluation Methods for a dsRNA Pesticide Product

Scott Teed¹, Michael F Winchell² and Miriam Frugis³, (1)Intrinsic Corp., Canada, (2)Stone Environmental, Inc., (3)Greenlight BioSciences Inc.

A new pesticide product for use on United States potatoes is being introduced. Ledprona Technical is a proposed active ingredient containing 1.4% ledprona. Ledprona consists of double-stranded ribonucleic acid

(dsRNA) that induces mortality of the Colorado Potato Beetle (*Leptinotarsa decemlineata*) (CPB) via RNA interference (RNAi). It is expected to be the first foliar-applied dsRNA active ingredient registered in the US. The EPA approved the Experimental Use Permit (EUP) for field testing in 10 States in May 2023. The CPB is considered one of the major pests in potatoes due to its ability to rapidly defoliate. Heavy pesticide use has resulted in CPB insecticide resistance. Ledprona was designed to have activity specific to the CPB. So how does this product fit into the EPA Endangered Species risk assessment paradigm? First, we must characterize Ledprona and RNAi technology: not chemically modified or encapsulated to increase its stability or uptake; applied at low application rates (0.0088 lb ai/A); applied only to post-emergent crops; formulation unstable in the environment (DT50 <3 days in soils, sediment, and water); vertebrates and insect orders have chemical and biological barriers that degrade the dsRNA molecules. In an EPA biological evaluation (BE) listed species range and critical habitat (CH) spatial data are overlaid with the action area to determine potential for exposure. Given the action area and a >1% overlap criteria, we found that 15 listed species overlapped the action area. Much of the remaining ESA analyses rely on determining risk (direct and indirect effects) and vulnerability. As a dsRNA product, risks associated with exposure are limited. Conducting the standard suite of ecotoxicity testing (e.g., OCSPP 850) is unlikely to result in anything but unbounded effect metrics given product specificity. Bioinformatics information is useful to predict the theoretical potential for gene silencing in susceptible organisms to environmental dsRNA. This along with a phylogenetic relatedness analysis of surrogate organisms provides information on whether non-target organisms like another coleopterans (e.g., Lady beetle) could be impacted. The development of dsRNA pesticide products appears promising. Particularly when addressing pests with resistance to chemical pesticides, that rapidly disseminate pesticides within colonies (e.g., bee hives), and where important ecosystem services are being delivered by non-target organisms.

5.06.T-04 A Higher-Tiered Risk Assessment Solution for the “Threatened” American Burying Beetle (*Nicrophorus americanus*) Using A Novel Dietary Life Cycle Toxicity Test in the Darkling Beetle (*Tenebrio molitor*)

Vincent J. Kramer and Patrick Havens, Corteva Agriscience

In a screening-level ecological risk assessment for US threatened and endangered (listed) species, terrestrial invertebrates are evaluated for potential risk using the US EPA BeeREX exposure and risk model, with honeybee effects endpoints and residue exposure estimates serving as a surrogate for all terrestrial invertebrates including beetles such as the American Burying Beetle (*Nicrophorus americanus*). The effects endpoints are derived from laboratory studies on honeybee adult and larval life stages. For the exposure estimation, honeybee daily dose is calculated based on dietary consumption rates for honeybees and estimates of maximum residue concentrations in nectar and pollen consumed by the bees. To refine the risk assessment for the Corteva Agriscience product Enlist™ One herbicide containing 2,4-D choline, two approaches were engaged: 1) Refinement of the exposure assessment utilizing measured 2,4-D residue decline in plant matrices and pharmacokinetic dissipation data in birds and mammals, and 2) refinement of the toxicity assessment with a lifecycle dietary toxicity study in the Darkling Beetle (*Tenebrio molitor*). The refined risk assessment demonstrates acceptable risk below the Agency’s level of concern and supports a No Effect determination for Enlist One herbicide. This work demonstrates how more species-appropriate effects and exposure information can give a more realistic estimates of risk to listed species.

5.06.T-05 Surveys of Agricultural Field-Edge Milkweed Habitats to Refine the Insect-Control Maize Risk Assessment for Monarch Butterfly

Joshua Fischer¹, Joshua Pritsolas², Randall Pearson² and Christopher Brown¹, (1)Bayer Crop Science, (2)Southern Illinois University Edwardsville

Due to declines in monarch butterfly (*Danaus plexippus* L) populations and their potential sensitivity to certain families of Bt-derived proteins expressed in insect control maize products, the monarch risk assessment has become increasingly important for the registration of plant-incorporated-protectants. Exposure to maize-

expressed insect control proteins occurs via pollen deposition on milkweed plants within the genera Apocynaceae; the primary food source for monarch larvae. Previous risk assessments for first-generation *Bt* corn products conducted over twenty years ago estimated that approximately 56% of monarch butterflies in Iowa originated from within maize fields. However, with modern weed control practices, milkweed populations are negligible within maize fields and potential exposure of monarch larvae to maize pollen is, therefore, limited to the field edge. To refine the current exposure model, it is necessary to determine the distribution of the milkweed at the edges of maize fields and how these field-edge milkweed populations contribute to overall habitat availability. In this study, roadside habitats were surveyed throughout the midwestern United States to determine the prevalence and distribution of milkweed stems adjacent to agricultural fields. Milkweed stems were found throughout midwestern states (IA, IL, IN, MN, MO, ND, SD and WI) with the smallest proportion typically present within 1m of the field edge. Furthermore, the cumulative distributions of field-edge milkweed stems were similar across states and years. Landscape-level modeling was used to estimate the contribution of field edges to the overall potential available habitat in agricultural and non-agricultural areas. These data will directly contribute to the refinement of off-field exposure models used in risk assessments for monarch butterfly.

5.06.T-06 Endangered Species Pesticide Risk Assessment: Opportunities and Implications for Surrogacy

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Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the registration of a pesticide requires compliance with the Endangered Species Act (ESA). To comply with ESA, EPA must evaluate the potential direct and indirect effects of a pesticide on over 1,600 ESA-listed species and over 700 designated critical habitats in a Biological Evaluation (BE). One of the first challenges that emerges in the BE is bridging taxonomic groupings in ecological risk assessments with those of ESA-listed species. For example, some of the unique ESA-listed taxa under the jurisdiction of the U.S. Fish and Wildlife Service that do not directly align with ecotoxicology standard taxa include ferns and allies, cycads and conifers, lichens, arachnids, terrestrial molluscs, and terrestrial crustaceans. The crosswalk to ecotox categories is further complicated by the ESA-listed species “terrestrial”, “aquatic”, or “both” lifeforms. Aside from amphibians and reptiles, species identified as “both” terrestrial and aquatic do not directly align with EPA’s separate terrestrial and aquatic assessments. Assumptions of ecotoxicity surrogates for species considered “both” may require a conservative approach, defaulting to the most sensitive taxa, however this can create inconsistencies across assessments. These assumptions and uncertainties are further complicated in the indirect effects assessment where a species prey, pollination, habitat, or dispersal agents must each be paired to a surrogate taxon used for risk assessments, often with limited data on the ESA-listed species biology to inform the pairing. There are certain taxonomic groups encompassing unique ESA-listed species diversity that consistently overlap with pesticide use sites which provide opportunities for refined surrogacy data. Conversely, other taxonomic groups of ESA-listed species are well represented by the current EPA surrogates. Here we will discuss these opportunities in the context of a high throughput screening approach using the AMMPS tool for a suite of insecticides, herbicides, and fungicides.

5.07.A.T Water Quality Criteria: Modeling Aquatic, Sediment, and Soil Toxicity Based on Mechanistic Chemical Interactions. Session Honoring Dr. Dominic Di Toro

5.07.A.T-01 Equilibrium Partitioning and its Application to Non-Ionic Organic Contaminants in Sediments

David R. Mount, U.S. Environmental Protection Agency

The development of Equilibrium Partitioning (EqP) as a framework for predicting the bioavailability and toxicity of sediment contaminants to benthic organisms has proven to be a foundational event in ecological risk

assessments for contaminated sediments. Given the geochemical and ecological complexities of the sediment environment, the daring simplicity of the approach almost guaranteed resistance from many when introduced. In the context of assessing non-ionic organic contaminants in sediments, two of the boldest assumptions were that 1) all organic carbon can be modeled as a homogenous pool with a single partition coefficient, and 2) at equilibrium, the path of chemical uptake (food vs respiratory or contact exposure) is immaterial to the estimated dose/effect. Though both of these can be shown to be untrue to an extent, the degree to which they are untrue appears small compared to the reduction in variability these simplifying assumptions can accomplish. One of the more important attributes of EqP is that its assumptions do not have to be accepted on faith, they can be evaluated directly by experimentation; partition coefficients can be measured, and toxicity resulting from a given exposure can be directly compared to that predicted from chemical activity in porewater and the results of water-only toxicity tests. Such experiments have identified important adjustments to the base EqP formulation that are needed to address certain contaminants or circumstances, such as the widely varying partition coefficients observed for PAHs among types of organic carbon. Through the years, the concepts underpinning EqP have survived and evolved in a number of ways, including passive sampling-based assessments and development of models with more than one partitioning compartment. *This abstract neither constitutes nor necessarily reflects US EPA policy.*

5.07.A.T-02 Equilibrium Partitioning (EqP) In Review: Metals - A Tribute to Dominic Di Toro

William J. Adams, Red Cap Consulting

The concept of equilibrium partitioning between sediment bulk chemical concentrations and the concentration of the chemical in the sediment porewater has been used to assess the potential for toxicity to benthic organisms. It is known that dry weight measurements of chemical substances do not correlate with toxicity. It has also been shown that the toxicity of chemical substances in sediments can frequently be related to the uncomplexed chemical concentration in the sediment porewater. The concept has been applied equally to organic and inorganic substances. The approach began with a demonstration that the toxicity of kepone to benthic organisms could be attributed to the concentration of kepone in the porewater. The supporting evidence was presented at a Pellston SETAC Workshop in 1983. This was soon followed with a publication by Di Toro et al (1991) entitled "Technical basis for the equilibrium partitioning method for establishing sediment quality criteria" and a report to EPA Science Advisory Board entitled "Equilibrium Partitioning Approach To Predicting Metal Bioavailability In Sediments And The Derivation Of Sediment Quality Criteria For Metals (1994). The concept of metal partitioning from sediments to porewater is now recognized and used worldwide in assessing possible toxicity from metal concentrations in sediments. Recently the approach has been extended to the use of the biotic ligand model to assess toxicity potential of metals in porewaters. A review of the application of EqP to metals is presented.

5.07.A.T-03 Lessons from an Electrical Engineer in Advancing Models to Support Water Quality Management decisions

Thomas Parkerton, EnviSci Consulting LLC

The development of practical, quantitative models has served as a critical aspect of water quality management decision-making. This presentation will provide an overview of Dr. Dominic DiToro's significant, early contributions in the field of environmental toxicology and chemistry that has advanced pragmatic models for assessing and improving water quality. Common themes that characterize this seminal work include: 1). identifying and drawing critical insights from key existing lab and field datasets; 2). integrating these learnings into a quantitative model framework; 3). invoking bold, simplifying assumptions; 4). testing these simplifying assumptions using existing and new data from appropriately designed studies; 5). leveraging appropriate statistical methods; and 6). honoring others for their valuable contributions. These themes will be illustrated using models for evaluating the role of nutrients in eutrophication, complex mixture toxicity from whole effluents and organic contaminants in sediments.

5.07.A.T-04 The Role of Physiological Data in the Development of the Biotic Ligand Model and its Successors

Christopher M. Wood, University of British Columbia

The Biotic Ligand Model (BLM) was the brain-child (one of many!) of Dom DiToro and his collaborators. The BLM is a computational tool for predicting metal toxicity to aquatic organisms on a site-specific basis as a function of local water chemistry. In various forms, the BLM and its successors such as simplified bioavailability models, look-up tables, and Multiple Linear Regression Models (MLRs) are an unparalleled success story. These approaches are now being used for risk assessment, guideline derivation, and regulatory decision-making in jurisdictions around the world. The BLM originated from a unique collaboration between physiologists, geochemists, and toxicologists. Physiological data provided the glue linking geochemical models to toxicological findings, as well as the mechanistic evidence that persuaded regulatory authorities that the concepts in the model were correct, thereby promoting its adoption. In modern versions of the BLM, the physiological component has been all but eliminated, yet the mechanistic underpinning based on pathophysiology endures. I will review, in a historical context, the key physiological findings with respect to metal toxicity that fueled the development of the modern BLM (NSERC).

5.07.A.T-05 The Role of Environmental Chemistry in Models of Aquatic, Sediment, and Soil Toxicity

Dominic M. Di Toro, University of Delaware

The explicit inclusion of a specific consideration of the environmental chemistry in models of toxicity from exposure in natural aquatic, sediment and soil matrices is an important feature of modern environmental toxicity models. This is a requirement since the chemical state and speciation greatly affect the resulting toxicity. The challenge is to include a sufficient but not overwhelming amount of detail. Examples will be discussed such as the use of thermodynamic quantities, e.g., chemical potential and fugacity, and metal speciation models using specific ligands as well as dissolved and particulate organic matter. For complex mixtures in sediments and soils choices need to be made to include only the dominant chemical controls and using extractions that are specifically designed for the model being used. For organic chemicals partitioning models have been shown to be necessary, for example ppLFRs, models using Abraham parameters. Finally, quantum chemistry calculations, which are not “real” chemistry but are useful if it is applied sensibly.

5.07.A.T-06 Reflections on Developing Useful Models of Aquatic, Sediment, and Soil Toxicity

Dominic M. Di Toro, University of Delaware

This presentation will be reflections on the choices that need to be made for the development of useful models to predict the toxicity of compounds. Useful implies that the models can be applied and have a sound scientific basis. Aphorisms, e.g. “The Perfect is the Enemy of the Good” and “All Models are Wrong, Some Models are Useful” capture some of the ideas. What specifically do they mean? Other important discussion points will be included: how to work with colleagues that know the science and regulatory situation, and how to choose between including everything in the model, versus just enough. Perhaps the most difficult choices involve simplifying and in some cases, what might be thought of as outrageous assumptions. Examples of both successful and not very successful choices will be discussed. For example, organisms respond to the thermodynamic chemical potential of the system, chemical equilibrium models can be used as models of organism response, and quantum yields are the same for all photoactive PAHs.

5.07.B.T Water Quality Criteria: Modeling Aquatic, Sediment, and Soil Toxicity Based on Mechanistic Chemical Interactions. Session honoring Dr. Dominic Di Toro

5.07.B.T-01 Application of a Mixture-based Biotic Ligand Model for Development of Sediment Remedial Goals for Metals

Robert Gensemer¹, Bjorn Bjorkman¹, Robert Santore², Aaron Redman³ and Steven S. Brown⁴, (1)GEI Consultants, Inc., (2)Windward Environmental LLC, (3)ExxonMobil Biomedical Sciences, Inc., (4)Anchor QEA

This study explores the application of a metals mixture-based Biotic Ligand Model (mBLM) to the development of bulk sediment remedial goals for each individual metal. The mBLM has been previously shown to be highly predictive of toxicity to the freshwater benthic amphipod, *Hyalella azteca*, when toxicity predictions are based on measurements of dissolved copper, cadmium, lead, nickel and zinc in sediment porewater from passive sampling devices (i.e., “peepers”), and on water quality characteristics in sediment porewater from centrifuged bulk sediment samples. Based on data from five sediment management sites, the mBLM correctly predicted toxicity (or the lack thereof) to *H. azteca* in 87.2% of the samples (based on Sum TUs). Sediment and porewater chemistry data from these sites were then evaluated in a series of steps to derive sediment remedial goals from mBLM predictions of 5th-percentile based final chronic values (FCVs). First, we used empirical metal partitioning relationships for individual metals to back-calculate bulk sediment concentrations that would equate to porewater metals FCVs as predicted by the mBLM. These sample-specific bulk sediment concentrations were then compared against paired porewater toxic units (TUs) to identify which samples from a study area or site would be associated with unacceptable adverse effects as predicted by the mBLM (i.e., TUs > 1). From these samples a single “remedial action level” (RAL) was then derived for each individual metal as the average bulk metal concentration in sediments with mBLM TUs ≥ 1. We suggest that this RAL can be used to identify which sediments would need to be remediated because they exhibit porewater conditions in which metals are most bioavailable and, thus, potentially toxic. For most of the five sites, RALs derived in this fashion provided an improved means of identifying remedial action needs compared to traditional bulk-sediment based sediment quality guidelines (SQGs) given that they incorporated site-specific porewater bioavailability conditions. For example, RALs from sites with relatively low metals bioavailability were higher than bulk sediment SQGs, whereas RALs from sites with high metals bioavailability were substantially lower than SQGs. Obviously, this approach may be less effective for samples or sites in which factors other than dissolved porewater metals are driving benthic toxicity (i.e., low DO, TDS, NH₃, organics).

5.07.B.T-02 A Simple Narcotic Toxicity Model Extended to Complex Environmental Toxicity Issues

Joy A Mcgrath¹ and Christopher J Fanelli², (1)GHD, (2)Mutch Associates, LLC

For narcotic chemicals, it is well-established that a linear relationship exists between the chemical’s acute toxicity and its octanol-water partitioning coefficient. Dominic Di Toro’s target lipid model (TLM) describes this relationship and assigns a universal slope across species and identifies the y-intercept as the critical target lipid body burden (CTLBB). The CTLBB is the concentration of total narcotic chemical in an organism’s lipid needed to cause an acute effect and varies for each species depending on sensitivity. More than 75 species-specific CTLBBs have been derived from a TLM database comprised of more than 1000 individual acute data points spanning over 150 different chemicals. Demonstration of the TLM-derived CTLBBs to ionic chemicals supports the broad application of the approach. The simplicity of the TLM is in the prediction of a chemical’s acute water-only toxicity using only the chemical’s log Kow value. Various statistical approaches have been applied to derive acute and chronic thresholds that are protective of 95% of species. Dominic Di Toro combined the TLM and the additive toxic unit (TU) concept for predicting the toxicity of mixtures of narcotics that act via the same mode of action. The application to mixtures is relevant for oil spills, where mixtures of polycyclic aromatic hydrocarbons (PAHs) are generated and often measured after oil spills. The TLM-TU approach is a recognized approach for predicting the toxicity of PAHs. Using the TLM and TU approach, Dominic presented a theoretical justification why the toxicity of oil decreases as it weathers and then used

empirical data demonstrating that the toxicity is reduced. Risk assessment tools for petroleum registration, oil spill models, and regulatory guidelines incorporate the TLM as the underlying toxicity framework for predicting the toxicity of PAHs and other petroleum-related chemicals. The TLM has been extended to complex toxicity issues including sediment toxicity, phototoxicity, and the impact hydrostatic pressure has on the toxicity of narcotic chemicals when exposed to deep-sea aquatic organisms. More recently, the TLM has been extended for predicting the acute toxicity of chemicals with varying mode of toxic action in the Target Site Model.

5.07.B.T-03 Target Site Model: Predicting Aquatic Toxicity of Organic Compounds with Various Modes of Action

Kathleen S Boone and Dominic M. Di Toro, University of Delaware

Estimating the aquatic toxicity for emerging contaminants is a challenging task. The Target Site Model (TSM) was designed to accomplish this using chemical parameters calculated from molecular structure information. The model estimates the LC50 is computed as the ratio of the organism critical target site concentration to the site-water partition coefficient of the chemical. The TSM was developed from a database of 2,049 chemicals, with 79 different aquatic genera, and 47 associated modes of action (MoA) compiled from literature. Chemical classes included alkanes, polycyclic aromatic hydrocarbons, pesticides, inorganic, and polar compounds. The model database was used to generate linear free energy relationships (LFER) to describe the toxicity for each specific MoA using multilinear regression analysis. The model uses chemical specific Abraham solute parameters to determine MoA specific solvent parameters. With this procedure, critical target site concentrations are determined for each genus. Abraham solute parameters are also used to predict the mode of action for each compound. Data is split into a calibration and validation groups (80/20) to apply a k -Nearest Neighbors (k -NN) methodology. Optimal prediction results were found in a voting platform ($k = 3$) with feature weighting. Using the predicted MoA, the appropriate polyparameter Target Site Model for that MoA is applied to calculate the LC50. Predicted LC50s for the validation database resulted in a root-mean squared error (RMSE) of 0.752. This can be compared to an RMSE of 0.655 for the same validation set using the reference MoA labels. This confirms that the classification model has sufficient accuracy for predicting the MoA and for determining aquatic toxicity using the Target Site Model.

5.07.B.T-04 Development and Applications of Phototoxic Target Lipid Model (PTLM) to Predict Photo-Enhanced Toxicity of PAHs and Petroleum

Solmaz Marzooghi¹ and Dominic M. Di Toro², (1)California State Water Resources Control Board, (2)University of Delaware

Phototoxic Target Lipid Model (PTLM) is developed based on target lipid model (TLM) of PAHs and other narcotic chemicals to predict phototoxicity of the chemicals. PTLM can predict either the LC50 (i.e. the concentration required to achieve 50% mortality) measured at a fixed duration of irradiance exposure, or the LT50 (i.e. the time required to achieve 50% mortality) measured at a fixed concentration. The inputs to PTLM include the molar adsorption spectrum of the chemical, the spectral irradiance of the light source, and the duration of exposure to light. These parameters are incorporated to calculate the energy absorbed by the PAH, P_{abs} (moles of photons absorbed by PAH per mole of PAH). The other input to PTLM is the critical target lipid body burden (CTLBB) obtained from the TLM which is the organism lipid normalized body burden that causes 50% mortality. CTLBB is the only species dependent parameter in PTLM. The model has two parameters that are fit by calibration to a data set compiled from the literature comprising 20 individual PAHs and 15 test species, including arthropods, fishes, amphibians, annelids, mollusks, and algae. The fitting parameters are a : an exponent that quantifies the nonlinear response of mortality to energy absorbed (P_{abs}) ^{a} , and R^* : the ratio of organism critical body burden for reactive phototoxic species (formed by the energy absorbed by the organism's PAH body burden) to the CTLBB. PTLM is successfully validated for 12 alkylated PAHs and other phototoxic chemicals in petroleum, binary and ternary mixtures of PAHs (by assuming additivity of the toxic units computed for each of the PAHs in the mixture), and for water accommodated fractions (WAF) of neat and

naturally weathered Macondo crude oil samples from the Deepwater Horizon oil spill sites. More recently, the model is validated for assessment of petroleum hydrocarbon phototoxicity to coral reefs. In an ongoing effort, British Columbia Ministry of Land, Water and Resource Stewardship is deriving phototoxic site-specific water quality guidelines for PAHs using PTLM. The model development and results of the calibration, as well as its subsequent validations and applications will be presented.

5.07.B.T-05 EqP: Evaluation of Biota Sediment Accumulation Factors (BSAFs) for PCBs and PCDD/Fs in New York-New Jersey Harbor

Kevin J Farley¹, Simon Vojta², Rainer Lohmann² and Jim Lodge³, (1)Manhattan College, (2)University of Rhode Island, (3)Hudson River Foundation

Laboratory 28-day bioaccumulation tests were performed for 66 sediment samples from New York-New Jersey Harbor using the dredged material test organism *Nereis virens*. Sediments and organism samples were analyzed for polychlorinated biphenyls (PCBs), and the 2,3,7,8-substituted polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Additional measurements were performed for lipid content, sediment organic carbon and black carbon content, and porewater concentrations (via polyethylene passive sampling). Results of the data analysis showed that BSAFs for PCBs increased with increased chlorination and then levelled off for the higher chlorinated PCBs. This is in contrast to PCDDs and PCDFs which both showed decreasing BSAF values with increasing degree of chlorination. More detailed analyses of the data were performed using porewater concentrations and derived equilibrium partitioning to sediment (K_D) and organisms (BAF) to examine the underlying reasons for differences in BSAF responses. Results for PCBs showed enhanced K_D values for lower chlorinated PCBs. This was described by binding of planar (non- and mono-ortho) congeners to black carbon (using the dihedral angle as a measure of planarity). Observed BAFs were found to be directly related to K_{ow} values. Taken together, reduced BSAFs for the lower chlorinated PCBs were attributed to enhanced binding of the more planar PCB congeners on black carbon. Further analysis of the PCDD data showed that K_D values were directly related to K_{ow} with no apparent effects of binding to black carbon, and BAFs were relatively constant with little or no relation to K_{ow} . Responses for PCDFs were very different with K_D values indicating a very strong affinity of higher PCDFs for black carbon, and BAFs increasing with increasing K_{ow} values. Based on these results, it appears that the similar declines in BSAFs for PCDDs and PCDFs are associated with very different factors. For PCDDs, declining BSAF values are likely associated with limited diffusion of bulkier PCDD molecules through organism membranes, whereas declining BSAF values for PCDFs are due to very strong binding of planar PCDFs to black carbon.

5.07.B.T-06 Development of Linear Free Energy Relationships (LFERs) for Predicting Speciation of Cobalt Organic Metal Salts (OMS)

Richard F. Carbonaro¹, William A. Stubblefield² and David Boyle³, (1)Manhattan College, (2)Oregon State University, (3)Cobalt Institute

Accurate speciation calculations in aqueous solution require a robust and complete thermodynamic database containing all relevant equilibrium reactions, as well as a computational framework to perform the calculations. Our previous work in developing models for estimating $\log K$ (stability constants) of metal complexes involved 1:1 complexes between metal ions and simple carboxylic acids and phenols. These methods are sufficient to estimate unknown constants for monocarboxylate ligands and perform speciation calculations at relatively low ligand concentrations where only 1:1 complexes are expected to form. In this work, a new LFER was developed to estimate the necessary 1:2 stability constants for cobalt complexes with monocarboxylate ligands present in Cobalt Organic Metal Salts (OMS). The LFER was tested and verified against known stability constants in the NIST Critical database and relies on a strong relationship between stability constants of 1:1 and 1:2 complexes. With estimation methods for stability constants of 1:1 and 1:2 complexes now available,

equilibrium speciation calculations of cobalt substances in environmental media e.g., hard and soft water, were performed for various OMS over a range of concentrations. Both simple and unknown or variable composition (UVCBs) OMS were modeled. The speciation modeling results indicate that 1:2 complexes of cobalt with monocarboxylate ligands are not important species until very high total ligand concentrations are achieved. Thus, speciation of cobalt OMS in systems of environmental relevance are dominated by “free” cobalt ion and inorganic complexes which has implications for bioavailability and toxicity.

5.07.P-Tu Water Quality Criteria: Modeling Aquatic, Sediment, and Soil Toxicity Based on Mechanistic Chemical Interactions. Session honoring Dr. Dominic Di Toro

5.07.P-Tu-202 Predicting Abraham Solute Parameters using Quantum Chemical Solvation Models

Anthony Sigman-Lowery, Dominic M. Di Toro and Olga Dmitrenko, University of Delaware

Poly-parameter linear free energy relationships (pp-LFERs) have been used to predict the equilibrium partitioning of environmentally significant chemicals in two-phase systems. However, current methods of computationally estimating the Abraham Parameters used in these two-phase solvation models can fall short for large and complex molecules that are outside of the training sets used by these programs. In this study, a method is presented for deriving the *LSABV* Abraham Parameters of a solute using only the 3-D molecular structure of the compound. Quantum chemistry solvation models are used to compute the solvent-air partition coefficients ($\log K_{SA}$) of more than three thousand solutes in multiple solvents. The Abraham *L* and *V* parameters are calculated using the quantum-chemically estimated hexadecane-air partition coefficient and McGowan Molar Volume of each solute, respectively. Remaining Abraham solute parameters *SAB*, which are descriptors of the solute's dipolarity/polarizability (*S*), ability to donate hydrogen-bonds (*A*), and ability to accept hydrogen bonds (*B*) are derived by multiple linear regression using the predicted $\log K_{SA}$, *L*, and *V* values for each solute. Work is ongoing to use these new *LSABV* Abraham Parameters to predict the $\log K_{SA}$ and solvent-water partitioning coefficients ($\log K_{SW}$) of these solutes, which are expected to agree well with experimental $\log K_{SA}$ and $\log K_{SW}$ values. This method will be used to predict the Abraham Parameters for several emerging contaminants, including novel brominated flame retardants.

5.07.P-Tu-203 Extension of TLM and PETROTOX to Characterize Toxicokinetics of Hydrocarbons and Oils

Aaron Redman, Thomas Parkerton, Abraham Smith and Cary Sutherland, ExxonMobil Biomedical Sciences, Inc.

Environmental exposures to hydrocarbons, either as individual chemicals or as mixtures like crude oils and related petroleum substance products, often are transient in nature. Ephemeral exposures in water are particularly characteristic of most spill scenarios. Therefore, we are working to adapt the target lipid model used widely in toxicity assessment to account for variation in exposure time by explicitly incorporating a first-order toxicokinetic model. This model was trained mainly using temporal effect observations compiled from constant, single test substance exposures in standardized aquatic toxicity tests. Based on this analysis, results in the observed toxicokinetic rates (k_e) range from 0.1 to 10 d⁻¹ with little dependence on organism type, chemical class, or substance hydrophobicity (e.g., $\log K_{ow}$). This framework and initial results are being further validated with pulsed exposure data for single chemicals. A novel extension of this framework is being performed for different oils that provide complex hydrocarbon mixture exposures to aquatic organisms. Since k_e appears to exhibit limited dependence on $\log K_{ow}$, PETROTOX-calculated steady-state toxic units (TU) of the test oils derived using comprehensive compositional data was used as the exposure metric for this evaluation. Preliminary results suggest that the magnitude and distribution of observed k_e for oils are similar in magnitude as found for the single chemicals, which supports the initial findings regarding lack of K_{ow} -dependence on k_e . Our findings highlight that the TU serves as a valuable exposure metric for predicting the time-dependent toxicity of oil derived aqueous hydrocarbon mixtures.

5.07.P-Tu-204 Modeling the Partitioning of Anionic Carboxylic and Perfluoroalkyl Carboxylic and Sulfonic Acids to Octanol and Membrane Lipid

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Perfluoroalkyl carboxylic and sulfonic acids (PFCAs and PFSA) have low pK_a 's and are, therefore, deprotonated under most experimental and environmental conditions. Hence, the anionic species dominate their partitioning between water and organic phases, including, octanol and phospholipid bilayers which are often used as model systems for environmental and biological matrices. However, data for solvent-water and membrane-water partition coefficients of the anion species are only available for a few PFAS. In this study, an equation is derived using a Born-Haber cycle that relates the partition coefficients of the anions to those of the corresponding neutral species. It is shown via a thermodynamic analysis that for carboxylic (CA) and perfluoroalkyl carboxylic (PFCA) and sulfonic (PFSA) acids, the log of the solvent-water partition coefficient of the anion, $\log K_{sw}(A^-)$, is linearly related to the log of the solvent-water partition coefficient of the neutral acid, $\log K_{sw}(HA)$, with a unity slope and a solvent-dependent but solute *independent* intercept within a PFAS (or CA) family. This finding provides a method for estimating the partition coefficient of PFCAs and PFSA anions using the partition coefficients of the neutral species, which can be reliably predicted using quantum chemical methods. In addition, we have found that the neutral octanol-water partition coefficient, $\log K_{ow}$, is linearly correlated to the neutral membrane-water partition coefficient, $\log K_{mw}$, and, therefore, $\log K_{ow}$ being a much easier property to estimate and/or measure, can be used to predict the neutral $\log K_{mw}$. Application of this approach to octanol- and membrane-water partition coefficients for PFCAs and PFSA demonstrates the utility of this methodology for evaluating reported experimental data and estimating anions property data for chain lengths that are unavailable. It is also envisioned that this modeling framework could be further developed to other partition-based systems—e.g., bioconcentration factors (BCFs) and toxicity data—. For the BCF system, for example, prediction of bioconcentration would be performed via the additive contributions from different biological compartments (e.g., lipids and protein), each of which would be modeled using a partition coefficient estimate, an approach that this research group has presented for fish and plant BCFs in previous studies for other substances of environmental concern.

5.07.P-Tu-205 Occam's Razor – Simplifying Toxicity Estimation for Neutral and Ionizable Surfactant Compounds and Mixtures Using the Target Lipid Model & Abraham pp-LFER Descriptors

Craig Warren Davis, *Exxon Mobil Biomedical Sciences, Inc.*

The Target Lipid Model (TLM), developed by Di Toro et al., has been used to describe the baseline narcosis toxicity of a wide range of neutral organic chemicals. Early on, chemical class corrections were required to describe differential behavior of some polar compounds (ketones, PAHs, etc...). The likely source of these correction factors is uncertainty or systematic errors in the models which estimate the partitioning between target lipid and the water phase. Later work by Kipka & Di Toro eliminated these correction factors by introducing an Abraham pp-LFER-based partitioning expression. Despite this simplification, the addition of chemistries outside the established domain of the TLM (hydrocarbons, simple non-polar molecules, PCBs) has been slow, despite the development and curation of several large experimental ecotoxicological databases. Recently, the performance of the TLM was validated for a large range of linear and branched alcohol ethoxylate (AEO) surfactants including binary and complex mixtures of alcohol chain length and ethoxylate number. The TLM coupled with pp-LFER estimates of $\log(K_{ow})$ was found to perform as well, without re-calibration, compared to bespoke acute toxicity QSARS previously developed for individual species. Further, toxic unit (TU) addition provided significant improvement over the use of representative structures for describing the acute toxicity of simple and complex AEO mixtures. For AEOs, HC5s derived using the standard acute to chronic ratio (ACR = 5.22) were found to be adequately protective for > 95% of available chronic AEO data. For many commercial anionic surfactants a large body of acute and chronic toxicity data exists. Mechanistic models for quantifying toxicity as a function of head group, chain length, or other physical chemical properties

are largely non-existent. Generic surfactant models (i.e., ECOSAR) may be used for these materials, with variable and uncertain accuracy and confidence. Preliminary analysis using the existing TLM demonstrates a strong trend with respect to the partitioning behavior, however systematic offsets between estimated log(Kow) and log(LC50) values are observed, varying with functional head group chemistry. Recent work by Droge et al have developed improved models for membrane – water partitioning for anionic surfactants, providing an opportunity to deconvolute these offsets and confirm baseline narcosis behavior and TLM applicability for this new set of chemistries.

5.08.P-Tu Addressing the Sustainability and Impact of Aquaculture from a One Health Perspective

5.08.P-Tu-207 Environmental Considerations for Drugs Used in Aquaculture

Andrew Miglino and Stephanie Mohandas, U.S. Food and Drug Administration

Aquaculture in the United States (US) is a \$1.5 billion industry that primarily supports the food supply; however, aquaculture also helps restore populations of threatened and endangered species, sustain wild stock populations, and aids in habitat restoration. For example, almost 25% of wild-caught Alaskan salmon and more than 70% of salmon caught in the Pacific NW were reared in a hatchery. Rearing food animals and wild stock relies on efficiently grown animals and requires resilience to infection and disease. Therefore, to support and sustain this industry, the Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM) Office of New Animal Drug Evaluation (ONADE) and the Office of Minor Use and Minor Species Animal Drug Development (OMUMS) work with various government agencies and aquaculture associations to increase the number of safe and effective drugs that can be used in aquaculture. Investigational use or approval of aquaculture drugs requires an evaluation of the potential for significant environmental impacts. This evaluation is typically performed via the preparation of an environmental assessment or a claim of categorical exclusion from the need to prepare an environmental assessment. In preparing these evaluations, drug sponsors and FDA conduct exposure analyses and risk estimations to support investigational and approved uses of aquaculture drugs. Unlike drugs used in terrestrial animals, the use of aquaculture drugs often directly introduces drugs into the aquatic environment. This direct introduction presents a unique challenge for regulatory decision making. Furthermore, the variety of aquaculture systems, routes of administration, and dosage regimens pose additional challenges for analyses that apply broadly to many use sites. In this talk, CVM's review process for aquaculture drugs, tools used by CVM's risk assessors, and the knowledge gaps associated with this process and these tools are discussed. Case studies will be presented exhibiting the range in aquaculture systems, conditions of use, and evaluation methods - from a simple semi-quantitative review to a complex exposure evaluation. Future goals for CVM's aquaculture review process and areas for growth will also be highlighted.

5.08.P-Tu-208 Water Quality Benchmarks for New Animal Drugs

Katherine Anne Johnson-Couch, Alexis Wormington, Holly Zahner and Andrew Miglino, U.S. Food and Drug Administration

Aquaculture has become the fastest growing form of food production worldwide. As the industry continues to grow, the use of animal drugs in aquaculture and their subsequent release into the environment are expected to increase. The Environmental Teams at the Center for Veterinary Medicine (US FDA) have developed an approach to derive Water Quality Benchmarks to refine the Environmental review process for aquaculture animal drugs of concern. Water Quality Benchmarks are scientifically derived ambient concentrations intended to protect aquatic life in freshwater or saltwater surface waters that may receive drug-containing effluent discharges or runoff. These benchmarks are similar to the Water Quality Criteria for Aquatic Life developed by the US Environmental Protection Agency (US EPA) and are derived using a slightly modified approach to that described in US EPA guidelines. The Water Quality Benchmarks are not effluent discharge limits but can be used by the National Pollution Discharge Elimination System (NPDES) or State effluent permitting authorities

to establish appropriate effluent discharge limits for individual aquaculture facilities, if needed, based on site-specific conditions. This approach may contribute to the integration of a rapidly growing industry and national environmental monitoring and protection efforts.

5.08.P-Tu-209 How a Prey Fish Can Cause Reproductive Failure in Its Predator: Discovery of De Novo Thiaminase I Synthesis in Fish

Catherine A. Richter¹, Allison Evans², John Field³, Nate Mantua³, Rachel Johnson³, Jacques Rinchar⁴, Scott Heppell², James L. Zajicek¹ and Donald Tillitt¹, (1)U.S. Geological Survey, (2)Oregon State University,

(3)National Oceanic and Atmospheric Administration (NOAA), (4)State University of New York, Brockport

Thiamine (vitamin B₁) deficiency is an emerging global challenge for ecological health, and a stressor for both wild and hatchery-reared salmonid populations. Thiamine is an essential co-factor for energy metabolism enzymes and is required by all organisms. Some organisms, including species of bacteria, ferns, mollusks, and fish, contain thiamine-degrading enzymes known as thiaminases, and consumption of these organisms can lead to thiamine deficiency complex (TDC) in the consumer. Effects of TDC may mimic and/or interact with effects of other stressors such as diseases and contaminant exposures. Ecosystems around the world have shown evidence of TDC in birds, fish, and wildlife, often correlated with anthropogenic changes such as food web alterations, invasive species, and global climate change. In the Baltic Sea, TDC has been implicated in spawning migration failures in Atlantic salmon. In the Great Lakes, low thiamine leads to elevated early life stage mortality prior to initiation of exogenous feeding in native lake trout (*Salvelinus namaycush*) populations that consume the non-native, thiaminase-carrying prey fish alewife (*Alosa pseudoharengus*). On the North American Pacific coast, native salmonid populations have shown signs of TDC associated with changes in the marine food web, likely related to record high ocean temperatures. Specifically, TDC in California salmon appears to be linked with reduced diversity and dominance of thiaminase-carrying northern anchovy (*Engraulis mordax*), in salmon diets. We present an overview of our current knowledge of potential causes of TDC and evidence for *de novo* production of thiaminase by some species of prey fish. Several thiaminases have previously been genetically characterized in bacteria and unicellular eukaryotes, and the source of thiaminase in multicellular organisms has long been hypothesized to be gut microflora. In an unexpected discovery, we identified and confirmed the biochemical activity of thiaminase I genes in zebrafish (*Danio rerio*) with homology to bacterial tenA thiaminase II. Genes homologous to the zebrafish tenA-like thiaminase I were identified in many animals, including common carp (*Cyprinus carpio*), zebra mussel (*Dreissena polymorpha*) and alewife. This new understanding of the sources of thiaminases in food webs may open new avenues for management practices to prevent TDC in wild and hatchery stocks.

5.08.P-Tu-210 Thiamine Supplementation Improves Survival and Body Condition of Hatchery-Reared Steelhead (*Oncorhynchus mykiss*) in Oregon

Freya E Rowland¹, Aimee N Reed², Jennifer A Krajcik² and Donald Tillitt¹, (1)U.S. Geological Survey, (2)Oregon Department of Fish and Wildlife Fish Health Services

Early rearing of steelhead (*Oncorhynchus mykiss*) in Oregon hatcheries is often problematic with fry becoming emaciated and dying between hatch and first feed. Thiamine (vitamin B₁) deficiency has caused early mortality in salmonines elsewhere, but the thiamine status of Oregon's steelhead populations is unknown. Of the 26 egg samples from three Oregon hatcheries in 2019, 20 (77%) had thiamine levels < 10 nmol/g, and 13 of those samples (50%) had levels < 6.5 nmol/g, suggesting the thiamine deficiency. To investigate if thiamine content was causally related to fry survival, females were injected with buffered thiamine HCl 50 mg/kg prior to spawning; additionally, a subset of eggs were supplemented via bath treatment with thiamine mononitrate (1000 ppm) at spawning. Cumulative fry mortality at 8 weeks post-hatch from thiamine-injected females was only

2.9% compared to 13.8% mortality without thiamine supplementation. Furthermore, condition factors were greater in thiamine-supplemented fry than in those that received no thiamine. These data identify thiamine deficiency in Oregon steelhead and suggest supplementation with thiamine can mitigate early mortality.

5.08.P-Tu-211 A National Approach to Aquaculture Research and Sustainable Seafood Using a One Health Approach

Janet Whaley, National Oceanic and Atmospheric Administration (NOAA)

Optimal implementation of One Health principles requires the development and coordination of programs, policies, legislation, and research in which multiple sectors communicate and work together to achieve better public health outcomes. Specifically, the concept focuses on ways to improve food security, quality, and safety; control of diseases, and manage environmental factors through harmonization and standardization. A One Health approach is specifically called out in the National Science and Technology Council Subcommittee on Aquaculture *U.S. National Strategic Plan for Aquaculture Research* (published February 2022). The authors recommend Federal research should be coordinated using One Health principles to achieve the following: 1. to improve aquatic animal and algal health management; 2. to ensure the safety of all biologics and therapeutics being used in domestic aquaculture; 3. to promote and ensure the safety and health benefits of consuming aquaculture products; 4. to minimize the potential for impacts on the environment for aquaculture facilities; 5. to restore endangered species and habitats; 6. to increase the socioeconomic health of communities; 7. to improve U.S. food and nutritional security; and 8. where appropriate, to minimize negative secondary impacts to human, animal, and plant health. In this presentation, opportunities will be explored and current examples highlighted where the National Oceanic and Atmospheric Administration (NOAA) is applying a One Health approach to tackle science gaps and unanswered questions relevant to domestic aquaculture and sustainable seafood production. This will include several notable areas ripe for a One Health approach such the *New Blue Economy* initiative, Aquaculture Opportunity Areas (AOAs), marine aquaculture medicines, seafood trade, and climate resilient aquaculture. For example, the U.S. imports 75-80 percent of seafood we eat, yet, the U.S. exclusive economic zone (EEZ) is the largest in the world, spanning over 13,000 miles of coastline and 3.4 million square nautical miles of ocean. NOAA is currently developing the NEPA analysis on several regional EEZ areas to support the AOAs and offshore marine aquaculture using One Health principles to expand healthy and nutritious seafood production grown domestically and lessen the reliance on imports.

5.08.P-Tu-212 Mussel Propagation and Conservation Research at the USGS Columbia Environmental Research Center

James L. Kunz, Jeff A. Steevens, Allison Sieja, David J. Soucek, Doug Hardesty and Eric Brunson, U.S. Geological Survey

The USGS Columbia Environmental Research Center (CERC) has more than 20 years of experience in working with freshwater mussels, mainly to develop, validate, and apply methods for assessing the effects of toxicants in water, effluent, and sediment. Research has recently conducted also evaluated mussel responses to non-contaminant stressors, such as elevated temperatures and suspended sediment. In the past decade, CERC has moved beyond laboratory testing to develop an active mussel propagation program in laboratory and ponds. With advances like the pulsed flow-through automated feeding system and the use of specialized pond grow-out systems, the program has seen high survival and growth rates of juvenile mussels from almost 30 species across 6 different mussel tribes, including difficult-to-culture mussels, such as *Margaritifera falcata* and federally endangered species *Epioblasma triquetra*, *Venustaconcha trabalis*. Long-term holding of adult mussels and culture of juveniles to sexual maturity has been accomplished using floating upweller systems in ponds. In addition to advanced wetlab facilities and a system of well-water-fed experimental ponds, the construction of a dedicated mussel culture facility has strengthened the program by allowing in-house inoculation of host species. Scientists at CERC are now working with Tribal and other management agency partners to apply these advances in propagation to support freshwater mussel restoration and reintroduction efforts.

5.09.P-Mo Assessing Risks of Using Resource Extraction Byproducts and Waste

5.09.P-Mo-183 Evaluating Treated Produced Water from the Permian for Potential Beneficial Reuse Opportunities

Aaron Redman¹, Josh Butler¹, Abraham Smith¹, Azivy Aziz¹ and James Rosenblum², (1)ExxonMobil Biomedical Sciences, Inc., (2)Colorado School of Mines

Substantial volumes of produced water (PW) result from oil and gas operations in water stressed areas including the Southeast US, which could potentially be reused following treatment to support other functions and services in the region. A screening risk assessment framework was developed to evaluate potential use types (e.g., irrigation, industrial, dust suppression, etc), address potential exposures based on comprehensive PW chemical characterization (e.g., 1000s of analytes), and address associated aquatic hazards and risk priorities by developing of hazard concentrations intended to provide 95% species protection (HC_{5S}) for a wide range of PW constituents. HC_{5S} were derived using the grouped Target Site Model (gTSM) across a wide range of neutral and organic chemical classes. This model accounts for variation in Mode of Action, as well as the partitioning properties of the molecules of interest. This framework is intended to provide a mechanistic and transparent basis for objectively evaluating the potential risks associated with treated produced water reuse scenarios. Since this approach requires substantial amounts of data and risk calculations, simpler, approaches are being evaluated including integrated passive samplers, and whole effluent toxicity testing (WET). WET test data are being used to confirm the intended protectiveness of the mechanistic risk assessment framework and to identify relevant toxicity test endpoints to practically monitor the suitability of treated PW for potential for beneficial reuse.

5.09.P-Mo-184 Assessing Risks of Using Resource Extraction Byproducts and Waste

LeeAnn Racz¹ and Kristy Richardson², (1)T ToxStrategies, LLC, (2)Colorado Department of Public Health and Environment

Operations that extract resources from the earth, such as metals mining, metals processing, natural gas extraction, and petroleum drilling, generate waste and byproducts such as tailings, chat, waste rock, slag, and brine. Whereas these materials may have been considered waste when initially processed, they still contain recoverable minerals or have other beneficial uses. Nevertheless, there may be inherent hazards associated with these materials, namely chemical toxicities and natural radioactivity. Sustainability initiatives must consider the entire life cycle of extracting such resources, including the ultimate fate and transport of constituents in waste and byproduct materials and the potential for human exposures to the hazards these materials pose. This presentation will introduce the session and the relevant issues. In particular, the presentation will highlight risk assessments of exposures to resource extraction byproducts and waste as they are used for beneficial purposes.

5.09.P-Mo-185 Radiation Exposures from the Beneficial Use of Alumina Production Residue

William Rish, Ph.D.¹, John Mauro² and Stephen Marschke², (1)ToxStrategies, LLC, (2)SC&A

It is estimated that the production of smelter and chemical-grade alumina has resulted in a combined stockpile of over three thousand million tons of alumina production residue (APR) in storage at over 100 locations throughout the world. Although the APR contains valuable minerals, a small percentage has been put to beneficial use. One of the concerns associated with the beneficial use of APR is that it contains naturally occurring radium-226 (Ra-226) and radium-228 (Ra-228). In this study, estimates of radiation exposure are developed over the life cycle of beneficial use in cement of an APR waste pile. The lifecycle includes radiation exposures that might be experienced by industrial workers involved in excavation and transport of the residue to cement plants, industrial workers at the cement plants, construction workers making use of the cement, members of the public who might be in the proximity of the cement products, and disposal of the cement at the end of its useful life. The results indicate that it is not reasonably likely for exposures related to beneficial use of APR waste in cement to exceed the acceptance criteria delineated in current radiation protection standards for

workers and members of the general public. The findings demonstrate that beneficial use of APR waste as a cement ingredient can be accomplished safely, with potentially significant benefits to management of the large volume of APR being stored around the world.

5.09.P-Mo-186 Assessing Sustainable Applications of Electric Arc Furnace Steel Slag as Construction Aggregate: Applications of Probabilistic Risk Assessment and Physiologically-based Pharmacokinetic Modeling

Deborah M. Proctor, Liz Mittal, Alexander Blanchette, LeeAnn Racz and Camarie S. Perry, ToxStrategies, LLC

Electric Arc Furnace (EAF) slag is a co-product of steel production, used primarily for construction purposes, including landscape aggregate and cover for unpaved rural roads. Steel slags are currently being evaluated in the National Science Foundation Product Category Rule for Environmental Product Designation and have been evaluated over the past 25 years in a series of characterization and health risk assessments (HRAs) to evaluate intended beneficial uses. Applications of EAF slag may involve residential exposures by direct contact and inhalation of airborne dust. In the most current assessment of potential health risks, metal concentrations in slag, leaching potential (including oral bioaccessibility [BA] by EPA Method 1340), and analysis of mineralogy were conducted. Metals in EAF slag exist in alkaline mineral matrices, and leaching of metals in environmental conditions is low. Antimony, arsenic, hexavalent chromium, iron, vanadium, and manganese (Mn) in EAF slag exceed EPA residential Regional Screening Levels and were evaluated using a probabilistic risk assessment (PRA) for residential EAF slag applications as driveways/landscape aggregate and for gravel on unpaved rural roads. Varying assumptions regarding particulate emissions, time spent at home, and time spent outdoors were considered, with exposure parameters from EPA guidance and measures of EAF slag-specific oral BA. For Mn oral exposure, relative bioavailability (RBA) measure of 14% was included from a recent *in vivo* rat RBA study. The PRA predicted cancer risk and hazard indices within acceptable regulatory guidelines. To further assess the potential for Mn neurotoxicity, a recently published physiologically based pharmacokinetic (PBPK) model, was used to predict levels of Mn in the brain (i.e., globus pallidus) for children and adults associated with residential exposure scenarios. The PBPK model predictions for Mn in the globus pallidus were slightly increased at the 90th percentile, as compared to diet alone and background ambient air, but were lower than no effect levels for neurological toxicity reported in the literature based on both human and primate studies. Overall, risk assessment supports the continued use of EAF slag for intended applications, including as residential roadway and rural roadway aggregate.

5.09.P-Mo-187 Towards a Transparent & Reproducible Framework for Risk Assessment and Evaluation of Produced Water for Beneficial Reuse

Craig Warren Davis¹, Josh Butler¹, Aaron Redman¹, Cloelle Danforth², Sean Thimons³, Michael Jahne³ and Pei Xu⁴, (1)ExxonMobil Biomedical Sciences, Inc, (2)Environmental Defense Fund, (3)U.S. Environmental Protection Agency, (4) New Mexico State University

There is growing interest in promoting the treatment and beneficial re-use of production water from onshore oil & gas operations, i.e., produced waters (PW). A significant challenge to re-use strategies is the variable and complex chemical composition of produced waters. PW may come from multiple wells, they often change in composition as the well ages, and may include intentionally added chemicals (e.g., biocides, solubilizing agents, lubricants), geogenic contaminants (petroleum hydrocarbons, naturally occurring radioactive materials), as well as high mineral and solids concentrations (total dissolved solids, major ions). As a result, partial or complete treatment of produced waters may be required to facilitate various beneficial re-use scenarios. As part of the assessment of suitability for PW re-use, a quantitative, risk-based evaluation which includes potential for environmental and human health risks should be included. To date, a harmonized quantitative risk assessment approach for evaluating untreated and treated produced water has not been developed. A quantitative framework is thus required which can address (1) compositional variability (e.g., organics, ionizables, and

metals/mineral chemistries), (2) exposure considerations, and which can adequately and reliably estimate hazard thresholds (e.g., predicted no-effect concentrations and derived no-effect levels), particularly when experimental data for many constituents is not readily available. The purpose of this work is to outline the compositional, exposure, effects, and methodological needs to perform robust, reliable risk assessment for PW and its constituents. This framework emphasizes the utilization (with appropriate modification) of existing exposure modeling (e.g., EUSES and TICKET-UWM for organics and metals, respectively), hazard estimation (e.g., target site modeling, toxic unit addition), emissions quantification (e.g., use maps and specific environmental release categories (SpERCs) approaches to leverage existing methodology & institutional knowledge. Ultimately, an objective of the scientific community in this space should be an engaged and trained community of practice centered around harmonized approaches and a user-friendly, freely available risk assessment toolbox. Disclaimer: The views expressed in this presentation are those of the authors and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.

5.10.P-Mo Emerging Techniques for Evaluating Ecological Risk at Contaminated Sites

5.10.P-Mo-188 An R-based Matrix Population Model Library Optimized for Risk Assessment

Timothy Alan Walker, Carolyn Meyer, Bonner Anthony, Holly McChesney and Emily Morrison, ARCADIS

Population models have become a popular tool for assessing vertebrate and invertebrate population-level risk resulting from exposure to environmental contaminants. The structure of stage-based matrix models is one method to link individual-based effects to population-level endpoints. However, currently available user-friendly population modeling tools often do not offer sufficient flexibility to consider site- and species-specific parameters necessary to more accurately model risk. The currently available matrix-based population models include standalone, relatively inflexible programs such as VORTEX and RAMAS, or function-based libraries that offer tools for constructing custom-built models that require a high level of skill to build (Excel Add-ins: PopTools, @Risk; R: popbio, demogR, SPop, stagePop). To provide a tool that is both accessible and flexible for the purposes of ecological risk assessment, we developed the R Population Model Library (RPML). The RPML offers simple R functions for construction, execution, and summarization of population models. These R functions include options for incorporating key model elements such as stage matrix structure, density dependence, catastrophic events, stocking events, metapopulations, dispersal, and sensitivity analysis. The library's construction tools are focused on the creation and subsequent modification of a baseline model. This property makes it especially suited for modeling differences between environmentally impacted and non-impacted scenarios in ecological risk assessment. The library can be further adapted to end-user needs using open-ended customization functions that link RPML to the vast array of existing open-source R libraries. Here, we provide validation of the RPML against an existing tool (RAMAS Metapop) with a case study on tiger salamanders. We demonstrate how RPML can be used to quickly develop a metapopulation model that evaluates risk to the salamanders in ponds impacted by selenium and then perform a sensitivity analysis to assess uncertainty in the results.

5.10.P-Mo-189 CSM-based Sediment Remediation Strategies Without Published State Standards

Nicholas Hastings, Jared Port, Dan Wolfram, Lucas Hellerich, Janet Robinson, Lisa M. McIntosh and Jeff Hamel, Woodard & Curran, Inc.

A number of states do not have published numerical criteria for constituents of concern (COCs) for the remediation of sediments. These states address sediment remediation through the understanding of the conceptual site model (CSM) and the use of sediment screening criteria, ecological risk assessments, discharge of groundwater to surface water standards, and surface water quality standards. Under this situation, development of sediment remediation strategies requires site-specific approaches. Three examples, based in the State of Connecticut, where sediment remediation approaches were developed without published sediment-based numerical criteria are presented. Each of the approaches relied on a combination of data acquisition to

refine the CSM and using sediment screening criteria and/or a site-specific ecological risk assessment. A robust set of sediment samples along with biota samples were collected at a tidally influenced river sediment site (Example 1). The concentrations of developed COC arrays were compared to the results of site-specific toxicity testing. Aggregation of the COCs allowed for the development of site-specific remediation criteria for metals and PAHs, as well as sediment lateral removal limits (depths were determined based on the biologically active zone). A forested wetland (Ex. 2) was characterized and a site-specific, toxicological-based remediation criteria for was established for nickel. The CSM was refined through the collection of additional sediment and wetland soil samples up to a depth of 2.5 feet to increase the density of data over the historic dataset. The remediation approach consisted of excavation and restoration to pre-existing grades, along with the preservation of large diameter trees to minimize the potential for erosion and to facilitate the re-establishment of plants and trees within the remediated area. PCBs were delineated within a bermed wetland (Ex. 3) horizontally to concentrations of < 1 mg/kg and vertically to an underlying low permeability silt/clay layer. The remediation consisted of a multi-phased approach designed to address source area contamination (Phase I) followed by a future ecological risk evaluation (Phase II). Ecological screening criteria and evaluation of the biologically active zone were used to establish the Phase I remediation excavation limits. The Phase I remediation approach consisted of excavation and restoration to pre-existing grades and native plant species within the wetland.

5.10.P-Mo-190 Addressing Uncertainties in Oral Bioavailability of Chemical Contaminants

Shenghong Wang, Zhizhen Zhang, Dingsheng Li and Li Li, University of Nevada

Understanding the oral bioavailability of chemical contaminants is essential for evaluating their exposures, toxicokinetic, health effects, and risks to humans. Most existing high-throughput physiologically based toxicokinetic (PBTK) modeling efforts rely on a constant gut absorption rate extrapolated from in vitro transmembrane permeability assays and ignore chemical loss due to first-pass metabolism. Such a simplifying approach may introduce uncertainty for certain chemicals. For instance, it does not account for the differences in conditions between the human intestine and transmembrane permeability assays, e.g., the aqueous boundary layer. Additionally, it assumes steady-state mass transfer, which may not hold for hydrophobic chemicals that require an extremely long time to reach the steady state, often exceeding the residence time of digesta in the gastrointestinal tract. This presentation introduces our improvement of the traditional PBTK modeling approach by accounting for non-steady-state gastrointestinal absorption of chemicals and pre- and post-absorption biotransformation. We evaluated our model using human oral absorption efficiency and time-dependent plasma concentration data, demonstrating its reliability and practicality. Inputs for the model include partition coefficients, molecular weight, and intrinsic hepatic clearance, all widely available or estimable using various quantitative structure-activity/property relationships, empowering this model as a practical tool for high-throughput screening of the multitude of chemicals in the market, or even before production. We also predict oral bioavailability for a wide range of chemicals of emerging concerns. The application of this new model improves our understanding of how chemical properties impact oral bioavailability and contributes to the development of New Approach Methods.

5.10.P-Mo-191 Toxicity Evaluations to Marine Benthic Organisms Exposed to Road Dust Collected in Kanazawa City, Japan

Seiichi Uno¹, Masatoshi Yamasaki¹, Kokushi Emiko¹, Kazuki Imamura¹ and Masato Honda², (1)Kagoshima University, (2)Kanazawa University

Road dust is composed with the complex components, and most of them derived from the automobile and road surface, for example, soot of exhaust gas, coating materials, tire wear particles, and asphalt dust. Those move into the river by the rain drainage, and at last achieve to coastal sediments. Therefore, benthic organisms in the coastal areas could be exposed to a certain amount of their road dust. Perhaps road dust includes some kinds of toxic substances, and possibly affect to organisms continuously. However, the information for their effects to aquatic organisms are limited in the field. Kanazawa city, Ishikawa prefecture, is a core city and most

population (460,000) at Hokuriku Region in Japan. Some pollutants discharged not only from center of Kanazawa city, but also to other countries as China have been detected in the atmosphere. Those pollutants are possibly settled on land and aquatic environments. In this study, we collected the road dust at 10 sites in the center of Kanazawa city, Japan. The individual dust was exposed to 12 marine amphipod, *Ptilohyale barbicornis*, for 10 days and evaluated the toxicity risks. The road dust collected 3 sites especially with the heavy traffics killed over half of amphipod 3 days after starting the exposure. Additionally, those collected at 6 sites also killed over half at 5 days. Those results suggest that road dust distributed in the big cities is potentially toxicities to the organisms. Some rivers flow into Kanazawa city and the aliquot of dust should easily flow into aquatic environment. The present study showed that the dust might already cause some effects even in the real aquatic environment around Kanazawa city.

5.10.P-Mo-192 Photocatalytic Degradation of Per- and Polyfluoroalkyl Substances (PFAS) in Landfill Leachate Using 3D Printed TiO₂ Composite Tiles

Andrew D McQueen¹, O'Niell Tedrow², Mark Ballentine¹ and Alan Kennedy³, (1)U.S. Army Engineer Research and Development Center, (2)Minnesota North College, (3)U.S. Army Corps of Engineers

Per- and polyfluoroalkyl substances (PFAS) are a complex group of recalcitrant substances that are present globally in many landfill wastewater leachates and have potential ecological and human health risks. Conventional treatment technologies have shown limited efficacy for many PFAS due to the stable C–F bonds. Therefore, there is growing interest in applying advanced oxidation processes (e.g., photocatalysis) to decrease the aqueous concentrations of PFAS in contaminated wastewater and mitigate risks. The goal of this study was to evaluate the photocatalytic performance of treating PFAS in landfill leachate using a novel photocatalyst composite. Treatment structures were fabricated using polylactic acid (PLA) and compounded with TiO₂ (15 wt%), and 3D printed into tiles. The appeal to 3D printing the photocatalytic composites for environmental applications is for limitless geometric customization for the application, including optimizing surface area and prototyping for research improvements prior to scale-up. A pilot-scale treatment system was designed to promote photocatalysis using 3D composite structures and UV irradiance intensity of 1.0 mW cm⁻² ($\lambda = 280\text{--}400$ nm) following 24- and 36-h hydraulic retention times. Photocatalytic degradation was achieved for seven of the 11 PFAS evaluated in this study: PFOS, PFOA, PFHPA, PFHxS, PFNA, PFDA, and PFOSAm. Greater than 80% removal of PFOS, PFNA, PFDA, and PFOSAm was observed after 24 h of photocatalysis. These results indicate photocatalysis using TiO₂ polymer composites can achieve beneficial levels of PFAS degradation. This study provides a proof-of-principle approach to inform the application of additive manufacturing of photocatalytic composites for use in the treatment of PFAS-contaminated wastewater.

5.10.P-Mo-193 Characterization of Molecular and Apical Effects of Legacy Contaminated Groundwater on Early Life Stages of Fathead Minnows

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Mechanistic toxicology approaches are seen as a promising alternative to traditional live animal testing in support of ecological risk assessments; however, the often-noted lack of predicted linkages between effects observed at molecular and apical levels curtails the adoption of such approaches. The objective of this study was to apply a novel transcriptomics tool, EcoToxChips, to characterize the effects of complex mixtures of contaminants in fish and to compare molecular response patterns to apical outcomes. Fathead minnow (FHM) embryos were exposed for seven days to increasing concentrations of groundwater collected from a legacy contaminated site. There was a concentration-dependent disruption of photo-dependent swimming response, spinal deformities, edema, and mortality after exposure to the groundwater mixtures. EcoToxChip analysis revealed a shift from a majority of upregulated genes at lower concentrations to a majority of downregulated

genes at higher concentrations. Many of the significantly dysregulated genes are involved in biological pathways that could be linked to the apical outcomes observed in FHMs. In particular, the main effects included induction of oxidative stress, activating of several metabolic processes and growth, cell death, and inhibition of signal transduction signalling processes. Overall, the results observed in this study demonstrated that focused transcriptomics approaches such as the EcoToxChip system could be supportive of risk assessment of complex contaminated sites.

5.10.P-Mo-194 Modeling PFAS Bioaccumulation Uncertainty Using Monte Carlo Methods

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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. This enables estimation of site-specific dietary doses for wildlife, a critical part of ecological risk assessments (ERAs) at PFAS-impacted sites. Using data from ten field studies, concentrations of PFAS in surface water and sediment at aquatic sites were used to generate predictions of PFAS in tissue (using food web models) that were compared to the measurements of PFAS in biota (invertebrates, fish, plants) from those same sites. Forage fish was the most well represented trophic level in the field studies, as this class had the highest number of data points (249); predatory fish, aquatic plants, benthic invertebrates, and pelagic invertebrates exhibited fewer data points at 104, 81, 80, and 44 data points, respectively. Perfluorooctane sulfonic acid (PFOS) had the highest number of data points, with Perfluorohexane sulfonate (PFHxS) and Perfluorooctanoic acid (PFOA) often a close second, depending on the biota type. Sediment and surface water field data from various studies were used as input parameters to an aquatic food web model and applied Monte Carlo modeling techniques to estimate a probability distribution for the modeled tissue values. The variability of bioaccumulation factors (BAFs) was considered, as well as variability in sediment and surface water PFAS concentrations. The model results showed that the contribution of BAFs and input sediment and surface water concentrations did vary results - specifically, the percent relative standard deviation (%RSD) for results, regardless of trophic level, ranged from 48% for Perfluorotridecanoic acid (PFTrDA) to 60% for PFHxS, with some variation depending on the specific trophic group. Benthic invertebrates displayed the largest variability of any trophic level, with %RSD between 58% for PFOA and 94% for Perfluorohelptanoic acid (PFHpA). Therefore, areas of continued uncertainty were identified through use of Monte Carlo simulation in food web modeling. Regardless of the uncertainties identified, most model results were within a factor of 5 to 10 of the measured tissue concentrations, especially at higher concentrations, which lends confidence to the model for use at sites impacted by PFAS.

5.11.P-We Soil Contaminants: Fate, Bioavailability, Environmental Toxicology in Ecological and Human Health Risk Assessment

5.11.P-We-111 Effect of Soil Properties on Bioaccumulation, Translocation and Potential Risk of Zinc Oxide Nanoparticles in Soil-Plant System

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Zinc oxide nanoparticles (ZnO NPs) are frequently used in commercial products and agriculture, and can easily be released into the soil environment. Once introduced into the soil, ZnO NPs can be transported to crops through biological uptake and can potentially cause toxicity in ecosystems and human health. The objectives of this study are: (1) to investigate phenotypic and physiological effects of ZnO NPs in soil-plant systems, with various soils, (2) to determine the comparative biological uptake and translocation of ZnO NPs on the effects of soil properties such as pH, organic matter concentration, cation exchange capacity and soil composition, (3) to further assess the potential human health risks concerning ZnO NPs in food chains. According to the pot experiment, the toxic effect and uptake of ZnO NPs in bok choy are influenced by soil properties. In particular,

plant growth in soils with high sand content and low organic matter (OM) is negatively affected, resulting in decreased leaf size and reduced root length, and the accumulation of ZnO from soil to plants is increased. In addition, in soils with high pH, the accumulation of ZnO is reduced, but the translocation of ZnO within the plant increase compared to other soils. Overall, the bioaccumulation and translocation of ZnO nanoparticles in bok choy are correlated with soil pH, electrical conductivity (EC), organic matter (OM), and cation exchange capacity (CEC). Furthermore, the potential human health risks associated with the accumulation of ZnO in bok choy may increase with higher intake levels. The information provided by this study is crucial for assessing potential ecological and human health hazard concerns associated with ZnO NPs in food chains.

5.11.P-We-112 Reproduction Toxicities of PFAS-Free Replacements of Aqueous Film-Forming Foams for Soil Invertebrates

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Legacy aqueous film-forming foam (AFFF) formulations containing per- and polyfluoroalkyl substances (PFAS) have been linked to accumulation in soil invertebrates and terrestrial plants in previous studies with the potential for biomagnification in terrestrial food-webs. Alternative PFAS-free surfactant formulations are being developed and evaluated for their ability to meet current US Department of Defense performance requirements. The relative toxicities of PFAS-free AFFF-alternatives, as compared with legacy AFFF formulations are not known. We have developed ecotoxicological data for seven candidate PFAS-free AFFF formulations and a legacy AFFF formulation by determining reproduction toxicity for soil invertebrates collembolan *Folsomia candida*, earthworm *Eisenia andrei*, and potworm *Enchytraeus crypticus*. Test species were exposed in separate studies to each formulation in a natural soil, Sassafras sandy loam, which has characteristics (low clay and organic matter content) expected to support high bioavailability for these materials. Toxicity data derived from this project will be used to develop Soil Ecotoxicological Risk Factors (SERF) to assess which PFAS-free AFFF formulations would exhibit lesser environmental toxicity, while meeting the current performance requirements.

5.11.P-We-113 Evaluation of Soil Background Values of Perfluoroalkyl and Polyfluoroalkyl Substances in Scandinavian Countries

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Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are ubiquitous in the environment and have been detected in a wide variety of environmental media such as water, soil, aquatic sediment, and organisms. Because of their widespread industrial application, persistence, and chemical properties, PFAS are routinely detected in environmental samples obtained from remote locations where a point source is not easily identifiable. The purpose of this study is to assess the ambient background concentrations of PFAS in soil in Norwegian countries and identify values for PFAS in soils that reflect the upper ranges of background conditions in Norway. These values, referred to as Background Threshold Values (BTVs), can be useful as criteria to determine if single measurements of PFAS in soil are likely attributable to background PFAS sources (i.e., sample result \leq BTV), or may be indicative of point sources of PFAS (i.e., sample result $>$ BTV). Publicly available sources of data for Sweden and Norway were reviewed generate BTVs. Based on available data, BTVs were estimated for the following six frequently detected PFAS compounds: PFOS, PFBS, PFOA, PFNA, PFDA, and PFUnDA. The 95-95 Upper Tolerance Limit (95-95 UTL) was selected as upper bound value statistic to represent the BTVs for this study due to the high coverage, confidence, and reliability of this statistic. The UTLs ranged from 0.87 (PFUnDA), 0.98 (PFBS), 1.3 (PFDA), 1.9 (PFNA), 4.3 (PFOA), to 5

(PFOS) ng/g in surface soil background samples from semi urban and remote background sampling locations. This review highlights the need to quantitatively evaluate ambient levels of PFAS in soil to investigate and manage PFAS at specific sites.

5.11.P-We-115 Widespread Climate and Emission Change Induce Losses of Selenium and Sulfur in Topsoil which Jeopardize the Soil Health and Human Nutrition on a Global and European Scale

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Studies predict that changes in climate and environmental conditions will decrease soil concentrations of essential nutrients, such as sulfur (S) and selenium (Se), thereby increasing the risk of malnutrition for people. Climate and environmental conditions contribute to biogeochemical cycling because they control a variety of processes (e.g., sorption, speciation, deposition) that control element retention in soils. Therefore, understanding these mechanisms is crucial for developing practices that mitigate S and Se soil element losses from climate and environmental change. We investigated the primary factors influencing the concentrations of S and Se in soils on global and European scales and assessed how change in these factors may affect future soil concentrations. We predicted current and future soil element concentrations using climate, soil physicochemical, and atmospheric deposition data at different spatial resolutions. On a global scale, soil Se concentrations were mainly influenced by interactions between climate and soil properties. Climate change scenarios for the period 2080-2099 at a resolution of 1° resulted in a global decrease in soil Se concentrations across 58%, with an average loss of 8.4%. These decreases were attributed to changes in redox conditions and soil organic carbon content. Refining our predictions for Europe at a higher resolution of 0.1°, while also considering anthropogenic emissions, we found that decreases in soil organic carbon and atmospheric deposition were the main drivers of S and Se losses. Conversely, increases in evapotranspiration contributed to gains in concentrations. The spatial patterns of S and Se changes were similar, with predicted gains and losses observed in northern and central/southern Europe. The global model for Se exhibited a similar spatial pattern, but with lower extent of soil concentration change, resulting in a mean loss of 5.5% for S and 4.0% for Se. Insufficient Se intake currently affects approximately 1 billion people globally, while soil S deficiency concerns up to 20% of agricultural soils worldwide. Therefore, the declining concentrations of S and Se in soils pose a threat to food security and may contribute to an increase in deficiency prevalence. Our results suggest that farming practices aimed at increasing soil organic carbon would increase S and Se retention, but further research needs to explore how nutrient uptake in crops is affected by soil management practices.

5.11.P-We-116 Phytoavailability, Toxicity, and in Planta Distribution of Coexisting Uranium and Microplastics Contamination in *Mentha arvensis*

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Plants uptake of microplastics and their subsequent role in transmission to human and non-human animals is of critical concern. Microplastics can induce a cascade of negative effects on the environment by changing the physicochemical quality and function of soil, invoking plant responses. Within the plant system, microplastics can either adsorb to the root surface affecting root activity and uptake of nutrients and water, and/or get into the transpiration pull causing high stress on the plant. Here, we investigated the bioaccumulation and toxicity of high-density polyethylene (HDPE) particles and uranium in *Mentha arvensis* (Wild mint) through the integration of plant exposure experiments, microscopy, and spectroscopy. Uranium was included in this study given that microplastics can adsorb toxic metals and metalloids, aggravating their potential harmful effect on the environment and human health. *Mentha arvensis* (quadruplicate) were exposed to HDPE (0.5% w/w to

soil), U (120 ppm), and a combination of U and HDPE in 500 g of soil for 14 days. The co-exposure to HDPE and U impaired the micro- and macro- elemental profile in the roots of mint plants by inducing a synergetic negative effect on the levels of Al, Fe, K, Mn, P, and S. Interestingly, Mg, Ca, Ba, Na, and Sr showed a more pronounced decrease in the roots when plants are exposed to U and HDPE separately. The impairment of nutrient elemental profile was also detected in the shoots with a synergetic decrease in P and Zn levels. These results give insight into the toxic ecological effects of U and HDPE on wild plants and the possible risks of food chain transfer in environments where those co-occur.

5.11.P-We-117 The Impact of Site History on Metal Concentrations and Bioaccessibility in Urban Parks in Toronto

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To assess the potential risk associated with exposure to metal contaminants in urban green spaces in Toronto, Canada, 75 surface soil samples were collected from exposed areas with high potential for human soil contact at 25 parks and playgrounds. A historical review indicated parks were situated on former landfills, industrial sites, or agricultural lands. The relationship between historical site information, metal concentrations and physicochemical properties, bioavailability was explored. Arsenic, antimony, cadmium, cobalt, mercury, and molybdenum concentrations in the samples were mostly below detection while the mean concentrations of chromium (40 mg/kg), copper (18 mg/kg), nickel (15 mg/kg), lead (43 mg/kg) and zinc (92 mg/kg) were all below the Canadian Council of Ministers of the Environment (CCME) soil quality guideline for parkland use. However, lead concentrations exceeding the CCME guideline were detected in samples from parks located on former agricultural or industrial sites with documented lead arsenate pesticide use. There was a strong correlation between lead and arsenic (Pearson $r = 0.933$, $p < 0.001$) suggesting a common origin. Chromium concentrations exceeded the guidelines in samples from a park built on a former reservoir while nickel levels from a park situated on a former harbour also exceeded the guidelines. There was no strong correlation between these metals suggesting different origins. The mean in vitro bioaccessibility were arsenic (25%), cadmium (68%), chromium (16%), copper (57%), lead (64%), nickel (18%) and zinc (43%). Incorporation of in vitro bioaccessibility data in the risk characterization using Health Canada human health risk assessment framework for incidental ingestion suggested the risk associated with exposure to metals in soils in most of the Toronto parks studied were low.

5.11.P-We-118 Site-specific Relationship Between Porewater Concentrations and Bioaccumulation for Polychlorinated Biphenyls (PCBs) in the Marine Wetland near Naval Air Station, Pensacola, Florida

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Wetland 64 at Naval Air Station, Pensacola, is an estuarine site located very near the confluence of Bayou Grande and Pensacola Bay. In the past, the site has had sediments that were contaminated with PCBs. According to recent investigations, PCB levels in surface sediments varied from 12 to 3100 $\mu\text{g}/\text{kg}$ in 2014. The main aim of this research was to compute and compare the PCB porewater concentrations from the passive samplers, interpret these results with the benthic organisms exposed to the sediments, and develop a site-specific relationship between porewater concentrations and bioaccumulation. Eight sampling locations (S5, S6, S10, S11, S12, S14, S15, and S22) were chosen in the wetland based on previous PCB results. Sediments from these locations were collected, homogenized, and a portion was used for lab experiments. Solid phase microextraction (SPME) samplers with poly dimethyl siloxane (PDMS) coating were deployed alongside sediment exposures for aquatic organisms in the wetland and lab. The test species for bioaccumulation were saltwater clams and marine worms. In-situ chambers were created using plastic buckets to house the samplers and organisms. The samplers and organisms were left in the wetland and lab for 28 days. Out of 209 biphenyl

congeners, a list of 135 PCB congeners was chosen for analysis. Total lipid content was also measured to obtain the lipid-normalized bioaccumulation. Sediment from the wetland had varying concentrations of bulk PCBs, ranging from 44 µg/Kg (S10) to 4286 µg/Kg (S11), where Hexachloro and heptachloro biphenyls were dominant. Most sediment samples had an average total organic carbon (TOC) percentage of around 2-3%, although S11 had a maximum of 41%. In-situ and ex-situ porewater concentrations (C_{pw}) generally agreed well, but occasional differences of up to a factor of 4.5 were observed. Due to limited recovery rates in the in-situ experiment, correlations between porewater and bioaccumulation were mainly established primarily for ex-situ clams and worms and in-situ clams. Strong correlations were observed between porewater concentrations and bioaccumulation in both organisms. The slope of $K_{ow}C_{pw}$ to lipid normalized concentration was found to be 2.86 for worms and 1.28 (lab), and 1.10 (wetland) for clams, where K_{ow} is the octanol-water partition coefficient. These findings suggest that porewater concentration can be useful for assessing bioaccumulation and supporting sediment site management and long-term monitoring.

5.11.P-We-119 Investigation of the Factors that Control and Predict Mercury Methylation in Soils and Sediments

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Mercury (Hg) can be transformed to methylmercury (MeHg) in aquatic sediments, which can bioaccumulate and biomagnify in the food web. The goal of this research is to develop a simple laboratory assay to experimentally assess biologically available Hg for methylation as well as identifying Hg methylation predictors in different soils and sediments. Hg methylation was evaluated in jar microcosms using seven different sediments from marine, freshwater, and terrestrial floodplain sources. Total Hg (THg) and net MeHg were measured in both sediments and porewater at 6 and 10 weeks designed to maximize MeHg formation. Porewater concentrations were measured via DGT devices and in filtered water. The primary results from the microcosm experiments showed that bulk THg was not a good indicator of MeHg production, however porewater THg was positively correlated with MeHg concentration for all sediments after correcting for colloidal/particulate dissolved organic carbon (DOC) ($r^2=0.94$ and $p < 0.002$). The effect of sediment particle size and Hg speciation (assessed by X-ray Absorption Spectroscopy) on methylation potential were also investigated. Two sediment samples were examined: one from a freshwater river (SS1) containing over 67% organic thiol-Hg ($Hg(SR)_2$), and another from a marine sediment (SS7) containing over 65% (β -HgS). The sediments were fractionated into four size fractions ($<0.5 \mu m$, $0.5-2 \mu m$, $2-45 \mu m$, $>45 \mu m$), and each fraction, were incubated with a pure culture of Hg methylator bacteria (*Desulfovibrio Desulfuricans* ND132) for 24 hours, followed by the evaluation of filtered THg, net dissolved MeHg, and unfiltered THg. Results revealed that nearly all dissolved mercury below $0.2 \mu m$ methylated. Thus, the distinguishing factor between SS1 and SS7 was the fraction of dissolved mercury relative to the THg in the system. The fractions of SS1, dominated by Hg-thiol species, demonstrated 1.5-4 times higher bioavailability compared to the size fractions of SS7, dominated by metacinnabar. Moreover, Hg methylation in SS1 and SS7 did not correlate well with the surface area indicating the complexity of the sediments. The results of the study highlight the significance of Hg species within the sediments, as they play a crucial role in controlling the dissolution of Hg and methylation process.

5.12.P-We General: Environmental Risk Assessment

5.12.P-We-120 Assessment of Allelopathic Plant Residual Effect of GM (Genetically Modified) Plants on an Indicator Plant

Jae Kim and Joshua Fischer, Bayer Crop Science

Allelopathy is a biochemical interaction between plants in which a donor plant releases secondary metabolites or bioactive molecules as volatiles, leachates, tissue residues, or root exudates that are detrimental to the growth of its neighboring plants. Allelopathic plant residual effect data inform on plant pest potential and contribute to the environmental risk assessment of genetically modified (GM) crops. Sensitive species such as radish can be

used to test for potential allelopathic impacts on plant growth and development. We discuss the development of an experimental design to generate robust allelopathic residual effects data that contributes as part of a weight of evidence approach to the environmental risk assessment of a GM crop.

5.12.P-We-122 Weight of Evidence Environmental Persistency Assessment for Data Poor Synthetically Modified Biopolymers

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The use of synthetic polymers in various industrial applications has become ubiquitous. In the area of cosmetic products, these applications enable the important functionalities of e.g., rheology modifying and film forming. However, established materials are often known to be persistent in the environment. In contrast, natural biopolymers e.g., Cellulose, Starch, Guar are biodegradable by definition but both their performance as well as their variety of resulting sensorial profiles are limited and not up to par to conventional technologies. Synthetically modified biopolymers are an interesting and promising alternative for cosmetic product formulations as they combine several aspects: Their natural polymer backbone is biodegradable and their functional hydroxyl groups allow a wide range of synthetic modifications and functionalities. The current screening tests to rule out persistency (e.g., OECD 301 and OECD 302) were not developed for polymers. As polymers are exempt from registration e.g., via REACH in the EU, data gaps are frequent. To ensure fundamental biodegradability a weight of evidence persistency assessment is proposed which takes into account OECD screening results, structural analysis of the respective synthetically modified biopolymer and higher tier testing results. The case studies presented are from the family of celluloses and starches.

5.12.P-We-123 Environmental Risk Assessment Screening Proposal for Data Poor Substances – Calibrated with Data Rich Substances

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When undertaking Environmental Risk Assessment (ERA), one routinely has to deal with data gaps. This is especially true for cosmetic ingredients as they are rather in the lower tonnage bands. Hence, e.g., EU-REACH requires less environmental hazard data for the registration of the respective substances. However, when ingredients are used additionally in other industries, tonnages, and environmental hazard data increase, respectively. Alcohol ethoxylates, an important class of non-ionic surfactants are used in e.g., laundry detergents, household cleansers, agriculture as well as in cosmetic formulations. The HERA (Human & Environmental Risk Assessment on ingredients of European household cleaning products) report for Alcohol Ethoxylates (version 2.0, September 2009) includes an extensive ERA for these chemicals. We developed an ERA screening for data poor chemicals and used the HERA data as a benchmark for our evaluation of Alkyl-PEGs. Our proposed ERA screening is based upon input data from consumer use and formulation concentration in cosmetic products. Predicted Environmental Concentrations (PECs) are derived for surface water and soil. Respective Predicted No-Effect Concentrations (PNECs) were derived for freshwater and soil applying standard methods and defaults. In a first step towards a refined ERA, we applied regional wastewater volumes and dilution factors for different countries. The risk quotients obtained by our screening methods were more refined than via default values. However, when comparing with the results of the data-rich HERA report, they are still conservative. By applying this proposed ERA screening method, we are confident that we can effectively identify critical ingredients within a larger portfolio of chemicals, leading to a more refined and accurate assessment of their potential environmental risks and ultimately supporting the development of more sustainable and environmentally friendly cosmetic formulations.

5.12.P-We-124 Estimating Nectar Contamination with Pesticides Using Leaf Tissue Measurements

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Exposure to pesticides is one potential factor contributing to the loss of pollinators and pollinator diversity

observed over the recent past. Pollinators may be exposed to pesticides by ingestion or contact when collecting nectar and/or pollen from contaminated flowers. Given that pesticides are necessary tools for economically viable ornamental plant production at large scales, pest management practices should be optimized to reduce exposures of pollinators as much as possible, while simultaneously providing adequate control of pests. Before practices can be optimized, an understanding of the influence of pesticide application and cultural practices on contamination of floral resources is needed. Few studies have specifically focused on the relationship between pesticide management during ornamental plant production and contamination of nectar, likely due to challenges associated with nectar sampling and analysis. This project evaluated the feasibility of predicting pesticide concentrations in nectar using concentrations measured in leaves in a study focused on evaluating the influence of pesticide application methods on contamination of nectar and leaves of *Salvia* x 'Indigo Spires'. This study used the systemic insecticide thiamethoxam as a model pesticide. Applications were made with tap water (zero rate) and at the lowest and highest labeled rates for the commercially available product for drench and spray applications. Nectar was sampled using 50 μ L microcapillary tubes, leaves were sampled by hand, leaves were extracted, and both analyzed by LC-MS/MS. Regression analysis was used to identify the best-fit equations for describing relationships between concentrations in nectar and leaves. Measured concentrations in the leaves were then fed into the best-fit equations solving for concentrations in nectar. Accuracy was then determined by comparing predicted values to measured values. Results indicate that analysis of leaves might serve as a good indicator of nectar contamination. More research is needed to evaluate the predictive accuracy for other pesticide classes. Results also indicate that concentrations of the systemic insecticide thiamethoxam and its metabolite clothianidin in nectar exceeded published LC50's for native bees and/or honeybees, with the highest concentrations occurring with drench applications.

5.12.P-We-125 Health Risk Assessment of Globally Consumed Shark-Derived Products

Laura Garcia Barcia¹, **Annette Valdes**¹, **Cindy Gonzalez**¹, **Stanley Shea**², **Yong Cai**¹, **Lanya Fanovich**³, **Demian Chapman**⁴, **Aljoscha Wothke**³ and **Ryan Mohammed**³, (1)Florida International University, (2)BLOOM Association, (3)Environmental Research Institute Charlotteville (ERIC), (4)Mote Marine Laboratory Shark meat and fin soup are seafood commodities exported and consumed worldwide. Because sharks are large and slow growing marine predators, they tend to accumulate a large amount of mercury in the form of methylmercury. Due to this well-documented biomagnification, many nations have blanket advisories urging citizens, especially pregnant women and children, to avoid consuming shark-derived products. Mercury levels, however, may vary greatly within and among shark species. Since shark-derived products can represent an important source of protein in some communities, assessing the consumption health risks of different shark species is needed. Here, we analyzed total mercury, methylmercury and selenium concentrations of 267 processed fin trimmings from Hong Kong Special Administrative Region and mainland China and 33 meat samples from Trinidad and Tobago. In conjunction with the most recent data from consumer practices surveys, and taking into account consumption rates, average body weight of consumers and the chemical interaction between mercury and selenium, we calculated the risks of consuming shark meat and shark fin soup from different shark species. We found that blanket maximum safe consumption limits applied to shark products are often overly restrictive because they do not take mode of consumption into account. Nonetheless, meat and fin products from large hammerhead species, *Sphyrna mokarran*, *S. zygaena* and *S. lewini*, need to be avoided by consumers, and not sold by retailers, due to high mercury levels. Species-specific advisories should also be issued for meat and fin products from oceanic whitetip and dusky smooth-hound sharks, which should be avoided by women of childbearing age.

5.12.P-We-126 Macro-Fouling of Cutlery and Other Single Use Plastics in a North Carolina Estuary

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Globally, over 8 million metric tons of plastic enters the ocean annually. This plastic is an open surface for

macro-fouling organism settlement. We exposed a variety of single use plastics on floating arrays in the tow 10 cm of the water column. Macro-foulers settled within days and common groups such as barnacles, bryozoans, ascidians and hydroids were reproductive in less a month. Communities developed on the different single-use plastics were different. Some of differences are related to critical surface energy. Other differences may be due to contaminants and additives that leach from the plastics. Single use plastic pollution provides dispersal mechanisms for members of the invasive ecosystems that dominate harbors.

5.12.P-We-127 Analysis of the Geographic Exposure of Reptiles to Pesticides in Brazil and Suggestion of Focal Species in the Context of Environmental Risk Assessment

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Exposure to chemicals is considered one of the decline causes of herpetofauna populations worldwide. In pesticide Environmental Risk Assessment (ERA) for reptiles, the protection goals are linked to the ecosystem services provided by these animals, which makes the conservation of the various species of this group crucial. In this work, we sought to estimate, for all Brazilian states, the exposure of reptile populations to pesticides and to identify possible focal species for ERA. In order to obtain an appraisal of reptile exposure to pesticides in Brazil, we integrated three variables: (1) the reptile species richness of each Brazilian state; (2) the number of endangered species per state; and (3) the ratio of pesticides sold in each state per area – considered here as an indicator of pesticide use. When working with species diversity, we considered only the species present in agricultural lands. States with high species richness, an increased number of threatened species, and large pesticide use were considered to be where the reptiles are more exposed and hence where the species are more vulnerable. The richest states in reptile species are Mato Grosso (289 species), Pará (258), and Bahia (255); those with the most significant number of threatened species are Bahia (17), São Paulo (9) and Rio Grande do Sul (8); and the states with the highest pesticide sales are: São Paulo (40.3 kg/ha), Paraná (37.18 kg/ha) and Rio Grande do Sul (26.16 kg/ha). This preliminary analysis indicates that São Paulo, Rio Grande do Sul, Bahia, and Paraná have the highest exposure index. Regarding the different groups of reptiles, the species with occurrence in more states are *Xenodon merremii* (25), *Leposternon microcephalum* (14), *Salvator merianae* (14), and *Caiman latirostis* (15) - which also occur in the states with the most exposure. This information should be taken into account when delimiting focal species for ecotoxicological studies, even though exotic species and those that have proven easy to reproduce in captivity can be used for this purpose. In these cases, however, it is important to consider extrapolation factors. The use of exposure indexes is greatly relevant in the definition of priority regions and the refinement of future ERA scenarios. Such scenarios should be as realistic as possible and scientific community must work together with decision makers to protect the entire group of reptiles.

5.12.P-We-128 An Ecological Risk Assessment of Perfluorooctane Sulfonic Acid (PFOS) Exposure to Piscivorous Receptors: Evaluating Sources of Uncertainty

Stephanie A. Maggio and William A Schew, GHD

Per- and polyfluoroalkyl substances (PFAS) are contaminants of concern that are detected ubiquitously in the environment and have been shown to cause toxic effects to exposed organisms. Ecological receptors can be exposed to PFAS through direct contact with contaminated environmental media and uptake via food web interactions. Ecological risk assessment often requires a multitude of data sources (i.e., surface water, sediment, soil, and biological tissue concentrations). When appropriate food chain data are lacking, bioaccumulation factors and, if necessary, conversion factors can be used to derive the missing food chain modeling input parameters. However, this introduces varying levels of uncertainty and can alter risk characterization and resultant understanding. To elucidate potential effects that these sources of uncertainty can have on ecological

risk characterizations, we present an evaluation of the exposure of piscivorous receptors to perfluorooctane sulfonic acid (PFOS) in a large river. Surrogate receptors include the green heron and mink, which feed on small forage fish. To evaluate risk to these piscivorous receptors, whole-body forage fish tissue concentrations are necessary to parameterize food web exposure models; however, available data are limited to surface water, sediment, and predatory fish fillet tissue concentrations. Bioaccumulation factors can be used to estimate fish tissue concentrations from environmental media, and conversion factors can be used to calculate whole-body fish tissue concentrations from fish fillet concentrations. Incorporation of these factors introduces different levels of uncertainty and in turn, result in varying levels of risk. Objectives of this exercise are to: 1) Derive whole-body fish tissue concentrations from surface water, sediment, and fish fillet tissue concentrations, and 2) Compare ecological risk to piscivorous receptors using the derived whole-body fish tissue concentrations. Results from this exercise are presented and discussed to evaluate how different sources of uncertainty have the potential to affect ecological risk characterizations.

5.12.P-We-129 General Approach for Selecting Analogues for Environmental Hazard of TSCA Chemicals

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Consideration of hazardous effects to aquatic and terrestrial organisms is essential to conducting chemical risk evaluations under the Toxic Substances Control Act (TSCA). When empirical data on the chemical substance being evaluated are not available to assess environmental hazard, new approach methods (NAMs) may be considered, or the Environmental Protection Agency's Office of Pollution Prevention and Toxics (EPA/OPPT) may request testing. Section 4(h) of the 2016 Frank R. Lautenberg amendment to TSCA identifies the following areas of information to be considered prior to making a request or adopting a requirement for vertebrate testing: available toxicity information, computational toxicology and bioinformatics, high-throughput screening methods and the predictions of those models; and encouraging the chemical grouping information. This work describes considerations that can be applied across OPPT programs (new and existing chemicals work) when identifying analogues for environmental hazard identification. Key elements relevant to environmental hazard read-across methodologies include structural similarity, similar behavior between analogue and target chemical substance in the environment (e.g., physical, chemical, and/or environmental fate), and similar behavior in vivo (e.g., hazard and/or absorption, distribution, metabolism, and excretion). This work describes general approaches on the lines of evidence for analogue selection, including structural, physical, chemical, environmental fate, and toxicological similarity. Analogue selection tools applicable to environmental hazard are discussed with an emphasis on NAMs identified in the TSCA Section 4(h)(2)(C) List of NAMs (e.g., Analog Identification Methodology [AIM] and OECD QSAR Toolbox) and EPA tools (e.g., Cheminformatics Modules and others). These approaches provide flexibility in the method by which an analogue is determined suitable for use in a risk assessment. The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency.

5.12.P-We-130 Survey and Organization of Test Guidelines Available for Environmental Hazard in TSCA Risk Evaluations

Christopher Green, John Allran, Jennifer C Brennan, Karen Eisenreich, Jeffrey Gallagher, Hector Malagon, Andrew Sayer and Emily Vebrosky Nolan, U.S. Environmental Protection Agency

Test guidelines (TG) serve to detail the materials, methods, and analysis needed to generate hazard data used within risk assessments. Navigating available TGs to ensure appropriate data are generated is important for scientifically sound environmental hazard characterizations. In addition, the evaluation of reasonably available literature within systematic review relies on understanding TGs and determining if studies adhered to the appropriate protocols for the production high quality information. The (OCSPP) Series 850 – Ecological Effects #SETACLouisville

Test Guidelines are intended to meet toxicity testing requirements of terrestrial and aquatic plants and animals under the Toxic Substances Control Act (TSCA); Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); and Section 408 of the Federal Food, Drug, and Cosmetic Act (FFDCA). The current work seeks to detail TGs available from OCSPP and the Organisation for Economic Co-operation and Development (OECD) by organizing their methodologies, durations, taxa relevance, and the relevance of the endpoints for risk assessment of environmental receptors. This work details the organization of aquatic toxicity TGs into groups of tests encompassing water column invertebrates, benthic invertebrates, aquatic vertebrates, and aquatic plants. In addition, terrestrial TGs are organized among soil invertebrates, terrestrial insects, terrestrial vertebrates, and terrestrial plants with further divisions among exposure durations. Species selection considers taxonomic and environmental relevance for species extrapolation (e.g., habitat, life stage, relative sensitivity). Furthermore, complementary toxicity data with acute and chronic durations of exposure for the same species allows for better comparison of toxicity and risks resulting from different exposure durations. Selection of the test duration should also be made based on the chemical profile (e.g., release information, chemical persistence). The creation of decision trees to map environmentally relevant OCSPP and OECD TGs aides in reviewing of reasonably available information and in identifying appropriate tests needed within TSCA risk evaluations.

5.12.P-We-131 Evaluating the Framework of a Metals Environmental Risk Assessment through the Lens of Climate Change

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Climate change is an extremely complex topic which will not only have major impacts on the physical and chemical constituents in the environment but could also alter the framework upon which environmental risk assessments are conducted. In early 2023, a collaboration between several metals associations and the International Council on Mining and Metals (ICMM) launched an initiative to evaluate how different aspects of metals environmental risk assessments may need to be reformed to account for the effects of our changing climate. Specific topics that were evaluated include: exposure assessments, effects assessments, bioavailability and modeling considerations, risk characterizations, sensitivities pertaining to the marine environment, uncertainty analyses, and classification schemes. The first phase of the project charged six experts to review the current guidance materials (e.g., MERAG documents, <https://www.icmm.com/en-gb/guidance/mining-metals/merag>) and to identify areas which may require additional considerations or changes to the guidance to ensure metals environmental risk assessments remain applicable and accurate as we navigate the future changing climate. This presentation will provide an overview of the conclusions stemming from each aspect of an environmental risk assessment. Being informed by these conclusions, the authors aim to generate a peer-reviewed scientific article highlighting the necessary changes to the Metals Environmental Risk assessment paradigm. This manuscript is targeted for completion in mid-2024.

5.12.P-We-132 Establishing a Geospatial Tool that Allows for Data-Based Tracking of Remediation and Assessments of Hazards Posed by Mined Lands in the Continental United States

Serena Mercedes Seawolf, Daniel K Jones, Brianna Williams, Stephanie E Gordon, Rachel M Atkins, Ashley L Whipple, Adrian P Monroe, Mark T Hannon and Kyle D Enns, U.S. Geological Survey

Mined lands, both surficial and underground, create a set of diverse environmental and socioeconomic impacts. Therefore, there is a large interest in accurately assessing hazards surrounding mines as well as systematically approaching remediation and reclamation of active and abandoned mined lands. To date, there is no robust

centralized dataset that addresses characteristics such as mine location, commodity, reclamation status, environmental features, and hazards for stakeholders and decision-makers to evaluate and prioritize remediation efforts. To address this, the USGS is developing a decision support tool combining national and regional data in an online, accessible, and interrogatable geospatial application for Department of Interior users. The application's aim is to aid stakeholders in identifying potential risks and hazards to vulnerable communities, as well as prioritizing and tracking remediation efforts, in an easy and transparent manner. Additionally, this tool will have the capability of generating hazard metrics which will guide users in the planning stages and progress of mine reclamation. Field activities in 2024-2025 will leverage application-derived metrics to prioritize locations for study in the Colorado River Basin and Appalachian regions. This project is a partnership between the USGS, Bureau of Indian Affairs, Bureau of Land Management, Bureau of Reclamation, National Park Service, and the Fish and Wildlife Service. Co-development with partners will ensure the effectiveness and usefulness of this web application which has the potential to guide well-informed decision-making.

5.12.P-We-133 Roadmap: Labcorp's approach to Next Generation Risk Assessment

Emma Danby and Sabitha Papineni, Labcorp Early Development

Current approaches for the safety assessment of human health and environmental risks due to chemical exposure have heavily relied on using animal models. The approach has generally been conservative and as such there has been a reluctance to change the way we perform risk assessments. However, given the amount of chemicals in need of assessment and the additional endpoints needing testing, such as endocrine activity, there has been a global awareness of the need for a paradigm shift towards moving away from animal models to more scientifically relevant hypothesis driven testing. Novel Approach Methodologies (NAMs) are being used to improve the process of chemical risk assessment activities by combining different advanced biological tools to make risk assessment decisions such as high throughput screening (HTS) in vitro assays, integrated omics techniques and molecular biomarkers with sophisticated computational techniques such as quantitative structure activity relationship (QSAR) modelling, in vitro to in vivo extrapolation (IVIVE) and physiologically-based pharmacokinetic (PBPK) modelling. As a result, next-generation risk assessment (NGRA) is becoming a reality. Many see these scientific advancements as the end of the traditional Contract Research Organisations (CROs) as businesses gradually rely less on animal testing and more on NAMs. However, at Labcorp we are helping to drive these changes and embracing NAMs to enable us to continue to support companies as we transition into the next phase of risk assessments in the 21st Century. Here we present the first look at how Labcorp already offer many alternatives following the 3R principles to reduce, refine and replace the use of animals in human health and environmental risk assessments and our roadmap for the future.

5.12.P-We-134 Pharmaceuticals and Personal Care Products (PPCPs) in the Terrestrial Environment: What the Fuss?

Oluyemi Ojo, University of York

Pharmaceuticals and personal care products (PPCPs) are perceived as contaminants of global concern due to their inherent ability to induce physiological changes in humans at low doses. Despite the increased use of PPCPs, their eco-toxicological profile in the environment is yet to be established. This study is focused on appraising the potential ecotoxicological effects of PPCPs on the terrestrial ecosystem. A systematic review of previous publications and ecotoxicological database revealed PPCPs have an inhibitory or lethal effect on the biochemistry, physiology, population and yield of some plants, soil microbiota and soil invertebrates. A total of 2678 publications with 857 soil entries were identified in the Umweltbundesamt database (UBA 2021) to develop the occurrence data. The most highly monitored antimicrobials in soils were tetracycline, oxytetracycline and chlortetracycline and 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 greater than 1 i.e (RQ > 1). Furthermore, several knowledge gaps have been identified. This include; toxicity data for chemicals and degree to which the ecotoxicological effects of PPCPs could disrupt ecosystem service delivery is yet to be well elucidated. The sorption, desorption, translocation and potential biochemical transformation of PPCPs in soils are yet to be described. The impacts of PPCPs on vital microbial functions at the soil-rhizosphere are also yet to be constrained. On-going field and laboratory studies are considering the toxicological effects of top fifteen most frequently occurring antibiotics on three terrestrial plants (wheat, rye, lettuce), microbes and a soil invertebrate; earthworm. Results from this study would inform policies on the management of potential threats from PPCPs.

5.12.P-We-136 Considerations for Reduced Animal Testing for Environmental Risk Assessments of Pharmaceuticals

Emma Danby, David Mayfield and Francis Crawley, Labcorp Early Development

Environmental risk assessments (ERA) for pharmaceuticals are necessary to fulfil global registration requirements. The data needed to describe environmental fate and aquatic or terrestrial hazards can lead to large testing programs and potentially high vertebrate usage. For example, both acute and chronic toxicity testing and bioaccumulation testing in fish species are often required after being triggered as part of the Persistent, Bioaccumulation and Toxicity (PBT) assessment. These tests alone can use in excess of one thousand fish depending on study design and replication. When the endocrine disruption endpoint testing is also required (e.g., Organisation for Economic Co-operation and Development [OECD] guidelines 230, 234, 240), this figure can increase exponentially. In consideration of the global goal of 3R (replacement, reduction, refinement) testing approaches, we have been working on strategies to provide the data required to fulfil the needs of an ERA while minimising animal use. In this analysis, we explore available strategies to limit or waive *in vivo* tests including: (1) literature-based weight of evidence assessments, (2) modifications to acute (OECD 202) and chronic (OECD 210) toxicity tests, and (3) adjustments to fish bioaccumulation tests (OECD 305). In addition, new approach methods (NAMs) (i.e., *in vitro* alternatives) will be discussed. We demonstrate these strategies through a series of case studies such that industry partners and regulators can explore options to reduce the use of vertebrates and expedite their ERA submissions.

5.12.P-We-137 Solid Media Sampling Considerations for Collecting Data Appropriate for Use in Risk Assessment

Julie Rothrock, Sarah Conlin, Donna Getty and Mark Follansbee, SRC, Inc.

Many Superfund sites are contaminated with metals as a result of historic smelting and mining operations, and metals at these sites are typically the contaminants of interest. While standard methods are available for collecting samples to establish the nature and extent of metals contamination in soil and sediment, those methods do not necessarily ensure that samples collected in the field will result in data that are appropriate for use in risk assessment. Site investigations to characterize the nature and extent of contamination for the Remedial Investigation/Feasibility Study (RI/FS) process often miss the opportunity to simultaneously collect samples for use in the risk assessment, leaving risk assessors and risk managers to either evaluate risk based on incomplete or inappropriate information or collect additional data. For those complete exposure pathways identified in the conceptual site model, factors such as sampling depth, particle size, and soil bioavailability of lead and arsenic are critically linked to exposure and are key to the applicability of the solid media sampling data for use in risk assessment. Ideally, these factors should be discussed in data quality objectives (DQOs) established prior to sampling. This poster provides an overview of solid media sampling parameters that should be considered when writing DQOs, Quality Assurance Project Plans, and Sampling and Analysis Plans prior to field sampling and data collection. This evaluation is geared towards sites on the National Priorities List for

evaluation under the Comprehensive Environmental Response, Compensation, and Liability Act, and assumes that Preliminary Assessment and Site Inspection activities have already occurred. This poster focuses on answering questions related to collecting solid media samples for use in human health risk assessment and/or ecological risk assessment as part of the RI/FS process to improve the data available to support risk assessments and avoid the need for additional site sampling activities. While this poster presents key solid media sampling considerations, site-specific needs should always be accounted for as well.

5.12.P-We-143 Developing Guidance for Performing Risk Assessments Applicable to the ASEAN Region

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In 2021, a virtual working group focused on chemical risk assessment was established to support the efforts of the Association of South-East Asia Nations (ASEAN) Regulatory Cooperation Project (ARCP) in promoting regulatory alignment in the southeast Asian region. The development of guidance documents that serve as relevant and useful resource references for ASEAN Member States (AMS) in the development of chemical management regulatory implementation is a key focus of ARCP. To support this initiative, the working group participants were charged with collating existing frameworks (e.g., EU REACH, UN GHS) into the development of a cohesive risk assessment guidance document that can be promoted and implemented across the ASEAN region. Through a series of workshops, inputs from regulators and industry from all AMS are being considered and discussed in the development process of the guidance documents to ensure its relevancy and fit-for-purpose. This guidance includes assessments for both human health and the environment, and specific chapters that have been collated for this effort include: problem formulation, data gathering, hazard identification, exposure assessment, hazard assessment, risk characterization, risk management measures, uncertainty analysis and documentation. Having a coherent regulatory environment allows the member states to export their products to the rest of the ASEAN with confidence. This is further achieved by having consistency in classification, labelling and risk assessment with existing systems across different global jurisdictions. A harmonized framework with consistent requirements, standards and processes allows member states who have lesser resources to enable the building of their foundation. This, in turn, will support a faster adoption of the existing regulatory framework, thereby creating a stabilized regulatory environment that is consistent with global standards.

5.12.P-We-144 Target Organ Toxicity in Sprague Dawley Rats Following Oral Exposure to Groundwater Mixture: Assessment of Dose-Response Relationships of Histopathological, Biochemical, and Behavioral Alterations.

Bright Boamah¹, Steven Siciliano¹, Natacha S Hogan¹, Mark L. Hanson², Markus Hecker¹, Patrick Campbell³, Rachel Peters⁴, Ahmad Al-Dissi¹, Cameron Morse¹, Dylan Olver¹ and Lynn Weber¹, (1)University of Saskatchewan, (2)University of Manitoba, (3)WSP E & Canada Limited, (4)Federated Co-operatives, Ltd. Groundwater collected from an industrial site containing a complex mixture of contaminants was characterized for toxicity via an oral exposure study with Sprague Dawley rats. Males and females were tested (n=5 /exposure) with drinking water (control group), 10% reference water (taken from edge of property) compared to 0.01, 0.1, 1 and 10% groundwater collected nearest known soil contamination (high impact) for 60 days. Plasma albumin was significantly reduced in the 10% high impact and reference groups compared to control. Plasma alpha 2 macroglobulin, an inflammatory biomarker was significantly reduced in all groups compared to the control. The histological score of hepatic inflammation was elevated in the 1% and 10% high impact groups

compared to control. Histopathological grading score was significantly increased in the 10% low and 10% high impact groups for acute tubular necrosis. The female rats showed a unique pattern of vacuolar degeneration along the cortico-medullary junction of the kidney. Plasma symmetric dimethylarginine (SDMA), a biomarker of glomerulotoxicity, was significantly elevated in the 1% and 10% high impact groups compared to control. A novel object recognition test after 60 days showed a significant reduction in the discriminatory index at 1- and 24-hr intervals within the 10% high impact group compared to control, indicating memory impairment. Carotid artery blood flow was observed to be significantly reduced in the 10% low impact group. Overall, the groundwater collected from both the high impact and reference wells at the study site induced hepatotoxicity, nephrotoxicity, and neurotoxicity. This pattern of effects is consistent with 2,4-dichlorophenoxy acetic acid and medium-high molecular weight hydrocarbons (F2 and F3) driving toxicity of groundwater throughout the site.

5.12.P-We-146 Comparing Water Quality Criteria from Around the World: Phthalates, Organophosphates, Carbamates, and Pyrethroids

Andrew James Hetrick¹, Ryan R Otter¹, Michelle Rau Embry² and Kristin A Connors³, (1)Middle Tennessee State University, (2)Health and Environmental Sciences Institute (HESI), (3)Procter & Gamble

Water quality criteria are threshold values established by entities (e.g., countries) to protect aquatic organisms. Key factors such as water type (freshwater or saltwater) and exposure length (acute or chronic) are considered by some, but not all entities. It is well established that deriving water quality criteria varies by region due to differences in minimum data requirements and the use of uncertainty factors. Water quality values are based on aquatic toxicity data, species sensitivity distributions (SSDs), or other probability distribution-based approaches on a chemical-by-chemical basis. The goal of this study was to compare the different available water quality criteria values used around the world and processes used to derive specific criteria for phthalates and multiple pesticide groups (organophosphates, carbamates, pyrethroids). In addition to chemical-specific comparisons, analysis of grouped data was performed using the ecological threshold of toxicological concern (eco-TTC) to predict a de minimis toxicity value for chemical groupings. This analysis was performed to explore the applicability of different approaches to derive water quality criteria depending on the amount of available data.

5.12.P-We-147 Evaluating Contaminant Dynamics in a Periodically Inundated Floodplain

Ashkan Alborzi¹, Hasti Ziaei Jam¹, Negar Shaghaghi¹, Amrika Deonarine¹, Benjamin Johnson², Sarah Greenfield³ and Danny D. Reible¹, (1)Texas Tech University, (2)GSI Water Solutions, Inc., (3)Oregon Department of Environmental Quality

Porewater passive sampling was conducted in a contaminated floodplain located at St. Helens, Oregon to understand the mobility and bioavailability of chemicals of concern (COCs). The site has been polluted with legacy contaminants by an industrial discharge since 1930s. Contaminant fate and behavior at the site is complicated by water variations due to tidal conditions as well as seasonal variations. Passive sampling was conducted at various porewater locations in different tidal and geological conditions as well as several surface water stations. The target depths for porewater samples are based upon the depth of bioturbation, the transition between oxic and anoxic conditions, and the anoxic zone. The samples were collected at three depths (6-in intervals) to analyze mercury, arsenic and organic contaminants as well as anions (Cl^- , NO_3^- and SO_4^{2-}) to facilitate the understanding of transport processes. The water level changes during the period of sampling and the resulting behavior of redox sensitive contaminants will be summarized. Use of the passive sampling for organics normally requires non-equilibrium sampling and the use of performance reference compounds (PRCs) to infer extent of non-equilibrium. The effects of water level variations and subsequent periods of passive sampler exposure to unsaturated soils on PRC release and target compound uptake was analyzed. Variations with depth and spatially across the site were evaluated and correlated with the water level fluctuations. A model was developed of the PRC release and target compound uptake to evaluate specifically conditions that will lead to misleading characterization of equilibration as a result of non-reversible release of PRCs. These conditions will be explored and summarized.

5.12.P-We-148 Validation of an Aquatic Food Web Model Using Site-specific Case Study Data

Jean Zodrow¹, Jason M. Conder¹, Marianne Batchelder¹, Caitlin Johnson¹ and Katherine Parakal², (1)Geosyntec Consultants, Inc., (2)Ohio State University

Food web models allow ecological risk assessors to predict concentrations of per- and polyfluoroalkyl substances (PFAS) in wildlife diet items, and thus are an important tool to calculate wildlife dietary exposures in ecological risk assessments (ERAs) at PFAS-impacted sites. An abundance of abiotic and food web data at many PFAS field sites have emerged in the last few years since food web models for PFAS were first developed, allowing a more robust validation of these models than was previously possible. Using data from ten field studies, concentrations of PFAS in surface water and sediment at aquatic sites were used to generate predictions of PFAS in tissue (using food web models) that were compared to the measurements of PFAS in biota (invertebrates, fish, plants) from those same sites. Forage fish was the most well represented trophic level in the field studies, as this class had the highest number of data points (249); predatory fish, aquatic plants, benthic invertebrates, and pelagic invertebrates exhibited fewer data points at 104, 81, 80, and 44 data points, respectively. Perfluorooctane sulfonic acid (PFOS) had the highest number of data points, with Perfluorohexane sulfonate (PFHxS) and Perfluorooctanoic acid (PFOA) often a close second, depending on the biota type. Model-predicted PFAS and measured PFAS concentrations from the studies were compared for all PFAS as a group as well as individual PFAS (e.g., PFOS, PFOA). Model-predicted concentrations of PFAS in biota (i.e., invertebrates, plants, and fish) were generally within a factor of 5-10 of the measured values. Model agreement was robust for forage fish, especially for higher PFAS concentrations that would be expected at Aqueous Film Forming Foam (AFFF) sites. For example, 44% of model-predicted concentrations of PFAS in forage fish were within a factor of 5 and 59% of the model-predicted concentrations were within a factor of 10 for the highest 50% of forage fish measurements in the case studies. Importantly, model predictions generally agreed with measurements in terms of identifying the biota with the highest concentrations of PFAS at the sites. This validation effort provides confidence in PFAS food web modeling and helps identify areas of continued uncertainty and refinement of modeling efforts.

5.12.V General: Environmental Risk Assessment

5.12.V-024 DEB-IBM-based effect modeling of thiamethoxam on field populations of the non-biting mosquito *Chironomus riparius*

Tido Strauss¹, Jana Gerhard¹, Josef Koch¹, Nika Galic² and Maxime Vaugeois², (1) gaiac - Research Institute for Ecosystem Analysis and Assessment, Germany, (2) Syngenta

Population models are powerful tools for predicting species population dynamics for different environmental scenarios under consideration of natural and anthropogenic stressors, and have been increasingly used in ecological risk assessment, often coupled with TKTD effect models. We developed a model concept for simulating populations of *Chironomus riparius* with the aim of extrapolating ecotoxicological laboratory data for the systemic neonicotinoid insecticide thiamethoxam (TMX) to field scenarios. This concept combines individual-based models (IBMs), based on physiological dynamic energy budget (DEB) modules and complemented by toxicokinetic-toxicodynamic (TKTD) effect models for lethal and sublethal processes, driven by dynamic environmental conditions. The core of the model is an individual DEB-TKTD model that has been parameterized for TMX using laboratory tests at different temperatures as well as experimental results on density dependence. In developing such complex models, simplified assumptions have to be made about the relevant processes in population dynamics to be represented in a model concept. In addition to external factors such as weather conditions and food availability, population dynamics relevant processes such as larval background mortality and adult loss rates in the field due to dispersal or predation need to be considered. While physiological modules or effect models such as the TKTD model are at least conceptually easy to parameterize with laboratory data, it is much more challenging to quantify relevant processes at the population or ecosystem levels, such as density-dependent growth and reproduction, or food availability, which ultimately determine the

dynamic capacity of the population throughout the year. Here we present a modular model framework for a population model of *Chironomus riparius* and apply it to a risk assessment of TMX under field conditions. The transferability of exposure multiplication factors (EMFs) estimated at the individual level using the moving-time-windows approach to field populations will be evaluated for relevant application examples.

5.13.P-Mo Late Breaking Science: Environmental Risk Assessment

5.13.P-Mo-206 Analysis of Biological Contamination in the Buffalo River Headwaters, Arkansas

Patrick Dyer and Jennifer Bouldin, Arkansas State University

The 3,500 square kilometer Buffalo River Watershed provides habitat, leisure, and drainage for north central Arkansas. Lands that are managed for agriculture and those for beauty and enjoyment have fundamentally different goals. When grazing and recreational uses compete, it can be hazardous to people. Fecal coliform testing is used to evaluate public risk from biological contamination, which in this case is primarily attributable to cattle. A seasonal division is used to indicate when there is a higher risk of injury (primary contact) versus a lower risk (secondary contact). For the years 2020–2022, four headwater streams draining to the Buffalo River underwent weekly fecal coliform testing. Two sites were chosen on each headwater representing upstream and downstream conditions. The provided results are the mean colony forming units per 100-milliliters of site water. This data has been paired with spatial analysis of drainages calculated using a 5-meter resolution digital elevation model (DEM). Initiatives to improve water quality aim to strike a balance between social and environmental concerns for equitable management, but can these areas continue to support dual-use?

5.13.P-Mo-207 Chronic Exposure to Thiamethoxam Amplifies the Respiratory Burst Response in Zebrafish Larvae

Nicole Kooij, Allie Fowle and Tisha King-Heiden, University of Wisconsin, La Crosse

Thiamethoxam is a second-generation neonicotinoid pesticide designed to alter neural transmission in invertebrate pests with minimal toxicity to vertebrates. Due to its high use, thiamethoxam is now found to be ubiquitous in the environment, causing emerging concern regarding their potential to cause adverse health effects in both wildlife and humans. While limited, research suggests that some neonicotinoid pesticides may impair the immune system. Potential links between exposure to neonicotinoid pesticides and outbreaks of infectious disease necessitate evidence to identify the potential immunological risks neonicotinoid pesticides may pose. Here, we tested the hypothesis that chronic exposure to sublethal concentrations of thiamethoxam would suppress the respiratory burst response of neutrophils, an important part of the innate immune response. We used zebrafish as a model to bridge the gap between toxicity to aquatic organisms and human health. Zebrafish embryos were exposed to various concentrations of thiamethoxam (0.02 µg/L - 2000 µg/L) for 5 days, after which we measured their ability to produce reactive oxygen species (ROS) in response to a proxy immunogenic challenge. Our data indicates that thiamethoxam interferes with the normal innate immune response by increasing the amount of ROS produced, but the effects were not dose dependent. Over-production of ROS could be an underlying factor associated observed adverse health effects in vertebrates, and our results suggest that its potential to alter the immune response should be studied further. Our data can be used to better understand the potential immunological risk that chronic exposure to thiamethoxam poses to human health as well as wild fish populations.

5.13.P-Mo-209 An Agent-Based Model to Estimate EMF Exposure From Offshore Wind Infrastructure to Benthic Marine Organisms

Daniel Dawson, Damian V Preziosi, Robert Pastorok and Conner Schultz, Integral Consulting, Inc.

Offshore wind (OSW) infrastructure (e.g., buried cables) generates both electric and magnetic fields (collectively referred to as electromagnetic fields, or EMF) that may interact with various benthic organisms living in proximity to OSW developments. In recent years, the potential risks from EMF exposure to marine

organisms have been increasingly assessed using a common method of chemical risk assessments: the comparison of effect thresholds against projected exposure estimates to estimate a hazard quotient. In this case, exposure estimates are typically based on maximum modeled EMF using planned project components. Observed effects of EMF exposure, meanwhile, are largely from experiments detailing short-term behavioral changes of marine organisms following EMF exposure. In many of these studies, however, behavioral changes revert shortly after EMF exposure, and the effects themselves (e.g., changes in direction, movement speed, avoidance behaviors) may not clearly relate to an effect at the population level. Thus, such threshold-based assessments may suggest a need for greater conservatism in minimizing EMF exposure than is warranted. In this poster, we present the development of an agent-based model (ABM) to simulate EMF exposure to select species and extrapolate how observed effects of exposure may translate into hypothesized population-level impacts. The model is constructed in Netlogo 3D, allowing for exposure quantified in 3-dimensional space. Using a species shown to be sensitive to EMF in experimental work as a test case (little skate [*Leucoraja erinacea*]), the model is population-based, ecologically detailed and includes an individual-level bioenergetics module. This allows for the exploration and evaluation of a variety of hypotheses to relate EMF exposure to population-level effects. Along with the model description, we present initial simulation results, and detail a pathway for the calibration, validation, and application of the model for EMF risk assessments of OSW projects.

5.13.P-Mo-210 Chronic Exposure to Imidacloprid Modulates the Respiratory Burst Response in Zebrafish Larvae

Allie Fowle, University of Wisconsin, La Crosse

Imidacloprid (IM) is an emerging contaminant of concern due to its frequent use in both urban and agricultural settings leading to exposure risks to wildlife and humans. Growing evidence suggests that while less susceptible, chronic exposure to sublethal concentrations can cause adverse health effects in vertebrates, including the potential to impair immune function. Here we use zebrafish as a model to determine the potential effects of chronic exposure to IM on the respiratory burst response, which is the primary line of defense against invading pathogens during early stages of development. Zebrafish were exposed to various concentrations of IM (0.02 $\mu\text{g/L}$ - 2000 $\mu\text{g/L}$) beginning just after fertilization for 5 days and we assessed their ability to produce reactive oxygen species (ROS) in response to a proxy immunologic challenge. Interestingly, exposure to 0.02-20 $\mu\text{g IM/L}$ caused an over-production of ROS, while exposure to $> 200 \mu\text{g IM/L}$ suppressed ROS production. While over-production of ROS could lead to generalized oxidative stress, suppression of the innate immune response could impact the ability to fight disease. Since outbreaks of infectious disease in wildlife have coincided with the increased use of neonicotinoid and other pesticides, our work suggests that further studies should evaluate the potential for neonicotinoid pesticides to alter the immune response in vertebrates.

5.13.P-Mo-211 Development of Meta-Analysis Techniques to Evaluate the Biomagnification Potential of Chemicals and Contaminants in Wildlife and Food-Webs For Environmental Risk Assessments

Kate Fremlin and Frank Gobas, Simon Fraser University

There are many examples of chemicals and contaminants in the literature that exhibit substantial variation in their estimated potential to biomagnify in organisms and food-webs, which often leads to considerable uncertainty in how best to characterize them and consequently make regulatory decisions for them. For example, field studies investigating the bioaccumulation of decabromodiphenyl ethane (DBDPE; CAS: 84852-53-9) in food-webs or wildlife reported biomagnification factors (BMFs) and trophic magnification factors (TMFs) for DBDPE that were highly variable, and thus did not provide conclusive information on the biomagnification potential of DBDPE. Hence, we reviewed the literature for studies reporting a field-derived BMF and/or TMF for DBDPE with the objective to (i) develop a methodology for a weight of evidence approach and meta-analysis that can be used to evaluate field-based biomagnification and trophic magnification studies and (ii) apply it to DBDPE with the aim to inform regulatory decisions on whether DBDPE has

biomagnification and/or trophic magnification potential. For the weight of evidence approach and meta-analysis, we developed several study criteria and benchmarks to evaluate the level of quality and confidence in the reported BMFs or TMFs. We also examined the linear relationship between reported BMFs and/or TMFs with the associated quality and confidence score to evaluate if the overall level of quality and confidence of the study was related to the reported values. The quality and confidence scores of the field studies generally increased as the magnitude of the reported BMF ($p < 0.05$; $R^2 = 0.37$; $F_{1,15} = 10.5$) or TMF ($p < 0.05$; $R^2 = 0.52$; $F_{1,8} = 10.6$) in the study decreased. Typically, field studies with moderate to high quality and confidence scores reported BMFs and TMFs that were less than 1, whereas field studies with low to moderate quality and confidence scores reported BMFs and TMFs greater than 1. Ultimately, the meta-analysis indicated that concentrations of DBDPE exhibit a limited potential to bioaccumulate and biomagnify in top predators and food-webs since the field studies with the highest quality and confidence scores consistently reported BMFs and TMFs that were less than 1.

5.13.P-Mo-213 Occurrence and Distribution of Total Mercury in Fish Across the State of New Hampshire *Tate Libunao¹, Alan S. Kolok¹ and Jonathan Petali², (1)University of Idaho, (2)New Hampshire Department of Environmental Services*

Aquatic ecosystems in the northeastern United States have been labeled “biological mercury hotspots”, thus necessitating continuous reconnaissance of mercury concentrations in recreationally caught fish. The New Hampshire Department of Environmental Services’ Fish Tissue Mercury Monitoring Program (FTMMP) has worked statewide with community anglers to crowdsource fish tissue sampling to monitor mercury concentrations from 1992-2021. The objectives of this study were to 1) evaluate the spatial and temporal trends of total mercury concentrations (THg) in fish captured across New Hampshire and elucidate spatial data gaps for future crowdsourced science efforts, and 2) conduct a risk assessment utilizing this data with recently collected fish consumption data. A database of 4,190 fish that comprised of 19 species, 14 HUC-8 level watersheds, 246 waterbodies, and spanning 29 years were analyzed. Fish species, waterbodies sampled, and sample size varied considerably from 1992-2021. We used a linear mixed effects model to determine the spatial and temporal variability of THg across watersheds and estimate the effects of waterbody and waterbody-year variability. There were significant differences observed in the geometric means of THg from 1992-2021 and an overall significant decrease in the geometric mean of THg from 1992 to 2021, as well as significant differences in THg in fish among the 14 watersheds. Additionally, there was a high degree of variability in THg in fish within and among the waterbodies ($GCV = 18.8 \pm 95\%$); however, in paired waterbody-years, the variation was considerably less ($GCV = 0.8 \pm 95\%$). Model results suggest that there is considerable spatial heterogeneity of THg across watersheds, and variation can be substantial from waterbody to waterbody. While THg showed reductions overtime, concentrations in several waterbodies exceeded risk-based thresholds for sensitive populations. The FTMMP provides valuable long-term information on the temporal and spatial patterns of THg in fish across New Hampshire; however, some select watersheds had less than ideal sample sizes and should be targeted in the future within the FTMMP to improve our understating of fish tissue THg across watersheds.

5.13.P-Mo-214 Toward a Risk-Based Framework for Rapid, Tiered Assessment & Prioritization of Additives and Polymer-Associated Chemicals (APAC)

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With the potential inclusion of polymers within the scope of REACH as well as UN activities surrounding additives in polymers and plastic articles, efforts to identify and prioritize substances of potential of concern have increased substantially. While attempts have been made to prioritize additives and polymer associated chemistries (APAC), these approaches largely focus on high-level hazard properties of constituents (e.g., GHS classification) and minimize or ignore physical-chemical properties which control the fate & transport of these

materials within and between polymer matrices and their surrounding environments (i.e., exposure). Further, limitations of chemical identifiers (e.g., CASRN) to facilitate capture of categorical and structural-analogue data presents a challenge to reliably identifying and separating truly data-poor substances from substances which may be adequately assessed via read-across or weight of evidence (WoE) approaches. The combination of “hazard-only” frameworks and incomplete data collection may result in a significant over-estimation of the number of chemistries for which adequate data to quantitatively evaluate risk is not available. The purpose of this work is three-fold: (1) A systematic workflow for the collection and synthesis of in-silico, in-vitro, and in-vivo hazard data for chemicals without established GHS classification and/or threshold values (i.e., TDI, ADI) is proposed which leverages existing QSAR models, experimental databases, and regulatory guidance. (2) a workflow for the collection and synthesis of environmental fate, migration, uptake, and exposure for aquatic organisms as well as man exposed via the environment (MvE) is proposed which provides a basis for establishing predicted environmental concentrations (PECs) and human oral dose estimates to be used in quantitative risk assessment and prioritization, and (3) A tiered framework is proposed which incorporates both exposure and hazard information to develop quantitative and categorical assessments of risk, which can be used to effectively prioritize and screen additives and associated chemicals in a systematic and consistent way. The utility of the framework is evaluated using a database of polymer and plastic-associated chemistries (N = 13,178) compiled by the United Nations in a recent publication. Risk characterization ratios (RCRs) as well as data completeness metrics are used to prioritize and evaluate the database of chemistries.

5.13.P-Mo-215 From the Ocean to the Road: A Pilot Project to Use Recycled Plastics in Hawaii’s Roads

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Recycling plastics into asphalt roadways is gaining momentum as a viable mechanism to divert plastic from waste streams and repurpose it in infrastructure. This has the potential to reduce plastic waste lost to the environment, as well as decrease greenhouse gas emissions by replacing virgin plastic that is commonly produced for polymer-modified asphalt binder. As an island state and a hotspot for marine plastic pollution, Hawaii is exploring the feasibility and environmental impacts of replacing a portion of virgin plastic in asphalt roadways with locally sourced, recycled plastic. On the island of Oahu, recycled plastic waste was incorporated into a trial asphalt road and monitored for performance and leaching of microplastic particles and plastic additive chemicals. The trial road was paved in three sections, each with a different asphalt mixture. The control section was paved with asphalt containing styrene-butadiene-styrene (SBS) polymer modified asphalt binder, which is standard practice for public roads in Hawaii. Two additional trial roadway sections incorporated post-industrial high-density polyethylene (HDPE) pellets, one with SBS-modified binder and one with unmodified binder. Water samples were collected after performance testing of the three asphalt pavement mixes and were analyzed for microplastics and plastic additives. The tests performed were mechanical performance testing using the Moisture Induced Sensitivity Test (MIST) and Hamburg Wheel Tracker and field testing by stimulating rainfall on the trial road. Triplicate samples per asphalt mix per test (plus blanks) were collected with high-purity, 0.2 µm filtered water. The water samples were filtered through 20 µm polycarbonate filters, density separated with a sodium polytungstate solution, and filtered for particle analysis using reflectance micro-Fourier transform infrared microspectroscopy (µFTIR). Preliminary results show no SBS and minimal HDPE particles leaching from any of the asphalt mixes tested in the laboratory with MIST and Hamburg Wheel. Based on µFTIR analysis, the number of HDPE particles from asphalt containing recycled plastics is not greater than the number from the control mix. Analysis to confirm these results with Pyrolysis-GC/MS analysis of HDPE mass in all samples is forthcoming. These data are a critical next-step in understanding the tradeoffs between the environmental risk and benefits of recycling plastics in asphalt roadways beyond Hawaii.

5.13.P-Mo-216 Temporal Trends in Removal and Transformation, and Environmental Risk Assessment of Priority Pharmaceuticals in a Biological Reactor

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Biological reactor (BR) in sewage treatment plants plays a significant role in elimination of organic contaminants. These substances, however, are not fully degraded, but often converted into transformation products (TPs). Incomplete degradation followed by discharge into the environment can potentially pose a risk to the surrounding ecosystem. In this study, we conducted target screening using LC-HRMS to identify pharmaceuticals, major contaminants of sewerage, and their TPs, and to track their removal and transformation over time in the process of BR. A composite sampler was employed to collect samples for a total of 8 hours, considering the retention time (6hr) in the BR. As a result of the analysis on 76 substances, 35 pharmaceuticals and 8 TPs were measured. The quantified substances were assessed for risk based on PNEC (Predicted No-Effect Concentration) values. Caffeine, sitagliptin, olmesartan, climbazole, niflumic acid, carbamazepine, diclofenac acid and valsartan acid showed concentrations higher than 100 ng/L, resulting in an RQ (Risk Quotient) greater than 1. Specifically, olmesartan was assigned a value of 140, representing the highest RQ. Caffeine exhibited a removal rate of approximately 90 %, indicating relatively efficient elimination. The removal rate of caffeine's TP, Paraxanthine and Theophylline, reached as high as 94 %. However, the removal rates of the other substances were all below 45 %. Sartans (Valsartan, Olmesartan, Losartan, Irbesartan), primarily used for hypertension, demonstrated removal rates: 42 %, 45 %, 43 %, and 30 % respectively. In the influent, they exhibited the highest concentration during the time period from 13:30 to 14:30, gradually decreasing afterward. In the effluent, it was observed that the concentration decreased by approximately 10 % to 50 % compared to the influent. Their TPs, valsartan acid, exhibited higher concentrations in the effluent, indicating the transformation of sartans is significant. The concentration ratio of valsartan acid to sartans increased 4.4 times in the effluent compared to the influent. Even with a higher concentration of valsartan acid, the RQ was showed to be 120 times lower than that of the parent compound. The combined RQ for sartans and valsartan acid in the effluent decreased by a factor of 1.8. In conclusion, sartans were not fully eliminated, but the TPs formed showed lower risk, which eventually reduces ecosystem risk.

5.13.P-Mo-217 Molecular Examination of Tundra Swan Feces to Determine if Sex and Diet May Influence Pb Exposure Level at a Contaminated Site in North Idaho

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Spring migrating tundra swans (*Cygnus columbianus*) use lead (Pb)-contaminated lateral lakes and wetlands along the South Fork of the Coeur d' Alene River as a refueling site. Although Pb exposure is known to occur via incidental sediment ingestion, differences in physiology and foraging behaviors associated with swan sex and diet may affect overall Pb exposure level. The influence of diet and sex requires examination in order to interpret Pb concentrations in swan fecal samples collected over space and time at the site and to guide remediation and restoration activities. We therefore collected and quantified Pb concentration in 34 fecal samples from wetland surfaces and six from captured swans at wetlands varying in sediment Pb concentration (<10-7000 mg/kg) in March of 2022 and 2023. Additionally, DNA was extracted from these fecal samples and sequenced to determine host species, its sex, and vegetation recently consumed via CO1, CHD, and rbcl genes, respectively. We found that 32 of 34 field collected fecal samples were tundra swan, molecular and morphological sex determination matched 5 of 6 times, and that the swan diet was highly unique to each wetland. Although sex did not significantly influence fecal Pb concentration, Cyperaceae (water sedges) were associated with lower Pb concentrations while Sagittaria (water potato) was associated with higher Pb concentrations. Furthermore, surprisingly, swans consumed a small fraction of their presumed preferred food item (water potato) and instead exhibited a large variation in plant species in their diet including Himalayan

horsetail (*Equisetum diffusum*). These data indicate that remediation and restoration activities may be able to consider vegetation that presents a lower exposure risk to tundra swans. However, further work is needed prior to instituting these findings in such a manner.

5.13.P-Mo-218 Exploring the Risk of Antibiotic Resistance Proliferation Through Sewage Sludge-Based Soil Fertilization

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For the last 20 years in EU-15 countries, the sewage sludge produced by wastewater treatment plants (WWTPs) has increased from 6.5 million tDM (dry matter) up to 9.5 million tDM (2000-2020). Applying sewage sludge (SS) to agricultural soil can improve the soil fertility, allows to recycle this biowaste and helps to meet crop nutritional needs. However, SS may contain various unregulated emerging contaminants, as well as mobile genetic elements (MGEs) and antibiotic resistance genes (ARGs), increasing the danger of the spread of antibiotic resistance. We looked at how applying thermally dried anaerobically digested SS affected the physical, chemical, and microbiological characteristics of the soil, and (ii) the relative abundance of 96 ARGs and 10 MGE-genes in the soil. Three factors—the dose of application, the technology of sludge treatment, and the amount of time since the last treatment—were different for each of the SS-amended agricultural fields from which soil samples were collected. ARGs and MGE-genes were both more abundant in soils fertilized with sewage sludge relative to non-amended soils in SS-amended soils, particularly in more recent SS applications and in soils supplemented by SS repeatedly thorough the last 5 years. The relative abundance of ARGs and MGE-genes was positively linked with some physicochemical factors, such as cation exchange capacity, nitrogen and phosphorus content. The primary explanation for the distribution pattern of ARGs and MGE-genes was the application of sewage sludge. We concluded that adding thermally dried anaerobically digested sewage sludge to agricultural soils without any additional treatments can enhance the chance of antibiotic resistance spreading. Much future research needed for the development of innovative treatments and technologies for SS in order to reduce the risk of further emergence and spread of ARGs and MGE-genes in SS-amended agricultural soils and afterwards, in crops. Until then, SS should be applied in agricultural soils with caution.

5.13.P-Mo-219 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 tool's 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 in order to maintain a high degree of environmental protection and support science-based decisions related to product formulations.

5.13.P-Mo-220 Evaluating the Risk of Climate Change and Chemical, Physical and Biological Stressors to the Great Barrier Reef, Australia

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Understanding the combined risk of climate change and chemical exposures is essential for improving ecological risk assessments of vulnerable ecosystems such as the Great Barrier Reef (GBR). However, there continues to be limited knowledge and study of the effects of multi-stressor exposures. Coral reef ecosystems in the GBR are under increasingly severe duress due to climate change pressures associated with rising ocean temperatures and increasing acidification. Near shore reef systems of the Mackay Whitsunday coastal zone are particularly at risk to climate change pressures as they are also being impacted by other anthropogenic stressors, including chemical, nutrient and sediment exposures associated with more frequent and large rainfall events that increase runoff of contaminated waters. To illustrate how climate change can be incorporated into ecosystem risk assessment frameworks, we first developed a conceptual model and illustrated collective effects of climate variables and the herbicide diuron on coral endpoints using an adverse outcome pathway (AOP) network. This informed the development of a Bayesian network to quantitatively compare the effects of historic and future climate on inshore hard coral bleaching, mortality, reproduction, and cover. We examined climate scenarios using an ensemble of 16 downscaled models representative of current climate conditions and future climate conditions assuming medium and high carbon emissions for two thirty-year periods centered on 2040 and 2085. Stressors included in the model were cyclones, temperature, acidification, total nitrogen, diuron, sediments, macroalgae and Crown of Thorns Starfish as an example of a coral predator. Both climate stressors and catchment-related stressors pose a risk to these inshore reef systems, with projected increases in coral bleaching and coral mortality under all future climate scenarios. The major risks identified could then be used to assist in the prioritisation of management interventions to build reef resilience for the future.

5.13.P-Mo-221 Advancing Animal-Free Environmental Safety Assessments of Cosmetics

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After use, cosmetic ingredients are often disposed of down the drain to sewage treatment plants or in some regions, directly released to the environment. Consequently, several tools exist to determine environmental exposure, fate and hazard of chemicals to identify their safe uses in cosmetic products. However, with a changing societal, scientific and regulatory landscape aimed at addressing evolving environmental challenges, new approaches are needed to ensure continued ability to assess safety. For example, animal tests on cosmetics are being increasingly banned around the globe. For some environmental endpoints, viable alternatives exist but for others, more need to be developed (such as for endocrine disruption). In addition, more fit-for-purpose exposure and fate methodologies are required to address increasing environmental challenges (such as for polymers and mixtures). The new global not-for-profit research organization International Collaboration on Cosmetics Safety (ICCS) is working to advance a 21st Century environmental toolbox to address environmental challenges through research, education, and regulatory engagement. This poster will summarize the following on-going environmental projects: 1) Tiered modelling framework for direct release of cosmetic ingredients to aquatic environments. 2) Experimental and in silico approaches for biodegradation/persistence assessment of polymers. 3) Weight-of-evidence persistence assessment tool (PAT- for details, see other ICCS poster). 4) International standard to calculate biodegradability of cosmetic formulations. 5) Identification of challenges and

strategies for broader regulatory acceptance of environmental non animal new approach methodologies. 6) Advancing development of an internationally recognised guideline for assessing acute and chronic coral toxicity in the marine environment.

5.13.P-Mo-222 Assessment of Toxicity of Fluorocarbon Refrigerants Due To Environmental Exposures

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As the demand and production of environmentally friendly refrigerants increase worldwide, one of the main challenges that remains is the assessment of their short- and long-term effects on the environment, as they are widely used in multiple Air Conditioning, Heat Pump and Refrigeration applications. Several generations of refrigerants have been developed to address environmental concerns such as ozone depletion and global warming. For example, in late 1989, hydrofluorocarbons (HFCs) replaced chlorofluorocarbons (CFCs) due to the lower ozone depletion potential (ODP). As global warming potential (GWP) became a bigger concern in the 2000's, hydrofluoroolefins (HFOs) and hydrochlorofluoroolefins (HCFOs) have been developed. These new molecules are highly reactive which in turn reduce their atmospheric lifetime, thus lowering their ODP and GWP. While ODP and GWP of these compounds is widely reported, less is known about their environmental toxicity. In this study, we review the literature on toxicity of 12 focal refrigerant compounds when used alone and in mixtures that are most likely to be used for stationary and mobile refrigeration applications worldwide. We consider the focal refrigerants and their degradation products that can arise from manufacturing processes, applications, and atmospheric transformation. For hazard and toxicity, the mammalian toxicity of focal refrigerants and degradation products are reviewed to assess the exposure scenarios and associated-toxicity endpoints. We considered acute and chronic inhalation studies from peer-reviewed studies and grey literature in this work. We found that the potential of focal refrigerants and degradation products to induce acute toxicity is low in rats, mice, rabbits, and dogs. Chronic exposures showed cardiac arrhythmias and hepatocellular responses at high concentrations for some focal refrigerants. Finally, toxicokinetic analyses indicate that focal refrigerants are rapidly absorbed and metabolized following oxidative and conjugation pathways. Predicted exposure nominal concentrations for human exposures to focal refrigerants and degradation products are well above 80,000 ppmV and 1,000 ppmV, respectively for some instances suggesting that these alternative refrigerants and their degradation products pose minimal health or ecotoxicity risks based on the literature available.

5.13.P-We Late Breaking Science: Environmental Risk Assessment

5.13.P-We-141 Dicamba and 2,4-D Concentrations in the Urine of Pregnant Women in the Midwest Obtained in 2020-2022 and Compared to 2010–2012

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The majority of soybean and cotton seeds sold in the US are now genetically modified to tolerate combinations of glyphosate, glufosinate, dicamba, and 2,4-D herbicides. Thus, the use of dicamba and 2,4-D has risen several-fold compared to 2010. As the use of these herbicides has increased, human exposure is also expected to increase. This study sought to compare levels of dicamba and 2,4-D in urine collected during pregnancy from women living in the US Midwest in 2010-2012 (N = 57) from nuMoM2b participants, in contrast to urine collected in 2020-2022 from Heartland Study participants (N = 86). Herbicide analytes were quantified using a validated method developed by the Quebec Toxicology Center (CTQ/INSPQ). Comparison of specific-gravity standardized concentration levels were conducted assuming the data are lognormal and specifying values below the LOD to be left-censored. The earlier samples were collected prior to commercial approval of dicamba-tolerant soybeans. Thus, our results provide preliminary information on whether and to what degree biomarkers

of dicamba and 2,4-D exposures have changed in the wake of adoption of dicamba-tolerant soybean technology. Dicamba was detected in urine samples at levels above the LOD (0.1 µg/L) in 28% (95% CI: 16%, 40%) of the 2010-2012 nuMoM2b cohort and 70% (95% CI: 60%, 79%) of the 2020-2022 Heartland Study cohort; therefore, the proportion of women with dicamba detected in their urine rose significantly, most likely as a result of approval and widespread planting of dicamba-tolerant soybeans. There was also a statistically significant increase in the SG-standardized geometric mean of dicamba concentration which was 0.066 µg/L (95% CI: 0.042, 0.104) in the nuMoM2b cohort and 0.271 µg/L (95% CI: 0.205, 0.358) in the Heartland Study cohort. Participant urine samples for 2,4-D concentrations were all above the LOD (0.01 µg/L) for both cohorts. The SG-standardized geometric mean concentrations for 2,4-D was slightly higher in the more recent Heartland Study cohort with a geometric mean of 0.442 µg/L (95% CI: 0.382, 0.511) vs 0.383 µg/L (95% CI: 0.321, 0.458) in the nuMoM2b cohort, but the difference was not statistically significant (p-value = 0.226). Increases in herbicide use in the Midwest warrants more effort in tracking changes in biomarkers of exposure levels and additional research is needed to elucidate the impacts of herbicide exposures on adverse pregnancy outcomes and health of the offspring.

5.13.V Late Breaking Science: Environmental Risk Assessment

5.13.V-024 Plastic Containers Usage for Take-Out Dining and Body Care Products in Malaysia, Creating a Potential Threat of Microplastics and Nanoplastics Exposure

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Background: The environmental plastic contamination was a well-recognized issue which affects the living organisms' health. The issue of microplastics and nanoplastics exposure to human is understated globally, though multiple plastic items are used for daily living and dining. The microplastics contamination was evident in the plastic take-out food containers, PET bottled mineral water and body care products worldwide including Malaysia. Habit for taking-out food and drink using plastic container or bag become popular across Malaysia, since the era of COVID-19 pandemic. However, the extent of plastic items usage for dining and living purpose is yet to be elicited. The current study examined the details information for the utility and the frequency of usage for plastic items to contain food, beverage, water and body care products aiming to uncover the possible risk of microplastics and nanoplastics exposure through daily dining and living. Methods and Results: The data collection was performed by convenient sampling through online questions. The total 385 consented participants, aged above 18 were involved within Malaysia. The percentage for the usage of plastic items for dining purpose included take-out food containers (90.9 %), plastic cutleries (53 %), PET water bottle (75.8%), plastic cup/ bag for beverage (84.4 %) and plastic straw (72.7 %). The majority of the participants used these plastic items 1-3 times per week. The (10.1 %) and (16.6 %) of participants reused twice for take-out food container and PET bottle respectively. The percentage of the usage for plastic items to contain body care products are; body wash (92.4 %), body lotion (48.6%), shampoo (85.7 %), conditioner (52.9 %), facial cleanser (68.5 %) and toothpaste (68 %). The majority of the participants used facial foam and toothpaste packed in plastic tube more than 10 times per week. The average number of participants using plastic items for the purpose of consuming foods, consuming drink and containing body care products were 277, 299 and 267 respectively. Conclusion: Majority of the people residing across Malaysia are using plastic products for

containing and consuming take-out foods, drinks and daily body care products widely. This study highlighted the existence of potential threat for microplastics and nanoplastics exposure through daily dining and living in this region. Further investigations for risk analysis is urgently required.

5.13.V-025 A Pharmacokinetic Model for Prediction of Human Exposure to Ciprofloxacin Following Consumption of Fish from Polluted Aquatic Environments

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Fluoroquinolones are broad-spectrum antibiotics that accumulate in the environment. To assess human exposure through the food chain, we developed a pharmacokinetic model of fluoroquinolone accumulation in fish and a human pharmacokinetic model to predict gastrointestinal concentrations of ciprofloxacin, the most common fluoroquinolone, following consumption of fish. At 70 ng/L ciprofloxacin, the average in North American surface waters, the fish steady state concentration was calculated to be 7.5×10^{-6} $\mu\text{g/g}$. Upon human consumption of the FDA-recommended portion of 113 g of fish with this level of ciprofloxacin, the predicted human intestinal concentration was 2×10^{-6} $\mu\text{g/mL}$. At 4×10^6 ng/L ($4 \mu\text{g/mL}$) ciprofloxacin, the highest recorded environmental measurement, the fish steady state concentration was $0.42 \mu\text{g/g}$ and the level in the human intestine was $0.1 \mu\text{g/mL}$. For consumption of a single serving of fish thrice a day for 10 days from the same source, the level in the intestine reached $0.2 \mu\text{g/mL}$. Thus, based on the ciprofloxacin MIC for *E. coli* of $0.13 \mu\text{g/mL}$, environmental pollution of ciprofloxacin may result in high intestinal levels that could cause gut dysbiosis. In all these scenarios, the predicted plasma concentrations of ciprofloxacin were negligibly low. The method described here can be used to assess the effects of environmental contamination on human exposure through the food chain for any drug. This approach will allow regulatory authorities to make long-term predictions based on environmental levels of drugs and patterns of fish consumption of local populations. The ability to derive gut and plasma concentrations of drugs over time and assess whether these are likely to reach toxic levels provides the basis for improved public information and balanced decision-making in response to environmental crises.

5.13.V-026 Background Soil Dioxin-Like Compounds (DLCs): A Pilot Survey Update

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Dioxin-like compounds are persistent, ubiquitous environmental contaminants that are formed during incomplete combustion activities and as byproducts of certain industrial chemistries. These compounds bind tightly to organic particulate matter, and are commonly detected at low levels in soils, sediments, and dust. Over the past several decades, regulatory efforts and technological advancements led to dramatic reductions in environmental releases of DLCs into the environment, and biomonitoring efforts in the U.S. demonstrate that human body burdens have been trending downwards. A comprehensive survey of background soil dioxin data published a decade ago found that background DLC concentrations across the U.S. are highly variable. The DLC concentrations in urban/suburban soils tend to be higher than rural soils, with levels in several areas exceeding state and federal health-based soil screening levels and remediation goals. The World Health Organization's (WHO's) Toxic Equivalency Factor (TEF) Expert Panel is updating the 2005 TEFs for DLCs, which had been adopted for risk assessment by regulators around the globe. This recent effort involved a systematic update of the underlying DLC relative potency (REP) database and integration of the data through a comprehensive workflow that incorporates machine learning-based quality category assignments, Bayesian dose-response fitting, and Bayesian meta-analysis. The WHO indicated that several DLC congener TEF values will change from their respective 2005 TEF values, based on this effort. It is expected that these updates will lead to a reduction in dietary TEQ concentration estimates, however, it is unclear what impact these changes will have on TEQ exposures from environmental media, which can have quite different DLC congener profiles

than dietary sources. In this pilot study we review the impact that TEF changes might have on the 2005 TEF-based DLC TEQ concentration estimates from three well-reported U.S. background soil surveys reporting DLC congener-specific data. The pilot study underscores the utility of an update of the broader U.S. background soil DLC survey database as a reference tool for regulators and stakeholders involved with sites where DLCs are suspected soil chemicals of potential concern.

Track 6: Engineering, Remediation and Restoration

6.01.P-Th-141 Field Demonstration of a Commercially Available Peeper Sampler for Measuring Metal Availability in Sediment Porewater and Surface Water

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Passive sampling using dialysis samplers (“peepers”) is a common approach for measuring the availability of metals in aquatic matrices. Peepers function by allowing sediment porewater or surface water to equilibrate with lab-provided 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 detail the results of a field demonstration in which commercially available peepers were deployed for 10 days in marine surface sediment and surface water at 10 stations located at Naval Base San Diego, San Diego, California (Diffusive Gradients in Thin-Films (DGTs) were also deployed in surface water). Lithium and bromide tracers spiked into the peepers before deployment indicated that more than 70% equilibration was reached for all target metals. Both tracers performed similarly. In future, the lithium tracer (rather than bromide) is recommended, as the use of a lithium tracer is most efficient in terms of method simplicity and analytical cost savings. Among the target metals, copper, manganese, and zinc were most frequently detected in sediment and surface water peepers at concentrations of 3-13, 5-240, and 4-33 µg/L, respectively. Differences between concentrations in sediment porewater and concentrations in surface water for copper and zinc at each station were minor (factor of 2 or less difference), indicating equilibration between surface sediment and surface water. In contrast, concentrations of manganese in sediment porewater were much higher (factor of 14) than surface water. Average concentrations of copper, manganese, and zinc in surface water indicated by DGTs were a factor of 2 to 6 less than those indicated by peepers. Ranges of the concentrations of metals in sediment porewater measured by peepers in this study overlapped with the ranges of concentrations indicated in a previous study that deployed DGTs in surface sediment in the study location, and both studies were in agreement that copper was the only metal that exceeded USEPA’s saltwater Criterion Continuous Concentration. Overall, this study indicated demonstrated a successful field deployment and retrieval of commercially available peepers to measure metal availability within an approximate 1-week timeframe.

6.01.P-Th-142 The (Sometimes Overlooked) Role of Temperature in Passive Dosing Experiments and Chemical Activity-Based Exposures

Sebastian Abel, Betty Chaumet, Sophie Steigerwald, Ann-Kristin Eriksson-Wiklund, Elena Gorokhova and Anna Sobek, Stockholm University

The chemical activity concept provides several advantages in the environmental risk assessment of hydrophobic organic contaminants (HOCs). Unlike concentration-based assessment, the thermodynamic or chemical activity (*a*) approach includes all factors affecting the actual HOC bioavailability. Per definition, *a* describes the pressure that a chemical exerts to partition from one environmental compartment, e.g., water, to another, e.g., biota. It is best approximated as a relative saturation of a compound in any matrix. Moreover, *a* is additive for a wide range of HOCs commonly found in the environment, thus, simplifying the assessment of complex mixtures. The resulting unitless scale for *a*, ranging from 0 to 1, provides straightforward means for risk

communication and management. A sometimes neglected factor when converting concentration-based data (e.g., EC50 values or threshold concentrations) is the effect of exposure temperature on the chemical activity. Often, thermodynamic properties at the standard temperature of 25 °C are utilized. However, the water solubility of HOCs is temperature-dependent, decreasing at lower temperatures, with a concomitant increase of their chemical activity. To address the issue, we extrapolated available temperature-dependent solubility data of several polycyclic aromatic hydrocarbons (PAHs) to obtain their saturation level at different temperatures. We found that chemical activity can change by as much as 25 % at a given aqueous PAH concentration with a 5 °C deviation from the standard 25 °C. We will validate these findings using passive dosing experiments conducted at 5, 17 and 25 °C, in which a silicone donor is loaded with PAHs to a specific *a*. Since using a well-defined silicone phase as PAH-donor allows the reliable setting of *a*, the observation of changes in aqueous concentrations at different temperatures can be used to quantify the impact on PAH-solubilities. Aqueous concentrations of PAHs will be measured by direct analyses of water samples equilibrated with the silicone donor for 6 days using liquid chromatography coupled with a photodiode array detector. With this presentation, we aim to raise awareness about the importance of temperature correction for chemical activity applications in laboratory experiments and field studies, where the water temperature can vary greatly.

6.01.P-Th-143 Historical Levels of Polychlorinated Biphenyls (PCBs) in the Sediments of Apalachicola Bay, Florida

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Estuaries are transitional environments between marine and freshwater systems. Estuaries receive pollutants from both point and nonpoint sources; thus, estuaries function as "natural reservoirs" of Persistent Organic Pollutants (POPs) and other polluting compounds. Polychlorinated biphenyls (PCBs) are persistent, toxic, bioaccumulative, and ubiquitous. Although PCBs are considered a "legacy" POP, there is still considerable interest in studying the fate of PCBs in the environment. Apalachicola Bay in Florida is known for high oyster yields and commerce. However, there has been a decline in the oyster fishery. It is unclear what environmental stressors may have contributed to the decline in oysters in the Bay. This research will help provide data for the better management of the Bay. This study analyzed the historical levels of PCBs in the core sediment from Apalachicola Bay. The vertical distribution of PCBs was measured in a dated core, and the total PCBs ranged from 167.37 - 228.30 ng g⁻¹ in the period 1927 to 2020. The historical trend of total PCBs in the sediment core indicated levels fluctuating according to historical usage patterns, increasing rapidly from the late 1920s to the mid-1940s and leveling thereafter. The penta- and hexa-PCBs dominated the vertical distribution for the chlorinated homologous group, while octa-PCB was not detected. The congeners PCB-20, PCB-180, and low-chlorinated PCBs dominated the sediment core. The dominance of low chlorinated PCBs suggests more distant sources (nonpoint), air deposition, or riverine input, probably due to Aroclor mixtures: Aroclors 1016, 1242, 1254, and 1260, majorly produced in the US. This research provides helpful information on PCBs' historical levels in Apalachicola Bay.

6.01.P-Th-144 Assessment of Dechlorination Rate of Chiral PCBs and TCE in Town Creek, SC Sediment Exposed to Selected Essential Metals

Catherine Sumner, Cindy M. Lee and Kevin Finneran, Clemson University

Microbial reductive dehalogenation of polychlorinated biphenyls (PCBs) can be an important step to remediate regions contaminated with the persistent organic pollutants. Reports suggest that reductive dehalogenases (RDase) can be an enzymatic pathway responsible for dechlorination of PCBs. However, the mechanisms underlying the enantioselective transformation of chiral PCBs are less explored. Field samples from around the world indicate non-racemic enantiomeric fractions of PCBs, demonstrating that one enantiomer is in higher concentration than the other. Commercial PCBs were released into the environment at racemic levels, so the observations propose the question as to how chiral PCBs degraded enantioselectively. Despite advances in

RDase mechanisms, one critical question remains: what step or steps in dehalogenation is enantioselective. There are several components, such as cofactors, that can promote enzymatic functions that could affect enantioselectivity. Recently, hazardous metals have been shown to inhibit RDase function, implying that metals play a role in the enzymes' pathway. Generally, metals have also been shown to serve as electron donors or acceptors and can form ligands that interact with enzymes. We are currently exploring how naturally occurring metals such as zinc and copper affect the dechlorination rate of trichloroethene (TCE) and PCBs. TCE in our project is used as a halo-primer to catalyze the dechlorination of PCBs. Due to low bioavailability and solubility of PCBs, halo-priming can be employed as a method to stimulate bacterial growth responsible for dechlorinating PCBs prior to spiking. Halo-priming is a method that uses a smaller, more bioavailable compounds, like TCE, to the bacteria with the goal to upregulate enzymes used to degrade a target chemical. Our research is being conducted using lab microcosms from sediment collected from contaminated areas of Lake Hartwell, SC, USA. This Superfund site is ideal for this prospective research because the only contamination is from PCBs, and previous research by our group has confirmed enantioselective degradation is occurring in the sediments. From when the source plant was opened in 1955 to its closure in 1977, it was estimated that over 220 tons of PCBs were released into Town Creek that feeds into Lake Hartwell. We will present the experimental design of our study and results designed to discover the relationships among essential metals and enantioselective dehalogenation.

6.01.P-Th-145 Quantification of the Impact of Sediment Particle Size Distribution on Sediment Resuspension and Release of Organic Contaminants

Betty Chaumet¹, Alyssa Azaroff¹, Sofi Jonsson¹, Elena Gorokhova¹, Stefano Bonaglia² and Anna Sobek¹, (1)Stockholm University, (2)Gothenburg University

Sediments are well known to act as a sink for contaminants. Due to organic matter content, sediments have a high affinity for certain chemicals such as PAHs and MeHg. When sediments are resuspended because of advective flow, the contaminants stored in the sediments can be released into the water column and induce toxic effects to aquatic organisms. The bioavailable fraction is proportional to the freely dissolved concentration, C_{free} , which can be assessed by using passive samplers. The sediment resuspension can be strongly affected by the particle size distribution: the finer the particles, the greater the expected resuspension phenomenon. The aim of this study was to assess and quantify the impact of sediment particle size distribution on first, the sediment resuspension by using turbidity as a proxy, and then how this affects the release into water of PAHs using passive samplers and MeHg with grab samples. For that purpose, we performed an experiment with cores containing spiked artificial sediments with four PAHs (acenaphthene, fluorene, phenanthrene and fluoranthene; 3 mg.kg⁻¹ each) and MeHg (5 µg kg⁻¹), brackish water and a polyethylene sheet used as a passive sampler for PAHs. Five types of sediments were prepared, ranging from fine to coarse sediments (fine particles <63µm: 10, 20, 40, 60 and 80%), and 2% of organic carbon. Passive samplers were collected after two weeks of exposure, and turbidity, dissolved organic carbon (DOC), and MeHg were assessed regularly in the water. The results highlighted that generally, the finest sediments lead to higher turbidity. However, surprisingly, the coarsest sediment presented the highest turbidity which was due to organic matter diffusion into the water, as supported by the DOC concentrations. This treatment, with only 10% of fine particles was also the one which released the most PAHs to water. Concentrations found for both PAHs in passive samplers and mercury in water decreased with the increase of the fine particle proportions (except at 80% of fine particles for PAHs). Moreover, as expected, a clear influence of PAH hydrophobicity was observed: the less hydrophobic the molecule, the more it was released from sediment to water. Finally, sediment particle size distribution had an impact on contaminant release, but not exactly for the reasons expected: a coarse sediment was more likely to allow the DOC to spread which is the main driver for contaminant release as it carries organic contaminants.

6.01.P-Th-146 Integrating Chemical Activity into Sediment-Water Bioassays with Benthic Invertebrates

Sebastian Abel¹, Jarkko Akkanen², Betty Chaumet¹, Ann-Kristin Eriksson-Wiklund¹, Elena Gorokhova¹ and Anna Sobek¹, (1)Stockholm University, (2)University of Eastern Finland Sediment-water bioassays for potentially harmful chemicals are based on several relevant guidelines (e.g., OECD and ISO) recommending artificial sediment formulations to assure comparable results. However, these recommendations do not standardize organic carbon (OC) sources in test sediments, even though OC properties can strongly impact the bioavailability of hydrophobic organic contaminants (HOCs) and, thus, the observed effects. This issue gets exacerbated when natural sediments, which can vary widely in OC quantity and quality, are used for bioassays. The impact of organic carbon properties on HOC bioavailability in bioassays can be circumvented if the chemical activity (a ; unitless) of HOCs is used instead of their concentration in the test sediment. Chemical activity can be approximated as the relative saturation of a compound in any phase reflecting its effective concentration in a given environment. As a thermodynamic property, chemical activity incorporates the external factors, including OC, that influence bioavailability, making it a highly reproducible dose metric. The experimental design employs sediment-water microcosms according to OECD guidelines, but using a as dose metric for the tested chemicals. Two benthic organisms with different habitats, *Lumbriculus variegatus*, a deep-dwelling oligochaete and *Chironomus riparius* larvae, inhabiting the sediment surface, are utilized as model organisms. We spiked artificial sediment with a mixture of 4 polycyclic aromatic hydrocarbons (PAHs) at a gradient from $a = 0.01 - 0.12$. The PAH concentrations for the target activities were determined in a pilot experiment and are additionally confirmed post-exposure using passive sampling methods. Preliminary results demonstrated that the experimental design was adequate for benthic exposure studies and indicated a higher sensitivity of *L. variegatus*, most likely due to the increased exposure through dietary pathways and proximity to the contaminated sediment particles. This assumption will be tested by determining the internal a within the organisms' tissues, which could indicate whether both organisms reached a thermodynamic equilibrium with the sediment. It is expected that a within the organism governs the observed effects. The long-term objective is to replace EC50 values with Ea_{50} values that can be acquired with our test setup and to further standardize the approach with regard to duration, feeding regimes and test species selection.

6.01.P-Th-147 How to Set Up Sediment-Water Bioassays for Hydrophobic Organic Contaminants Using their Chemical Activity as Dose Metric

Sebastian Abel, Manuela Ospina Arboleda, Ann-Kristin Eriksson-Wiklund, Elena Gorokhova and Anna Sobek, Stockholm University

The chemical activity concept provides several advantages in the environmental risk assessment of hydrophobic organic contaminants (HOCs). Chemical activity (a) incorporates all environmental factors affecting HOC bioavailability. It is easiest approximated as a relative saturation of a compound in any matrix, indicating the pressure it exerts to partition from a source into other environmental compartments, such as biota. However, this approximation implies challenges when working with non-aqueous environmental matrices for which HOC solubilities are unknown, particularly, when determining the effect activities (Ea_{50} values) based on the observed adverse effects of chemicals. We provide an alternative approach to load artificial sediments for bioassays directly based on thermodynamic properties instead of converting test concentrations to their corresponding a values. The basic concept relies on an initial saturation of an organic carbon source for the sediment formulation (e.g., peat) with a chosen HOC. A peat suspension is equilibrated with a saturated, aqueous HOC solution. To prevent HOC depletion in this solution, we developed a crystalline HOC reservoir enclosed in a polyethylene (PE) membrane, which is permeable to dissolved but not solid HOCs. The loading process was tested with four polycyclic aromatic hydrocarbons (PAHs), whose concentrations within the peat were monitored over 18 weeks by exhaustive extraction using acetonitrile. The extracts were analyzed using liquid chromatography coupled to a photodiode array detector (LC-PDA). After a thermodynamic equilibrium was reached between the peat and the saturated loading solution (5-17 weeks), the PAH-saturated peat was diluted with clean peat. The target chemical activity value was obtained by adjusting the clean-to-saturated peat

ratio. Next, the saturated and diluted peat samples will be analyzed using passive sampling methods, which can directly measure a , to validate the assumption that a can be approximated as the relative saturation at both low and high PAH concentrations. If this assumption is met, our loading design can provide a simple and reproducible approach to creating sediment bioassays with higher environmental relevance than the current test designs based on total sediment concentrations.

6.01.P-Th-148 Decreasing Uncertainties in Assessing Environmental Exposure, Resilience, and Ecological Implications of Dredging Near Coral Reefs in the Honolulu Harbor

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Hawaii's coral populations face multiple stressors, including increased water temperatures, higher predator populations, and deteriorating water quality. The process of dredging, necessary for maintaining harbor facilities, can further exacerbate these challenges by causing sedimentation and temporary sediment plume gradients. This research aims to enhance coral impact studies by investigating the link between sediment plume monitoring parameters and the concentration of sedimentation effects on coral. This study focuses on evaluating sediment plume turbidity data as a proxy for sediment concentration near corals in the Honolulu Harbor entrance channel. A water quality monitoring program conducted during a maintenance dredging operation in Honolulu Harbor in 2021 provided an opportunity to study the relationship between sediment plume turbidity and coral health. The primary objective of this study was to assess the sediment concentration in the water column near coral by analyzing sediment plume turbidity data. Additionally, laboratory-scale experiments were conducted to compare the results of sedimentation (0-control, 6, 30, and 60 mg cm⁻²) and associated turbidity with coral health indices, offering insights into the potential effects of sedimentation on corals. Findings suggest that sediment plume turbidity can serve as an indicator of sediment concentration near corals, albeit with limitations. The laboratory experiments revealed corals exposed to sediment concentrations >30 mg cm⁻² had increased tissue loss, emphasizing the importance of understanding the impact of sedimentation. This study's relevance lies in its potential to advance the understanding of the impacts of dredging activities on coral populations in nearshore environments. By identifying the relationship between sediment plume parameters and coral health, it offers valuable knowledge for decision-makers and stakeholders involved in coastal management and harbor operations. The findings can guide the development of mitigation measures and monitoring protocols to minimize the adverse effects of dredging on corals and support the sustainable management of Hawaii's coastal ecosystems. Furthermore, this study highlights the need for further research and investment in cost-effective methods to directly measure sedimentation on coral surfaces, considering its importance in assessing the impact on coral health.

6.01.P-Th-149 Using Sediment Toxicity Tests to Develop Remediation Goals for PAHs at Manufactured Gas Plants

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Polycyclic aromatic hydrocarbons (PAHs) are often contaminants of concern in sediments adjacent to former Manufactured Gas Plant (MGP) sites. Laboratory sediment toxicity tests can be used to develop remediation goals for the protection of sensitive ecological receptors such as benthic invertebrates. Data from MGP sites will be used to examine the relationship between various exposure metrics and toxicity to the standard test species, *Hyalella azteca*. Exposure metrics include bulk concentrations of PAHs in sediment, concentrations of PAHs in porewater measured with solid phase microextraction (SPME), and Toxic Units (TU) based on the U.S. Environmental Protection Agency's equilibrium partitioning sediment benchmarks for PAH mixtures. Issues related to the development of remediation goals from regression models, including treatment of replicate

data from toxicity testing and treatment of outliers will be discussed. An alternative approach to developing remediation goals from toxicity tests, the Receiver Operating Characteristic Curve (ROC) methods will also be discussed.

6.01.P-Th-151 Multiple Lines of Evidence to Predict Sediment Toxicity to Invertebrates Due to DDT and its Metabolites

Phyllis C. Fuchsman, Sydney Kruse, Shuo Yu, Kiersten McMahon and Kyle Fetters, Ramboll

Sediment toxicity to invertebrates due to DDT and its metabolites (collectively DDx) has been studied relatively extensively, through spiked sediment toxicity tests and site-specific sediment toxicity and benthic community assessments. Additionally, DDx is amenable to toxicity prediction by extrapolating aquatic toxicity data to sediment using the equilibrium partitioning approach. Ample aquatic toxicity data are available for DDT, but discerning the relative toxicity of metabolites is more challenging. We evaluate the utility of the Target Site Model (Boone & Di Toro 2019, ETC 38:222-239) for this purpose. The model represents an extension of the Target Lipid Model for additional modes of action, including the “diphenyl sodium channel modulation” mode of action applicable to DDx. We also review modern (passive sampling) estimates of DDx organic carbon/water partitioning to extrapolate from aquatic to sediment toxicity. Taken together, these lines of evidence provide a strong mechanistic and empirical basis to interpret risks to benthic invertebrates related to DDx in sediment.

6.01.P-Th-152 A Sustainable and Low-Impact Approach to Contaminated Sediment Remediation

John Collins and Moses Ajemigbitse, AquaBlok, Ltd

Sediments contaminated by hydrophobic organic compounds (HOCs) pose a threat to human and ecosystem health and the vitalization of recreational space. Contaminated sediments can sustain undesirable levels of bioaccumulation and biomagnification, especially in areas where traditional sediment remediation practices of dredging and/or capping are challenging to implement. Demonstrating an approach that greatly reduces ecological risk either due to low ecosystem impact and high effectiveness, while remaining low-cost, is attractive to stakeholders and regulators. In situ sediment remediation is a focused approach to address contamination in the biologically active zone (BAZ) of sediment dwelling and feeding organisms with the goal of demonstrating rapid improvement in contaminant porewater concentrations and subsequent improvement in body burden and uptake by select indicative benthic macroinvertebrates. Targeted treatment of contaminants is achieved using amendment materials delivered into the BAZ. Through extensive lab and field research, activated carbon (AC) has emerged as a leading amendment for in situ remediation of sediments contaminated by HOCs. Unlike traditional approaches for sediment remediation, in situ treatment with AC can be implemented without significant destruction or disturbance to the existing benthic ecosystem. This low impact benefit is expressed in rapid response and recovery of the benthic ecosystem. In addition, the relative reduction in material use and transportation nets reduced construction activities, providing a secondary benefit towards sustainability and resource conservation. This talk will present data on the effective use of powder activated carbon in conjunction with a delivery system for in situ treatment at several contaminated sediment sites. Supporting data on porewater concentration reductions and subsequent reductions in uptake and body burden concentrations of test organisms, such as *L. variegatus*, will be presented from a recently concluded project. Considerations of the secondary effects of using AC on benthic community will also be discussed. The use of PAC along with a delivery mechanism can be (i) low impact on benthic community, (ii) effective for reducing porewater concentrations and thereby contamination risk, and (iii) provide a lower carbon footprint than traditional approaches.

6.01.T Contaminated Sediment Toxicity, Emerging Contaminants, Risk Assessment and Management, Remediation, Restoration, Sustainability, Climate Change

6.01.T-01 Re-Use for Restoration: Beneficial Use of Dry Sediment in a Nearly Freshwater Wetland

Wendy Hovel, Lisa Van Tassell, David Umberg and Jay Merani, Geosyntec Consultants, Inc.

Wetland remediation and restoration programs often require clean sediment or soil as backfill and topsoil. However, in many regions of the U.S. it can be challenging to meet the stringent standards required for use in wetland restoration projects. This investigation involves a restoration program in the San Francisco Bay region that was struggling to find soil that met regulatory standards when a low carbon footprint opportunity came along involving sediment from a flood control channel (only 4 miles away). The challenges were: (1) the flood control channel sediment was dry for the majority of the year, (2) the flood control channel was freshwater, and although the placement site was a freshwater wetland area, the underlying groundwater was sometimes brackish, and (3) the regional beneficial use testing program was designed for marine sediment placement in a regional marine wetland (i.e., not dry freshwater sediment in a pseudo freshwater wetland). Thus, a site-specific, and phased testing program was designed with regulatory input to follow the process for beneficial use testing prescribed for the San Francisco Bay region, but with modifications needed to assess the freshwater sediment and placement. Sediment samples were collected, composited, and analyzed in accordance with regional guidance, with composites representing lateral and vertical extents of material in each (~50,000 cy) area to be excavated. Composite sample chemical concentrations were evaluated against the minimum of marine and freshwater screening levels to assess suitability for surface or foundation material. Samples met the minimum standards and thus freshwater toxicity testing (benthic and elutriate, per the Inland Testing Manual and regional guidance) was performed as the water ponding in this area was typically freshwater. An alternate toxicity testing program was also prepared in case the dryness of the channel sediment led to test deviations. Toxicity tests passed their requirements and the site-specific and conservative testing program showed that the unexpectedly clean, flood control channel sediment met stringent requirements for nearby wetland placement; the majority (~99%) of the 132,000 cy of sediment was found suitable for use as wetland surface or foundation material. Results from this investigation could support similar regional beneficial use programs, potentially leading to more restoration programs with low carbon footprints.

6.01.T-02 Contaminated Sediment and Climate Change – Sediment Desiccation, The Unthought About Hazard for Caps and Monitored Natural Recovery

Jason Dittman, Philip Spadaro and Michael J Bock, TIG Environmental

Climate change poses an increasing threat to remedy effectiveness at Superfund Sites, especially those located in waterways. Many contemporary remedies were designed or constructed decades ago without considering the myriad effects of climate change. Although sea level rise is often considered, forecasted increases in temperatures will lead to longer and more frequent heat waves and potentially chronic, long-duration droughts, which can cause surface water levels to drop, resulting in exposed and desiccated sediment. Our objective was to evaluate how increasing air temperatures, increased drought potential, and sediment desiccation affects existing and future sediment remedies such as subaqueous sediment caps, monitored natural recovery (MNR), and enhanced monitored natural recovery (EMNR). We focused on physical and chemical changes to contaminated sediment and the performance of subaqueous sediment caps and MNR/EMNR during drying and rewetting cycles. We also evaluated unanticipated risks, such as shifting exposure pathways from desiccated contaminated sediment from inhalation, ingestion, dermal contact, and impacts to new ecological and human receptors. Damage to remedies at Superfund Sites can lead to releases of contamination. Existing and future remedies must account for the effects of climate change, such as increased temperatures and increased drought risk. As water levels drop, sediment caps exposed to the atmosphere can desiccate and crack, ultimately decreasing the cap's ability to cover or sequester contamination and resulting in migration of previously contained contamination to ground or surface water and/or resuspension of contaminated sediment. Sediment

desiccation can also change the biodegradation potential as contaminants shift from anaerobic to aerobic conditions. Lower water levels can make caps unsuitable in working rivers and harbors if additional dredging is required to allow for a deeper navigational channel. Because MNR/EMNR uses an assumed sedimentation rate of new uncontaminated material, decreased water flow due to drought could decrease the deposition rate of new material and impact the success of MNR/EMNR as a remedy. Desiccation can also change exposure pathways, such as exacerbating the exposure and inhalation risk posed by wind-blow contaminated particles. Higher temperatures, increased drought risk, and sediment desiccation must be considered when remediating contaminated sediment sites.

6.01.T-03 Natural Resource Damage Assessment Habitat Restoration and Sediment Cleanup Coordination – Vigor Shipyards, Seattle, WA

Amanda McKay and Kate Snider, Floyd/Snider

Between 2004 and 2006, a contaminated sediment cleanup was completed at the Todd Shipyards facility in Seattle, now owned by Vigor Shipyards, Inc. As part of the cleanup action, contaminated sediments and shipyard waste were removed from open-water areas of the facility. Dredging was performed at the site to depths where contaminant concentrations were less than compliance criteria defined in the U.S. Environmental Protection Agency Record of Decision that governed the site. During the site characterization process, sediments under structures (piers and shipways) were not sampled, but it was assumed that they may be contaminated. Therefore, temporary sand caps were placed to meet the remedial action objective in these areas where no dredging occurred, with the requirement that permanent cleanup would be performed when the structures were demolished at the end of their serviceable life. Between 2021 and 2023, Vigor completed construction of a new off-channel aquatic habitat for juvenile salmonids to settle Natural Resource Damage liabilities associated with historical releases of contamination discharged over prior decades to the industrial Duwamish Waterway. The construction of the habitat project required demolition of two piers and shipways. This provided Vigor the opportunity to implement the final cleanup of sediment contamination remaining within the footprint of the habitat area, through dredging and permanent cap placement, as an integral part of habitat construction. With the sediment cleanup completed, the new approximately 2.7-acres of subtidal, intertidal, and riparian habitat improves degraded habitat in this industrially zoned but ecologically important area. The project is a precedent-setting example of how to design and construct protected near-shore intertidal and marsh aquatic habitat in an industrial context. Vigor will maintain the habitat area in perpetuity, as part of the working shipyard.

6.01.T-04 How Old is Too Old? A Comprehensive Evaluation of Historical Sediment Data Representativeness to Support Development of Risk-Based Benthic Thresholds

Rachel Zajac-Fay, Wendy Hovel, Jennifer Arblaster, Jean Zodrow, Cathy Crea and Jen Huha, Geosyntec Consultants, Inc.

This client confidential site (Site) is a former specialty chemical manufacturing plant located in southeastern Massachusetts where environmental investigations have been on-going under the Resource Conservation and Recovery Act (RCRA) since the 1990s. The Site features three aquatic areas of concern (AOCs), which include freshwater and brackish environments. A screening-level ecological risk assessment (SLERA) was completed for the Site in 2021 which relied on bulk sediment chemistry data collected between the 2000s and 2021, and historical toxicity testing data from the 2000s. Based on screening level exceedances of risk-driving contaminants of concern (COCs; PCBs, mercury, and chlorobenzenes) in sediment, a baseline ecological risk assessment (BERA) was required for each of the AOCs using newly collected bulk sediment chemistry, porewater, and toxicity test data. However, as part of work plan development, an in-depth review of the historical toxicity and analytical data quality in two AOCs was performed and results showed that historical risk assessments were spatially and analytically comprehensive, used multiple lines of evidence, and data used were high-quality. However, questions were raised about the representativeness of these historical data for current

conditions at the Site. Thus, historical representativeness was assessed using spatial assessment tools and statistical analyses. Except for bioavailability data (which showed spatial data gaps in both AOCs), the spatial plots of COC concentrations in AOC-2 (a brackish water wetland) showed no spatial differences among temporal data and no statistically significant differences among historical and more recent COC concentrations. Thus, preliminary sediment chemistry and historical toxicity testing data were used to develop risk-based threshold concentrations (RBTCs) protective of the benthic community, with a plan for additional collection of bioavailability data. In AOC-5 (a freshwater creek), significant spatial and temporal data gaps were found in some portions of the creek, and it was determined that more data were needed to accurately assess whether there were changes in chemical concentrations and bioavailability factors over time. This presentation provides an overview of the work conducted to assess the historical sediment data quality, conduct the benthic risk assessment, and plan for fulfillment of key data gaps that will inform development of benthic RBTCs and future remedial decisions.

6.01.T-05 PAHs Release from Resuspended Sediment: Comparison of Accumulation Between a Passive Sampler and Biota

Betty Chaumet¹, Hoi Shing Lo¹, Elena Gorokhova¹, Sofi Jonsson¹, Stefano Bonaglia² and Anna Sobek¹, (1)Stockholm University, (2)Gothenburg University

Sediments store a wide range of contaminants, particularly hydrophobic ones, such PAHs. Shear stress on the sediment induces sediment resuspension causing higher turbidity, which may lead to the release of contaminants into the water column. There, freely dissolved PAHs are bioavailable to aquatic organisms. PAHs and other hydrophobic contaminants can cause baseline toxicity in algae. To assess the freely dissolved – bioavailable - concentration (C_{free}) of PAHs, previous studies demonstrated the efficiency of polyethylene passive samplers. Moreover, the C_{free} of a chemical is directly related to its chemical activity, which is the molecule's potential partitioning and reaction in a matrix (e.g. water) and can describe the bioaccumulation and potential for baseline toxicity. In practice, it can be calculated as the ratio between the chemical concentration (e.g. C_{free}) in a defined phase and its solubility in this phase. One advantage of using the chemical activity concept is that the chemical activity of different compounds is additive and therefore it allows for the assessment of contaminant mixtures. The aim of this study was to compare C_{free} to the bioaccumulated concentrations in biota and to the biological response and assess how the uptake and the toxicity vary under different turbidities. We performed an experiment with a system containing contaminated artificial sediment, PAHs, brackish water and the model algae species *Ceramium tenuicorne*. The artificial sediment was spiked with 4 PAHs (acenaphthene, fluorene, phenanthrene and fluoranthene; 100 mg.kg⁻¹ each) representing a range in log K_{ow} from 3.9 to 5.2. Two turbidities were continuously applied by creating sediment resuspension using a stir bar. The whole system was exposed for 7 days, and all the compartments (biota, water and sediment) were sampled for further analysis. The PAHs will be extracted from all the compartments and quantified by GC-MS. Lipid and protein contents will be determined by ATR-FTIR (Attenuated Total Reflectance – Fourier Transform Infrared Spectroscopy). Endpoints and biomarkers such as photosynthesis inhibition, total antioxidant capacity (ORAC) and pigments will be measured. By coupling data on bioaccumulation and toxicity in the algae with chemical activity measured in the passive samplers at varying turbidities, we will provide a better understanding of how passive samplers can be used to assess the risk caused by release of PAHs from contaminated sediments.

6.01.T-06 Mitigation of Porewater Sulfide in Wood Waste-Impacted Sediments by Reactive Amendments: Geochemical and Spectroscopic Observations in Bench-Scale and Field Pilot Studies

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Historical log handling and wood mill operations have led to the accumulation of wood waste deposits in the

subtidal area of Esquimalt Harbor, British Columbia, Canada. An excess amount of sedimentary organic matter from wood waste decomposition creates anoxic conditions and induces sulfate reduction resulting in elevated porewater sulfide concentrations (up to ~200 mg/L), which is highly toxic to benthic organisms in the sediment bioactive zone. Surface sediment and sediment porewater were extensively studied to evaluate the geochemical relationships between key geochemical parameters including porewater sulfide, labile organic carbon (LOC), bulk iron in sediments and to delineate wood waste impacted areas. Diffusive gradients in thin films (DGT) monitoring captured significant seasonal variability in “bioavailable” porewater sulfide concentrations in surface sediments. A bench-scale mesocosm study was conducted over a 1-year period with site sediment and surface water to evaluate the effectiveness of placing a thin cover layer of sand (with and without various reactive amendments) to suppress bioavailable porewater sulfide generated from underlying sediments. The sand cover reduced the flux of dissolved sulfide from the underlying sediments as a result of the reactive iron of the sand. A siderite ($\text{FeCO}_3(\text{s})$) amended sand cover suppressed dissolved sulfide concentrations not only in the cover layer porewater but also in underlying sediment porewater throughout the test duration. X-ray absorption (XAS) results indicated that sulfide was sequestered in iron sulfide solids by reacting with ferrous ions (Fe^{2+}) dissolved from siderite. After that, a field pilot study was conducted at several test areas to evaluate placement methods of thin cover layer (30 cm thick), cover physical stability, and effectiveness at mitigating sulfide under dynamic field conditions. Over two years of monitoring, DGT results show no sulfide breakthrough in the upper 15 cm of sand cover and siderite-amended sand cover except a few locations whereas elevated sulfide concentrations were observed in control areas (no cover). Sediment profile imaging, diver observations and sampling, spectroscopic analyses such as X-ray fluorescence (XRF), X-ray diffraction (XRD) and XAS on selected cover materials confirmed the biogeochemical processes responsible for the performance of cover layers in the field pilot study.

6.02.T Natural Resource Damage Assessment: Injury Determination in Multi-Contaminant, Multi-Stressor Systems

6.02.T-01 Discussion - Injury Determination in Multi-Contaminant, Multi-Stressor Systems

Sarah E. Allan, Amy A. Merten, Marla Steinhoff and Cathy Laetz, National Oceanic and Atmospheric Administration (NOAA)

The chairs will lead a discussion about challenges, successful approaches, and new ideas for injury assessment in multi-contaminant, multi-stressor systems. The focus of the discussion will be on case studies from ongoing or recently settled Natural Resource Damage Assessment (NRDA) cases and novel approaches for addressing the challenges inherent in these types of assessments. It may also include considerations for evaluating lost human uses in these environments, especially in disadvantaged communities experiencing environmental injustices. The platforms will provide examples of different laboratory and field studies, data analysis methods for new and historic data, and injury determination and quantification approaches. The discussion will provide an opportunity to highlight key take-aways from those case studies and share ideas about ways to improve injury assessments at urban waste sites and other areas where multiple contaminants or stressors present scientific challenges for determining liability through the Natural Resource Damage Assessment process.

6.02.T-02 Growth of Pacific Staghorn Sculpin (*Leptocottus armatus*) is Reduced at Contaminated Sites in the Lower Duwamish River, Washington

Cathy Laetz, National Oceanic and Atmospheric Administration (NOAA)

The Lower Duwamish River is a highly industrialized waterway flowing into the densely urbanized Puget Sound waterfront of Seattle, WA. The river has been profoundly altered from its natural state over more than a century of channelization, recurrent dredging, shoreline armoring, and pollution discharges. As part of a Natural Resource Damage Assessment addressing historical pollution at three designated Superfund sites (i.e., the assessment area), juvenile Pacific staghorn sculpin (*Leptocottus armatus*) were sampled throughout the lower

river in order to evaluate injury from exposure to pollutants including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDTs), and butyltins (BTs). Sculpin live in close association with the river sediments within and upriver of the assessment area. Fish were collected for analysis of contaminant concentrations in composited whole bodies and stomach contents, as well as individual fish health metrics including daily somatic growth rates measured from otoliths. Sediment contaminant concentrations were also measured at sites near to fishing locations. Fish growth rates varied from 0.65 to 1.05 mm/day, and were significantly lower at unremediated downriver sites compared to upriver and remediated locations. Sculpin growth rates were negatively correlated with concentrations of PCBs in fish bodies, PAHs in stomach contents, as well as PCBs, DDTs and PAHs in sediment. Fish growth rates were positively correlated with water temperature, which generally increased upriver. Overall, these results support an evaluation of injury to resident fish from contaminant exposure in the Lower Duwamish River. Furthermore, this study demonstrates the utility of using paired biological and chemical indicators of pollutant-induced injury in a resident fish to inform a complex natural resource damage assessment and associated restoration efforts.

6.02.T-03 Dietary Exposure to Environmentally Relevant Levels of Chemical Contaminants Reduces Growth and Survival in Juvenile Chinook Salmon

Jessica I Lundin¹, Paul M Chittaro², Irvin Schultz², Mary R Arkoosh², Mary C Baker², David H Baldwin², Tracy Collier³, Barbara French², John Kern⁴, Jana S Labenia², Tiffany L Linbo², Amy A. Merten², Cameron M Schuster⁵, Karl B Veggerby⁵, Gina M Ylitalo², Nathaniel Scholz² and Joseph P Dietrich², (1)National Research Council Research Associateship Program, (2)National Oceanic and Atmospheric Administration (NOAA), (3)Western Washington University, (4)Kern Statistical Services, (5)Industrial Economics, Inc.

Portland Harbor and the Lower Duwamish River are urbanized waterways in the Pacific Northwest that have been designated Superfund sites due to extensive pollution in receiving waters. Both sites have generally similar industrial histories resulting in similar contaminants of concern; specifically, mixtures of polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), and polycyclic aromatic hydrocarbons (PAHs). The presence of chemical contaminants is a driver of poor water quality that is limiting the recovery of wild Pacific salmon populations that are listed as threatened or endangered under the U.S. Endangered Species Act (ESA). Although many legacy persistent organic pollutants have been phased out, and their environmental levels have decreased in many habitats, the effects of these present-day exposure levels have not been well characterized. Specifically, information on adverse health outcomes associated with juvenile salmon exposure to mixtures of these contaminants at current levels is lacking. The research reported herein determined the effects of contaminant exposures on the growth and disease susceptibility of juvenile Chinook salmon. The salmon were dietarily exposed for 35 days to mixtures of DDTs, PCB, and PAHs at multiple doses with chemical proportions that reflected the levels of contaminants found in the stomach contents of juvenile Chinook salmon collected from Portland Harbor in 2018. Salmon exposed to current environmentally relevant levels of contaminants, as well as greater doses, had significantly reduced fork lengths and otolith microstructural growth indicators. Similarly, salmon fed the environmentally relevant dose for 35 days had reduced disease resistance, as evinced by higher rates of mortality when subsequently challenged with the pathogen *Aeromonas salmonicida*. These results show that dietary exposures to mixtures of industrial contaminants, at present-day levels, remain a concern that should be considered through the Natural Resource Damage Assessment process, as they can cause injury via adverse effects on salmon growth and disease resistance.

6.02.T-04 Site Specific Mixed Metal and Polycyclic Aromatic Hydrocarbon Toxicity Models Using Field Collected Sediments and Laboratory Bioassays

Jeffrey M. Morris¹, Michelle Krasnec¹, Fabrizio Bonatesta¹ and Gregory F Baker², (1)Abt Associates, (2) National Oceanic and Atmospheric Administration (NOAA)

A mixture of hazardous substances including metals and polycyclic aromatic hydrocarbons (PAHs) are present at elevated concentrations and different compositions in freshwater sediments at an active Natural Resource Damage Assessment (NRDA) site in the Midwest. In support of the injury assessment, we collected sediment samples from the site across a range of mixed metal and PAH concentrations and a range of organic carbon concentrations. We then conducted laboratory bioassays on *Hyaella azteca* and *Chironomus tentans* using the site-specific sediments and fit individual hazardous substance concentration data to the toxicity data. To allow for the assessment of a mixtures of hazardous substances, we also calculated toxic units (TUs) for the hazardous substances and fit concentration response curves to individual TUs and sums of TUs. We were able to fit dose response curves to mortality data for multiple individual metals as well as individual PAH sums. Our ability to fit dose-response models for multiple individual contaminants may be due to the co-occurrence of elevated concentrations of metals and PAHs in Site sediments; with the observed toxicity being due to exposure to multiple hazardous substances in these sediments. The combined TU mixture models also fit the data well, consistent with the observed toxicity resulting from the combined effects of multiple hazardous substances. These results can be extrapolated to samples previously collected within aquatic habitats at the site, to assess injury at the site. We will also discuss a preceding pilot study we conducted in the laboratory to determine appropriate sediment equilibration time needed prior to adding organisms to the exposure chambers.

6.02.T-05 Exposure to Contaminants in Sediments from the Portland Harbor Superfund Site Reduces Growth and Survival in Benthic Invertebrates: An Analysis of Historical Sediment Toxicity Data

Kaitlin Lieb¹, Sarah E. Allan¹, John Kern² and Katherine Zarada³, (1)National Oceanic and Atmospheric Administration (NOAA), (2)Kern Statistical Services, (3)Industrial Economics, Inc.

In the Natural Resource Damage Assessment (NRDA) process, natural resource trustee agencies must seek to determine whether contamination injured natural resources and, if so, quantify that injury over time and space. Establishing a relationship between sediment contaminant concentrations and toxic effects in benthic species and communities is important for determining and quantifying injuries to aquatic habitat and resources resulting from contamination. Sediment chemistry and benthic invertebrate toxicity data from two large studies conducted in the Portland Harbor Superfund site in 2004 and 2007 were compiled using NOAA's DIVER (Data Integration Visualization Exploration and Reporting) data warehouse. The relationships between contaminants and survival and growth endpoints in *Hyaella azteca* and *Chironomus dilutus* were evaluated to determine if contaminated sediments in the Superfund site have caused toxic injuries to benthic invertebrates. Multiple regressions, correlation matrices, and visual methods were used to evaluate the relationship between toxic effects to benthic invertebrates and both single and mixtures of contaminants. Increased contaminant concentrations in sediments from the site are associated with decreased growth and survival in benthic invertebrates. Comparing the average growth and survival of benthic invertebrates in concentration ranges above and below established sediment quality guidelines reveals distinct differences and serves as an effective metric for evaluating benthic injury for NRDA. These results indicate that the contaminants present in Portland Harbor are likely adversely affecting the survival and growth of benthic invertebrates.

6.02.T-06 Approaches and Case Studies for Assessing Injuries at Legacy NRDA Sites with Environmental Justice Considerations

Nadia Martin, Adam Stack and Robert Unsworth, IEC

Determining and quantifying natural resources injuries and selecting and scaling restoration actions to address those harms can demand specialized approaches at legacy hazardous waste sites with long histories, multiple contaminants and stressors, and complex physical and biological environments. For example, consideration may

need to be given to harms to nearby communities of color or economically disadvantaged communities. Harms to communities with environmental justice concerns may be amplified by their unique attributes, institutions, burdens, and vulnerabilities. There are approaches to incorporating cultural service losses and environmental justice considerations in NRDA. For example, when quantifying natural resource injuries (including service losses), examining the socioeconomic, cultural, and historical characteristics of communities can provide insight into the inequitable burden of human use losses stemming from the release of hazardous substances. Further, developing restoration options through meaningful engagement with communities to address both natural resource injuries and associated service losses is required for a more complete restoration of lost services. We will draw from several case studies and examples to highlight approaches in this context.

6.03.P-Mo PFAS Regulatory Investigations – Actions and Lessons Learned

6.03.P-Mo-195 Non-Targeted Analysis of Emerging PFAS in Site Investigations

James McCord, Mark Strynar and Jacqueline Bangma, U.S. Environmental Protection Agency

Industrial producers and users of per- and polyfluorinated alkyl substances (PFAS) have been a major source of chemical contamination to nearby communities due to historical releases of legacy PFAS. Following the general phase-out of legacy PFAS such as PFOA/PFOS, industrial usage has shifted to alternative PFAS and it has been the domain of non-targeted analysis (NTA) to identify replacement species. Environmental and public health organizations have a significant interest in identifying emerging chemical contaminants such as PFAS due to concerns for potential widespread release and the persistence and bioaccumulation of PFAS species. Over the last several years, the US Environmental Protection Agency's Office of Research and Development (USEPA ORD) has applied non-targeted analytical approaches alongside traditional targeted PFAS quantitation techniques to expand the coverage of PFAS information in uniquely impacted locations. USEPA partnerships with state and regional governments have examined novel PFAS classes including novel PFAS fluoroether species exhibiting ether linkages and acid head groups, chlorinated perfluoropolyethers (CIPFPECAs), and polyfluorinated side products of polyfluorovinylidene (PVDF). Specific replacement chemicals used in applications such as metal plating and PFOS-free AFFF have been identified in local media, including biota. These results have been used to evaluate the effectiveness of various PFAS remediation techniques on legacy and novel PFAS. This presentation describes case studies of recent work by EPA ORD in NTA investigations of PFAS sources. Examination of effluent impacted media from industrial sources indicates that in the absence of treatment, effluent-derived contaminants are frequently present, even in the case of "non-contact" processes. Source examination reveals both intentionally added chemical species and production byproducts as common origins for emerging PFAS. Non-targeted interrogation of water treatment approaches for emerging contaminants indicates that existing PFAS treatment technologies can be effective controls for many undescribed PFAS species. We will also discuss efforts to ensure the consistent application of NTA approaches, transparent reporting of NTA results, and decision making using non-targeted analysis.

6.03.P-Mo-196 PFAS Legal Enforcement Tools

Jeffrey Speir, Laurence Libelo and Lianna Kardeman, U.S. Environmental Protection Agency

The U.S. Environmental Protection Agency (EPA) has used a variety of legal enforcement tools to address per- and polyfluoroalkyl substances (PFAS) contamination. EPA has used its information gathering authority across statutes to better understand PFAS manufacturers' operations. EPA has also entered into a number of Safe Drinking Water Act administrative orders with entities responsible for PFAS contamination, most recently in November 2022, to require characterization of PFAS in drinking water and the provision of alternative water. EPA also acted on alleged violations of the Clean Water Act, entering into an administrative order in April 2023 which seeks compliance and characterization measures. These actions emphasize the Agency's commitment to holding polluters accountable for their actions, as additional efforts are underway to promulgate regulations related to PFAS. EPA's actions help to ensure that polluters bear responsibility for characterization and

remediation efforts and prevent future releases of PFAS. EPA remains committed to addressing PFAS contamination, as noted in the recent Federal Register notice inviting public comments on National Enforcement and Compliance Initiatives for Fiscal Years 2024-2027, which include a proposed initiative titled, “Addressing PFAS Contamination.”

6.03.P-Mo-197 Extraction Efficiency and Partitioning of Fluorotelomer Alcohols in Soils

Marina Evich¹, John Washington¹ and Du Yung Kim^{1,2}, (1)U.S. Environmental Protection Agency, (2)Oak Ridge Institute for Science and Education Research (ORISE)

Fluorotelomer alcohols (FTOHs) are widely used in applications of fluorochemical manufacturing. FTOHs are precursor compounds of perfluorinated carboxylic acids such as perfluorooctanoic acid (PFOA). Since FTOHs are volatile and sparingly water soluble, liquid extraction with organic solvent is recommended. Although FTOHs are extractable with organic solvent, differing hydro- and lipophobicity with carbon chain length of FTOHs requires assessment of soil/solvent ratio or serial extraction. In this study, FTOHs in contaminated or sludge applied soils are extracted with various volumes of methyl *tert*-butyl ether (MTBE), and partitioning characteristic between MTBE and soil was investigated. FTOHs in soil were extracted with soil/MTBE (mass/volume; g/ml) ratio of 5/2, 1/3, 1/10, and 1/20 overnight by rotation at room temperature. To find out the extraction efficiency, soil samples were extracted serially up to 4 times. Positive chemical ionization gas chromatography mass spectrometry (GC-MS) was used for quantitation of 4:2 to 18:2 FTOHs and 5:2 to 11:2 secondary FTOHs (sFTOHs). The extracted mass of FTOHs generally increased with increasing MTBE volume. In a series of 4 serial extractions, more than 90% of the total recovery for longer chain FTOHs (10:2 to 18:2 FTOHs) was observed in the first extraction step and the total extracted mass varied relatively little across a range of soil/MTBE ratios. Conversely, the extraction efficiency of shorter chain FTOHs (4:2, 6:2, and 8:2 FTOHs), which was <50% of total FTOH mass recovered, increased with MTBE volume. Shorter chain FTOHs have less hydrophobicity and higher affinity to absorb to the soil phase relative to MTBE. In the environment, these different partitioning properties of varying chain length might affect sorbed-water partitioning and leaching in soil. The result of extracted FTOHs mass with soil depth profile will be helpful to elucidate transport of FTOHs in soil and groundwater systems.

6.03.P-Mo-198 PFAS Risk Assessment for a Defence Military Training Base

Olivia Henderson, Christopher J. McCarthy and Barrie Selcoe, Jacobs Engineering Group Inc.

A Human Health and Ecological Risk Assessment (HHERA) was completed for exposure to PFAS (namely PFOS, PFHxS and PFOA) at a military base. The HHERA sampling program included collecting soil, sediment, surface water, groundwater, terrestrial biota (home-grown fruits, vegetables, chicken- and duck-eggs, grass, and fodder for livestock feed) and aquatic biota (fish and crustaceans). Risk to human health was assessed for statistically based ‘typical’ and ‘upper limit’ exposure intakes to represent a reasonable worst case. Published read-across data were used to inform the risk estimates from PFAS other than PFOS, PFOA and PFHxS. Several exposure pathways presented an elevated risk including the consumption of duck-eggs, the consumption of home-slaughtered sheep meat, the consumption of fish from private dams, potential future consumption of home-slaughtered pigs for meat consumption, and the future consumption of milk from dairy cattle. The screening-level ecological risk assessment identified over 40 threatened or endangered species potentially inhabiting the site. Aquatic and terrestrial food-web models across multiple habitats identified complete exposure pathways from the base of the food web (primary producers) to apex predators. To evaluate lower order species (plants, terrestrial/aquatic invertebrates and fish), detected PFAS concentrations were compared to screening levels. Indirect toxicity to higher order species was evaluated via a holistic assessment of complete (and significant) exposure pathways, as well as assessing dietary composition and available food sources within the Study Area. The potential for exposure was characterized considering factors such as specific habitat requirements, feeding strategies and behaviour, migratory patterns, and the species-specific foraging range or home range. These factors were considered in relation to the habitat conditions in the PFAS source areas and

flow pathways. Taken together, these data provided a qualitative understanding of the likelihood that wildlife obtained a substantial proportion of their food (for indirect exposure and bioaccumulation/biomagnification) from within the contamination footprint within the Study Area. The findings of the ecological risk assessment concluded that "potential adverse effects to ecological receptors can't be excluded". However, risks were likely overstated for those receptors that receive only a portion of exposure (and therefore diet) from the site.

6.03.P-Mo-200 PFAS and EPA Clean Water Act Enforcement and Compliance Inspections

Lianna Kardeman, Ivy Koberlein and Sean Ireland, U.S. Environmental Protection Agency

PFAS contamination released to air, soil, and water is impacting our ground water and surface water, and most importantly our drinking water. EPA Administrator Michael S. Regan stated, "I have directed EPA staff to use every enforcement tool at our disposal to require manufacturers of PFAS to address potential endangerment to the public and to compel them to characterize, control, and clean up ongoing and past PFAS contamination." This presentation will describe water-related investigations including the review of documents, communication with facility representatives, site visits, sampling, and analysis. Enforcement tools, including inspections and sampling activities, are critical for understanding current PFAS pollution and its impacts on human health and the environment. During inspections, on-site operations and maintenance procedures are evaluated to understand the processes and effects of chemicals used and produced, both intentionally and unintentionally, which may contribute to discharges. Wastewater, stormwater, soils, and drinking water may be collected and analyzed by targeted and non-targeted methods to identify and quantify fluorocarbon chemicals present and to identify potential noncompliance during sampling investigations under the Clean Water Act and Safe Drinking Water Act. Past water-related investigations pertaining to PFAS-related compliance and general results will be discussed.

6.04.P-We General: Engineering, Remediation and Restoration

6.04.P-We-149 Potential for In Situ Biodegradation of Pyridine Derivatives at the Indianapolis Reilly Tar and Chemical Superfund Site

Gerald K. Sims¹ and Edward J. O'Loughlin², (1)New Mexico State University, (2)Argonne National Laboratory

In 1984, the Indianapolis Reilly Tar and Chemical property was placed on the USEPA National Priority List due to aquifer contamination with benzene, pyridines, and ammonia. The site is situated in a diverse, low-income community. EPA approved (1992) *ex-situ* treatment (pump and treat). After 30 years of treatment, several contaminants, including alkylpyridines, exceeded tolerances. In 2021, EPA issued a ROD to change the remediation strategy to barrier biosparging. In the lead up to the 1992 decision, we conducted studies to determine if the sediment's aerobic microbial community could degrade pyridines. Owing to its relevance to the 2021 ROD, we report herein a series of previously unpublished studies performed on sediments from the site in 1988-1990. Sediments (10 m) were found to be iron-reducing and contaminated with dozens of compounds, including 16 alkylpyridines, detected by GC-MS. Aerobic dissipation rates of seven alkylpyridines were monitored in sediments (within and outside the contaminant plume) using capillary GC-TSD. Indigenous degraders of pyridine, 2-, 3-, or 4- methylpyridine were enumerated by a most probable number method (MPN) using resazurin dye as a growth indicator and colorimetric detection of ammonium to confirm biodegradation. MPN enumeration demonstrated enrichment of degraders within the plume. Five isolates degrading 2-methylpyridine, the primary contaminant, were recovered by elective culture and putatively assigned to the genera *Enterobacter*, *Pseudomonas*, *Xanthomonas* and two members of the genus *Arthrobacter* (one of which was recently sequenced). All seven alkylpyridines were degraded by indigenous populations (with release of ammonia) at greater rates within than outside the plume. Mono-substituted pyridines exhibited significantly faster degradation than di-substituted analogs, consistent with the outcome of 30 years of treatment. Anaerobic incubation lengthened $t_{1/2}$ relative to aerobic incubation. Sorption effects on bioavailability were limited, attributable to the high solubility of pyridines as well as the coarse texture and low organic carbon content of

the sediments. Collectively, results indicate conditions in the aquifer were conducive to aerobic *in situ* bioremediation at the onset of treatment. The results support EPA's decision to switch to this technology, which should prove more effective for resolving the environmental justice concern at the site, now approaching 40 years on the Superfund list.

6.04.P-We-152 Seasonal Sulfur Redox Cycling in Two Constructed Wetlands with Insight on How They Age

Cher M Lindelien^{1,2}, *Xiaoyu Xu*³, *Anna Knox*³ and *Erin Peck*³, (1)NCASI, Inc., (2)National Council for Air and Stream Improvement, (3)University of Georgia

Long-term metal remediation in wetland treatment systems (WTSs) involves facilitating dissimilatory sulfate reduction to produce sulfide and mineralize metals in deep sediments. We evaluated seasonal sulfur cycling in two constructed wetlands (Maintained WTS constructed in 2007, and the Unmaintained WTS constructed in 2000) on the Savannah River Site in Aiken, SC that were originally amended with inorganic sulfate to produce anoxic sulfide and mitigate metals more permanently in deeper layers of the sediment. There was a significant interaction in the sulfide concentration between the sediment depth range, season, and wetland ($F=4.64$, $df=11$, $P=3.28 \times 10^{-5}$). Specific seasonal differences were significant at the 0 cm depth in the Maintained WTS ($t=-2.66$, $P=9.70 \times 10^{-3}$). This indicated that dissimilatory sulfate reduction would most likely be dominant in the surface sediments of the Maintained WTS during the warm season, while the seasonal change was not evident in the Unmaintained system. The concentration of sulfate in the pore waters across both WTSs changed seasonally ($F=7.84$, $df=1$, $P=6.50 \times 10^{-3}$); however, the concentration was significantly higher in the warm season compared to the cool season ($t=-2.80$, $P=6.60 \times 10^{-3}$), contrary to what was hypothesized to be occurring in these systems. Sulfate concentrations in the pore waters would be expected to be depleted as sulfate reduction is known to dominate in warmer conditions. Sulfur limitation in the sediments of the Unmaintained WTS during the warm season aligned with increased assimilation of total sulfur content in the giant bulrush during the productive warm season. The lower concentration of sulfide in the surface sediments reported in the Unmaintained WTS demonstrated what would occur in WTSs over time. The Maintained WTS can be managed to address the issues of erosion, decreasing pH, and sulfur limitation observed in the Unmaintained WTS.

6.04.P-We-154 Development of QAPP for AROMA-VOC

*Hong Cheng Tay*¹, *Anthony Miller*² and *Kelly Pennell*¹, (1)University of Kentucky, (2)Entanglement Technologies, Inc.

Real-time VOC monitoring technology has become an important tool for site assessment, especially at vapor intrusion (VI) sites. Site professionals often use traditional passive sorbent-based samplers and/or using Summa canisters for assessing vapor intrusion risks. Real-time monitoring techniques can also provide useful information about concentration fluctuations. Several studies have identified that concentrations of volatile organic compounds (VOCs) can fluctuate throughout the day at a VI site due to seasonal and temporal variations. Real-time monitoring technologies, such as HAPSITE, portable Gas Chromatography (GC), Trace Atmospheric Gas Analyzer (TAGA), have been used at many sites. In the present work, a Quality Assurance Project Plan (QAPP) is developed for a new technology, AROMA-VOC, to determine the performance and reliability of the instrument. AROMA-VOC uses cavity ringdown spectroscopy coupled with thermal desorption to analyze 9 chemical compounds at ppb and ppt levels. This instrument is fairly user-friendly and does not require an operator with a scientific background compared to the other real-time monitoring alternatives. This study produces calibration curve with 9 different compounds that exhibit correlation above 0.99. The quality assurance parameters and values are compared with GC-MS obtained from literature. The results of this study are significant because GC-based technology is by far the most applied technique for site assessment at vapor intrusion sites and comparison with AROMA-VOC QA/QC data provides a meaningful benchmark.

6.04.P-We-155 Planning for Disaster: A Case Study Based on the East Palestine, Ohio Train Derailment
Bruce W. Vigon¹, Scott C. Smith², Donald P. Brown³, Beatrice A. Golomb⁴ and Peter DeCarlo⁵, (1)Breveja Environmental Consulting LLC, (2)ECO Integrated Technologies, Inc., (3)Brown Energy and Environmental Consulting and Development, LLC, (4)University of California, (5)Johns Hopkins University

In early February of 2023, a train carrying multiple combustible and hazardous chemicals derailed in the town of East Palestine, in eastern Ohio near the border with Pennsylvania. In addition to the immediate fire and physical damage to the railcars, a decision was made by the on-site commander to burn the contents of several of the tank cars, ostensibly to avoid a potential catastrophic explosion. At the time of the derailment, various organizations, with differing levels of experience and expertise, sprang into action to mitigate damages and ultimately to prepare for remediation efforts. This presentation will not evaluate or judge the appropriateness of the actions taken. Rather, it will focus on planning for a scientifically informed response to future incidents. Train accidents are very common. Responders may not have been able to train and understand what information should be available or could be collected immediately following the incident to provide improved decision-making. Based on the actual events, the authors have identified a number of elements of the response that would have been more optimal had a better response plan been implemented. Data collected after the derailment and burn will be used to “backcast” knowledge that could have been used to improve decisions. In addition, monitoring equipment choices, access to chemical properties and toxicology information, and other potentially beneficial decision support elements will be examined. Had state of the art, proven remediation technologies been used, the dispersion of chemical contaminants presumably would have been minimized. Community and regional health effects, environmental damages, and legal and financial liability for the responsible party would have been mitigated. Hopefully, recommendations can be incorporated into future responses that result in better organized response plans put in place with commensurate training.

6.04.P-We-156 Effects of Spatial Variability of Macroinvertebrate Communities on Assessment of Remediation and Restoration Efforts at Great Lakes Area of Concern (AOC) Sites

Roger Yeardley, Marc Mills and James M. Lazorchak, U.S. Environmental Protection Agency

The U.S. EPA’s Office of Research and Development (ORD), in working with its multiple state and federal partners, is tasked with helping to determine if conditions have improved after remediation and restoration at Great Lakes AOC sites. Environmental agencies of the Great Lakes states have long used macroinvertebrate community metrics and indices in bioassessment, and more recently for delisting of Beneficial Use Impairments at AOCs. Spatial variability affects the ability to detect community differences pre- and post-remediation and restoration. Though duplicate samples of media for chemical analyses have long been collected as part of bioassessment efforts, it is not as common to collect duplicate community samples, especially by long-term deployment of artificial substrate samplers. Since 2020, ORD has been deploying duplicate moorings at AOC sites to assess spatial variability in macroinvertebrate communities and how that will affect the ability to detect changes. We assessed small-scale spatial variability from community data from duplicate moorings (each with multiple Hester-Dendy (HD) samplers), deployed and retrieved from 17 Stations at 9 Sites (Ottawa River, Otter Creek, Swan Creek, Lower Maumee River, Pickle Pond, Loons Foot Lake, Erie Pier Pond, Clough Pond, Ashtabula River), and located at 3 AOCs (Maumee River (Toledo, OH), St. Louis River (Duluth, MN, Ashtabula River (Ashtabula, OH)). We examined the relative spatial variability of a suite of structural and functional metrics and indices by comparing the means of the relative percent differences (RPD) of these metrics for the pairs of samples from all stations from all sites. Variability was metric/index dependent. For example, the common metrics, total taxa richness and percent dominant taxon, had RPDs of 15% and 24% respectively. The mean RPD for abundance/ density was 41%. The lowest mean RPDs were those of metrics/ indices that incorporated tolerance values, the Hilsenhoff Biotic Index (3.3%) and the mean tolerance value (3.2%). At Ohio AOCs, 2 versions of HD samplers were deployed, an ORD version and an Ohio EPA version.

No significant difference (paired t-tests, $p \leq 0.05$) was seen for any metric or index when RPDs from both types of samplers were compared. Multivariate analyses (Nonmetric Multidimensional Scaling) will also be presented as another line of analysis to compare communities retrieved by the duplicate moorings.

6.04.P-We-158 Utilization of Biochar for the Removal of Phosphorus and Chloride in Water

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Phosphorus (P) and chloride (Cl) pollution in water have created serious and widespread problems on the environment. P is one of the limiting nutrients to control eutrophication, which has various detrimental effects such as increase of water treatment cost, decrease of oxygen content and recreational use of water. High chloride concentration can cause 1) corrosion and scaling of pipelines facilities, 2) salinization of freshwater and soil, and 3) inhibition of plant growth. Therefore, it is urgent to remove P and Cl from water. Different technologies are available to remove P and Cl from water. However, as a low-cost adsorbent, biochar derived from a combination of various wood chips has been rarely investigated to remove P and Cl. This study focuses on the removal of P and Cl from water by batch adsorption experiments using biochar derived from a combination of various wood chips (spruce, pine, and fir). Adsorption characteristics of P onto biochar were evaluated as a function of biochar dosages, initial P concentrations, and pH using synthetic water. Adsorption of Cl was evaluated as a function of initial Cl concentrations. During batch tests, the mixture was shaken at a speed of 150 rpm using a Platform Shaker at room temperature for a specified period. Samples were collected at regular interval and filtered through 0.45 μm syringe filters. The filtered samples were then analyzed to determine the residual P and Cl concentrations using a HACH DR 2800 Spectrophotometer. Results demonstrated that the percentage of P removal increased consistently with the increase of biochar dosages. The highest P removal was 75% at a biochar dosage of 100 g/L. P removal deteriorated with the increase of initial P concentrations. The maximum P removal was obtained at a pH value of 2. The adsorption isotherm followed the Langmuir model and adsorption kinetics followed a second order kinetic model for P adsorption onto biochar. On the other hand, the percentage of Cl removal was 19% for an initial Cl concentration of 100 mg/L at a neutral pH value. This study indicated that the biochar has the potential to remove P and Cl from water. Further experiments are in progress to investigate the optimum parameters for the removal of Cl.

6.04.P-We-160 Impacts of Rainfall Pulses on Efflux of Cu and Zn from a Constructed Wetland

Dylan Ricke¹, Andrew C. Lydeard, Christina Fulghum, Paul Stankus and Dean Fletcher, University of Georgia

Constructed wetlands (CWs) are widely used to buffer pH and sequester harmful pollutants, such as metals, from downstream waterways and resident organisms. Although CWs may have a net effect of sequestering metals, stormwater events are known to mobilize a portion of sequestered metals, resulting in discontinuous patterns of metal efflux from CWs over time. Importantly, metal efflux is the product of changing metal concentrations and the volume of water flowing through the system, which is often overlooked. In the current study, we monitored the concentration and flux of total and dissolved Cu and Zn, as well as total suspended solids, through the H-02 CW. This CW was built to buffer pH and sequester metals originating from the National Nuclear Security Administration's Tritium Processing facility at the Savannah River Site, Aiken, SC, USA. Water sampling for measuring metal flux was focused on the periods of time shortly before, during, and after major rain events. Programmable water samplers and monitoring sensors immediately upstream and downstream of the treatment cells were used to sample water and measure rainfall, discharge, and a set of water quality parameters (pH, turbidity, conductivity, dissolved oxygen, and oxidation reduction potential) at 15-minute intervals. Samples and measures were composited to calculate average hourly values for each metric. Our study revealed patterns between rainfall, metal flux, and metal loading through a CW over multiple rain events. We found that the onset of rain (the "first flush") often coincided with an initial spike in metal efflux through H-02, with a heightened proportion of metals associated with suspended solids. As events proceeded, metals transitioned from particulate to dissolved phase. This was especially true for more intense rain events.

Despite spikes in metal efflux following rain, overall, H-02 still captured a greater amount of metal from its influent than was expelled as effluent (its metal sequestration efficiency), demonstrating effectiveness of the system. Although sequestration efficiencies for total Cu and Zn were usually positive, they declined in the hours after the onset of rain. Our results contribute to a quantitative foundation for testing additional drivers of metal sequestration over time, including seasonal changes in water quality and time since last rainfall. These factors could potentially be used to parameterize models for guiding future decisions in CW management and design.

6.04.P-We-161 Soil Contaminated with Mining Waste Abandoned 50 Years Ago in La Planta (San Juan, Argentina): From Socio-Environmental Assessment to In Situ Phytoremediation

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Adverse effects on human and wildlife health by exposure to mining waste have generated public and scientific concern. Abandoned mines cause environmental pollution due to changes in the landscape and the persistence of metals. The goal of this study was to assess the degree of soil pollution caused by mining waste and to evaluate different strategies to (phyto)remediate it to achieve long-term ecosystem restoration working together with the community of La Planta town. Since 2013, our research team has evaluated the occurrence of metal(oids) and the physicochemical properties in soil, vegetal diversity and cover, ecotoxicological effects using aquatic and terrestrial organisms, and the perception level of the peasant community that inhabits the surroundings of the site. A contamination plume was detected up to 2.1 km away and correlated with the concentration of several metal(oids), pH, conductivity, toxicity, vegetal diversity and soil cover. The most contaminated site was located in the discharge point of the abandoned treatment plant which was characterized by acidic pH (2.7), high conductivity (42.9 mS/cm), and high concentration of Pb (450), Cu (826), Cd (19), Zn (2227), Mn (707) and As (2383 mg/kg). A sensitivity distribution profile showed that 52% of the endpoints had values of EC50 less than 1%. Using a combination transects in field and satellite images, a decrease of 50% in plant richness (13 spp.) and 70% in vegetation cover (bare soil=42%) were found (Shannon index=0.02). As an outcome, four native trees and shrubs were identified as potential bioaccumulator plants. A series of surveys and workshops carried out with the peasant community allowed a successful exchange of knowledge in social, economic and environmental aspects. After remediation trials, a combination of soil with 5% compost and 18% dolomite allowed to increase the pH, organic matter and nutrients, and reduce the metal availability and toxicity. Chronic exposure of the four native plants to amended soil demonstrated their successful ability to grow and phytoextract metal(oids). Two ongoing experimental 500 m²-plots were installed and are being monitored together with the community. Plants are growing faster in the less contaminated plot (1-y). Therefore, this socio-environmental study shows the ability to long-term articulate between interdisciplinary and inter-institutional research groups together with a peasant community throughout a restoration process of the ecosystem.

6.04.P-We-162 Equilibrium Passive Samplers for Short-Term Measurements of Polychlorinated Biphenyls

Oindrila Ghosh, *Louis Cheung*, *Nathalie Lombard* and *Upal Ghosh*, *University of Maryland*

Hydrophobic organic contaminants (HOCs) like polychlorinated biphenyls (PCBs) have strong bioaccumulation potential that pose threats to human and ecological health even at trace concentrations of ng-pg/L. Polymeric passive sampling allows measurement of the freely dissolved concentrations that control bioavailability of HOCs. Short-term surface water measurements are often critical in assessing inputs from episodic events for which passive sampling is currently not available. In current literature there exists a knowledge gap of optimizing equilibrium passive samplers for short-term measurements of PCBs. In this study we fill this gap by optimizing the designs of two short-term polymeric samplers (PE sheet and PDMS fibers) using a combination

of mathematical modeling, in-lab physical experiments and finally field demonstration. The conceptual model was built using one-dimensional Fick's diffusion equation and the exchange kinetics of PCB molecules through PE sheet and PDMS fibers was simulated. To experimentally verify our modeling results, we designed an in-lab artificial flow chamber with a pump controlling the flow within a closed sampling station. Samplers loaded with a known concentration of performance reference compounds were placed within the sampling station and the fractional loss of these compounds tracked at 3 flow velocities (6, 15, 25 cm/s). We observed through these experiments that short-term measurements using an 18- micron (0.7 mil) thick PE sheet is possible in a 24- hour exposure during a high flow storm event. In a pilot field study, we deployed the optimized samplers in the Lower Beaverdam Creek and two storm outfalls, right before and collected right after a storm event so that the exposure period encompasses the duration of the storm. The freely dissolved concentrations were measured over the high flow period after a major rainfall event (short-term measurement) were compared with concentrations measured over a longer duration time integration of 2-3 months (long-term measurement). We observed that the short-term measurements at site showed slightly higher concentrations than long-term measurement which could be the influence of the storm event.

6.04.P-We-163 Use of Forest Residues for the Removal of Pesticides Present in Water

Rodrigo Andrés Sarria-Villa, José Antonio Gallo-Corredor and Mónica Marcela Collazos Ramirez, Universidad del Cauca

Water is an essential component for human life and in recent years it has become a limited resource, since the growth of the population and industry have generated a decrease in its quality, directly associated with pollution problems. A variety of contaminants can reach the water, such as pesticides, which come mainly from agricultural activities and through runoff and leaching processes from the soil, reach water sources. In Colombia-South America, the water resource is very abundant, but human activities and the lack of adequate treatment systems lead to its contamination. For these reasons, different decontamination processes for pesticides in water have been proposed, including precipitation, electrochemical processes, ion exchangers, biological treatments, advanced oxidation processes, membrane treatments, adsorption, among others. In this work, it was proposed to use agroforestry residues to elaborate an adsorbent for the retention of the organochlorine pesticide DDD present in water. Experimentally, this material obtained from natural renewable sources has shown retention percentages higher than 60% and adsorbent capacity comparable to those obtained with other types of materials such as activated carbon and lignocellulosic materials without prior treatment. References García Ríos, A., Martínez, A. S., Londoño, Á. L., Restrepo, B., & Landázuri, P. (2020). Determination of organochlorine and organophosphorus residues in surface waters from the coffee zone in Quindío, Colombia. *Journal of Environmental Science and Health, Part B*, 1–6. doi:10.1080/03601234.2020.180218 Ibrahim, A., Yaser, A. Z., & Lamaming, J. (2021). Synthesising tannin-based coagulants for water and wastewater application: A review. *Journal of Environmental Chemical Engineering*, 9(1), 105007. doi:10.1016/j.jece.2020.105007

6.04.P-We-164 Optimization of Electrocoagulation for Removal of Chlorides in Galvanizing Industry - Protecting the Wetland Systems

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Environmental pollution from galvanizing industry effluent is a global concern. The effluent contains a high concentration of chlorides, which poses environmental, health and regulatory challenges. Due to its efficiency and cost-effectiveness, electrocoagulation is a promising technique for the removal of chlorides. In this study, stainless steel and aluminum plates were used as electrodes in a parallel plate configuration. Process parameters such as current density, pH, conductivity, and electrolytes concentration were optimized to achieve maximum Chlorides removal efficiency. Samples were obtained from a galvanizing effluent plant. Current density, conductivity and pH were determined using a DC Supply, conductivity meter and pH meter. Ultraviolet visible

and Mohr method were used to measure Chlorides from the effluent. Aluminum plates achieved a higher chloride removal efficiency of 53.2% under optimal conditions; pH 8.8, inter-electrode spacing of 2 cm, operating time of 60 minutes, voltage of 13.2 V, and current of 3.01 mA/cm². Stainless steel electrodes achieved 44.79% removal under optimal conditions: pH 7.5, inter-electrode spacing of 4 cm, operating time of 20 minutes, voltage of 12.1 V, and current of 1.99 mA/cm². Aluminum electrodes had a higher chlorides removal efficiency but did not comply with local discharge limits. The release of untreated effluent into the environment can lead to water pollution, ecological disruption, and risks to human health. In order to comply with regulatory requirements, additional treatment techniques such as membrane and advanced oxidation could be applied.

6.04.P-We-165 Photocatalytic Degradation and Removal of Organic Contaminants Using Polydimethylsiloxane (PDMS) Composite Sponge with Titanium Dioxide

Milena Ceccopieri, Brian Ng, Kassidy Troxell, Natalia Quinete and Piero Gardinali, Florida International University

The increase in industrialization and urbanization has led to a significant influx of pollution into water sources, including hazardous chemicals, pharmaceuticals, and pesticides. These organic contaminants pose health risks and deteriorate water quality, emphasizing the need for their effective removal to mitigate potential adverse effects on aquatic organisms and human health. Polydimethylsiloxane (PDMS) is a silicone-based polymer known for being efficient in the adsorption of organic contaminants due to its oleophilic and hydrophobic nature. When made into a sponge, the increase in surface area can potentialize the PDMS adsorption properties. A previous study from our group had successfully demonstrated the adsorption potential of a simple and inexpensive PDMS sponge in the removal of organic contaminants from urban waters, with greater affinity for compounds with higher Log K_{ow} values. The study also shows that the functionalization of the PDMS sponge with charcoal further enhanced its adsorption efficiency, and the incorporation of metallic copper into the PDMS sponge provided effective antimicrobial properties against *E. coli*. In the present study, we continue testing the potential applications of PDMS sponge hybrid composites in environmental remediation by functionalizing the polymer with the addition of TiO₂ for the photocatalytic degradation of organic compounds. The tests are being conducted with a set of environmentally relevant chemical compounds (Caffeine, Lincomycin, Sulfamethoxazole, Trimethoprim, Norcocaine, Carbamazepine, Diltiazem, Atrazine, Diphenhydramine, Fluoxetine, Sertraline, Clotrimazole) that cover a wide range of Log K_{ow} (0.16 to 6.26). The experiment consists in adding a single 1x1x1 cm³ PDMS sponge composite with TiO₂ to a solution containing a mixture of the compounds (1 ng/mL), under both UV light and dark conditions. At specific time intervals (0, 0.5, 1, 2, 4, 8, 12 and 24 h), a sample (10.5 mL) is taken out for analysis using online SPE HPLC-HESI-HRMS (Orbitrap). Our hypothesis is that the combination of the unique properties of PDMS and TiO₂ will enhance the degradation and removal of these selected organic compounds under the influence of light.

6.04.P-We-166 Contaminants of Emerging Concern in Coastal Waters in St Croix, US Virgin Islands

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There have been few reports in the peer-reviewed literature on the levels of contaminants of emerging concern (CECs) in surface waters, groundwater and coastal waters from the Caribbean region. Because coastal damage, flooding and drought are predicted to worsen in the Caribbean region over the coming decades, it is important to identify the sources of these contaminants so that mitigation measures can be implemented. Having these data will inform coastal management units and watershed managers on the best approaches for designing resilient engineered and nature-based solutions to mitigate runoff due to storms and/or reduce seepage from septic systems during dry periods. Furthermore, these data are important for protecting coral reefs and inshore fisheries in the region. In this preliminary study, we deployed Polar Organic Contaminants Integrated Samplers (POCIS) at three locations within the nearshore coastal zone in St Croix, US Virgin Islands. Of the CECs

analyzed, caffeine, the pharmaceuticals, acetaminophen, propranolol, metformin and sulfamethoxazole, and the natural hormones, estrone and androstenedione were detected at all three sites. Interestingly, the artificial sweetener, sucralose and the antidiabetic drug, metformin were detected in μg amounts in POCIS deployed at one of the sites. Since these sampling sites were not located near centralized wastewater treatment plants, it is assumed that the source of these contaminants is leakage from household septic systems and/or dumping of sewage from boats. This further highlights the importance of finding solutions to mitigate the transport of CECs into the marine environment. Overall, more sampling is required but these preliminary data indicate that there is potential for impacts on the marine environment in St Croix and throughout the Virgin Islands from CECs discharged into the coastal zone.

6.04.V-018 *Lemna minor* mitigates effects in plasma and neurotoxic parameters in a Neotropical fish exposed to silver

Vanessa Bezerra, Giovanna Ivy Prado Santos, Beatriz Nazo Mônico, Lígia Silva Martins, Eduarda Avani Rodrigues and Juliana Delatim Simonato Rocha, State University of Londrina, Brazil

Phytoremediation has emerged as a green and low-cost alternative for removing contaminants from soil or water. However, few studies have evaluated whether the sublethal effects caused by exposure to contaminants are also mitigated in the aquatic fauna after phytoremediation. Silver is a non-essential metal and causes concern as a contaminant in aquatic ecosystems. Thus, the present study aimed to evaluate the effectiveness of *Lemna minor* remediation through physiological biomarkers in the Neotropical freshwater fish *Prochilodus lineatus* exposed to silver. *Prochilodus lineatus* juveniles ($n = 64$, 4.8 ± 1.4 g, 7.5 ± 1.0 cm, mean \pm SD) from a local fish farm were acclimated to laboratory conditions for at least 7 days. After acclimation, the animals were divided into 4 groups for a 48-h exposure test. In the control group (CTR) the fish were exposed only to tap water; in the *L. minor* group (Lm) duckweed was added on the water surface; in the Lm+Ag group the animals were exposed to $20 \mu\text{g L}^{-1}$ of silver with duckweed on the water surface; and in the Ag group the fish were exposed only to silver. After 48 h, the fish were anesthetized with benzocaine for blood collection. The blood samples were centrifuged to obtain the plasma used to evaluate glucose, sodium, potassium, and chloride concentrations. After the blood sampling the fish were euthanized by cervical transection for the collection of brain and muscle samples, which were used to assess acetylcholinesterase (AChE) activity. The concentration of sodium, potassium, and chloride did not differ between groups. Only the Ag group presented an increase in glucose concentration and a decrease in AChE activity in the muscle and in the brain. Hyperglycemia indicates that the animals exposed to silver were probably under a stress response. In addition, the reduction of AChE activity causes hyperstimulation of cholinergic pathways in the central and peripheral nervous system, which may lead to behavioral alterations in fish. No changes were observed in the Lm+Ag group compared to the CTR group. Thus, we can conclude that phytoremediation with *L. minor* is an effective alternative for mitigating the effects of silver in plasma and neurotoxic parameters in *P. lineatus*.

6.05.P-Mo Treatment and Remediation of Micropollutants (Including PFAS, microplastics, microorganisms)

6.05.P-Mo-199 Substantial Defluorination of Polychlorofluorocarboxylic Acids Triggered by Anaerobic Microbial Hydrolytic Dechlorination

Bosen Jin, Huaqing Liu, Shun Che, Jinyu Gao, Yaochun Yu, Jinyong Liu and Yujie Men, University of California, Riverside

Chlorinated polyfluorocarboxylic acids (Cl-PFCAs) derived from the widely used chlorotrifluoroethylene oligomers and polymers may enter and influence the aquatic environment. Here we report the substantial defluorination of Cl-PFCAs by an anaerobic microbial community via novel pathways triggered by anaerobic microbial dechlorination. Cl-PFCAs first underwent microbial reductive, hydrolytic and eliminative dechlorination, with the hydrolytic dechlorination leading to the highest spontaneous defluorination. Hydrolytic

dechlorination was favoured with increased Cl substitutions. An isolated, highly enriched, anaerobic defluorinating culture was dominated by two genomes that were most similar to those of *Desulfovibrio aminophilus* and *Sporomusa sphaeroides*, both of which exhibited defluorination activity towards chlorotrifluoroethylene tetramer acid. The results imply that anaerobic non-respiratory hydrolytic dechlorination plays a critical role in the fate of chlorinated polyfluoro chemicals in natural and engineered water environments. The greatly enhanced biodegradability by Cl substitution also sheds light on the design of cost-effective treatment biotechnologies, as well as alternative polyfluoroalkyl substances that are readily biodegradable and less toxic.

6.05.P-Mo-201 Advanced Clay-based Adsorbents for Reducing Bioavailability of Per- and polyfluoroalkyl Substances in Water

Raj Mukhopadhyay¹, Binoy Sarkar² and Carrie A McDonough¹, (1) Carnegie Mellon University, (2) University of South Australia

Per-/polyfluoroalkyl substances (PFAS) are highly persistent contaminants widespread in water resources. PFAS are used in many industries including textiles and leather processing, surfactants, and plastic manufacturing. PFAS are extremely persistent and bioaccumulative which make their remediation in water challenging. PFAS bioaccumulation in edible crops such as rice, wheat, and vegetables through PFAS-contaminated irrigation water and biosolid fertilization is a growing concern. Treatment strategies for adsorptive removal of PFAS using clay minerals and oxidic clays are inexpensive, eco-friendly, and efficient for bulk PFAS immobilization due to high surface area, porosity, and high loading capacity. Here, we discuss the potential of these materials to reduce the bioavailability of PFAS in agriculture. Clay-based materials adsorb PFAS through hydrophobic and electrostatic interactions. Based on a comparison of the partition coefficient values, high PFAS removal performances are obtained when clay minerals are modified with surfactants, polymers, and amines, i.e., organo-clay minerals, compared to pristine clay minerals. Adsorption is dependent on pH and PFAS molecular structure. Dose of adsorbents, temperature, ionic strength, and dissolved organic matter content of the medium also impact adsorptive removal of PFAS from water. Clay adsorbents minimize the transport of PFAS to edible crops through decontamination of irrigation water and reduce PFAS bioavailability. Advanced clay modification approaches such as clay-biochar composites and microorganism-loaded clay-based photocatalysts hold potential to improve PFAS remediation via enhanced adsorption-transformation mechanisms and therefore reduce PFAS bioavailability. However, regeneration and safe disposal are issues to overcome for using clay-based PFAS adsorbents at scale.

6.05.P-Th Treatment and Remediation of Micropollutants (Including PFAS, Microplastics, Microorganisms)

6.05.P-Th-153 The Impact of Pesticides and Human Use Pharmaceuticals on Nitrogen-Removal Processes with Wetland Treatment Systems: A Mesocosm Study

Emily Nottingham¹ and Tiffany Messer², (1)University of Kentucky, (2)University of Nebraska Lincoln

Wetland treatment systems are used extensively across the world to mitigate surface runoff. While wetland treatment for nitrogen mitigation has been comprehensively reviewed, the implications of other contaminants of potential concern such as common-use pesticides and pharmaceuticals on nitrogen reduction remain relatively unreviewed. Therefore, this study sought to determine the impact that imidacloprid, atrazine, caffeine, acetaminophen, sulfamethoxazole, and sulfate have on nitrogen removal processes (e.g., microbial denitrification, plant uptake). The contaminants of potential concern were determined based on their appearance across Kentucky surface waters in both urban (imidacloprid, caffeine, and acetaminophen) and rural (atrazine, sulfamethoxazole, and sulfate) environments. Two types of constructed wetlands, floating treatment wetlands, and free water surface wetlands, were tested to determine the impact that wetland design has on treatment removal potential along with planted and un-planted controls, equating to 24 mesocosms with replicates. Five

10-day experiments were conducted between May through August 2023 to determine if the contaminants of potential concern impacted nitrate removal when they appeared together in various mixtures. Preliminary results are currently being processed. By understanding the impact that different types of contaminants have on nitrogen removal processes within these systems, treatment wetlands can be specifically designed to treat and manage contaminants of potential concern.

6.05.P-Th-154 Microplastic Detection and Mitigation in Local Crops

Claire Yvonne Bucklin, North Toole County High School

Microplastics, plastics the size of 5 millimeters or less, are an issue of global concern with the majority of research being heavily directed towards the aquatic environment; however, microplastics have been noted in the terrestrial environment as well as have been detected and measured in various foods and within the human body. My previous research detected microplastic contamination in snow samples in rural mountain ranges around Montana as well and microplastics in and around bark samples taken from trees growing in isolated, rural areas. The question addressed in this study was, has microplastic contamination infiltrated into the spring wheat crops of Northern Montana and, if so, does the position of the crop in relationship to the local Interstate (East and West Sides of I-15) change microplastic levels in the crops samples? If so, can I engineer a device designed to minimize the microplastic contamination stemming from I-15? The question was tested by obtaining six wheat crop samples from both sides of I-15, testing them for microplastic contamination and then creating a device to mitigate this source of contamination. Following initial testing, using visual counts from extracted samples, and analysis via unpaired t-Testing, it was determined that the difference in microplastic levels between the two sample areas was statistically significant, indicating the interstate did contribute to microplastic contamination in crops near and downwind of the roadway. A device was created from chicken wire, hay, wood, and metal poles. Five grams of microplastics were induced into the device through controlled, simulated wind, microplastics were collected and weighed on the other side of the device. The amount of microplastics weighed after the device was used was extremely statistically significant compared to the weight induced, implying that the device was able to minimize microplastic contamination. Therefore I concluded that microplastic contamination was prevalent in all wheat samples, with increased contamination levels on the East side due to wind patterns, and a device could be constructed that would reduce microplastic contamination from reaching local crops. This is relevant in today's society as microplastic contamination is discovered in increasingly diverse locations and my research provides a solution to minimize the problem.

6.05.P-Th-155 The Effects of Nanopesticides on Wetland Ecosystems

Kiley Power and Tiffany Messer, University of Kentucky

In recent years, agricultural engineers and chemists have turned to nanomaterials to improve traditional agriculture practices, including pesticide development. Nanopesticides are thought to be a promising course of action for reducing agricultural impacts on the environment as nanopesticides are able to effectively target pests with smaller quantities and less frequent applications compared to conventional pesticides. However, little is known regarding the fate and transport of nanopesticides, specifically their influence on wetland ecosystems. The objective of this study was to assess the implications of nano-Cu fungicides (Kocide 3000) and neonicotinoid insecticides (imidacloprid) on downstream wetland habitats. The experiment was conducted using fifteen established mesocosm wetlands, each containing local soils and native wetland plant species. Mixtures of nitrogen, Kocide 3000, and imidacloprid were applied to the mesocosms at low, medium, and high concentrations. Water samples were regularly collected to determine nanopesticide transformation and degradation rates as well as quantify denitrification; plant and soil samples were collected before pesticide application and after the experiment was concluded to measure nitrogen plant uptake. The complex interactions between nitrogen, Kocide 3000, and imidacloprid were found to impact nutrient kinetic rates, and the chemical and biological processes observed in wetland ecosystems resulted in altered nanopesticide transformation and

degradation rates. Findings from this study can provide insight on the fate of nanopesticides in downstream wetland habitats, as well as provide guidance for the design of best management practices for managing agroecosystem pesticide loads.

6.05.P-Th-157 Assessing the Impact of Co-Occurring Contaminants of Emerging Concern (CECs) in Constructed Floating Treatment Wetlands

Matthew Vincent Russell, University of Nebraska-Lincoln

Natural and constructed wetlands are frequently used across the United States for mitigating nitrate losses to surface and groundwater. Though the use of wetlands as a treatment approach for nitrate in runoff is well-established, other active contaminants regularly co-occur with nitrate, potentially affecting the efficacy of nitrate-N removal. For example, veterinary pharmaceuticals have been observed in runoff originating from fields that receive livestock facility animal waste. In 2022 and 2023, a total of five floating treatment wetland mesocosm experiments were conducted to evaluate the combined effects of 4 common-use veterinary antibiotics (VAs) (chlortetracycline, sulfamethazine, lincomycin, monensin) and a commonly used nitrification inhibitor (nitrapyrin) on nitrate-N reduction efficiency in wetlands. We hypothesized veterinary antibiotics would significantly impact nitrate-N removal through changes in denitrification processes within wetland ecosystems. To test this hypothesis, we simulated two pulse-flow storm events (2.5mg NO₃-N/L, 7.5 mg NO₃-N/L) and quantified the combined effects of trace-level antibiotics (1.0 mg/L) and nitrapyrin (1 mg/L) on the wetland nitrogen cycle processes. 2022 mean NO₃-N concentration demonstrated that there is a significant difference (p-value=<0.0001) between treatments amended with veterinary antibiotics and those without. 2023 data is currently being analyzed and evaluated for the implications of nitrapyrin to nitrate-N removal. Findings from these experiments will provide new insight into whether contaminant residues from animal feedlots will have a substantial impact on nitrogen removal in wetland environments and potentially impact proposed mitigation strategies for controlling nitrogen losses from fertilized crops and managing nitrate contamination of ground and surface water.

6.05.P-Th-159 Advanced Clay-based Adsorbents for Reducing Bioavailability of Per- and polyfluoroalkyl Substances in Water

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Per-/polyfluoroalkyl substances (PFAS) are highly persistent contaminants widespread in water resources. PFAS are used in many industries including textiles and leather processing, surfactants, and plastic manufacturing. PFAS are extremely persistent and bioaccumulative which make their remediation in water challenging. PFAS bioaccumulation in edible crops such as rice, wheat, and vegetables through PFAS-contaminated irrigation water and biosolid fertilization is a growing concern. Treatment strategies for adsorptive removal of PFAS using clay minerals and oxidic clays are inexpensive, eco-friendly, and efficient for bulk PFAS immobilization due to high surface area, porosity, and high loading capacity. Here, we discuss the potential of these materials to reduce the bioavailability of PFAS in agriculture. Clay-based materials adsorb PFAS through hydrophobic and electrostatic interactions. Based on a comparison of the partition coefficient values, high PFAS removal performances are obtained when clay minerals are modified with surfactants, polymers, and amines, i.e., organo-clay minerals, compared to pristine clay minerals. Adsorption is dependent on pH and PFAS molecular structure. Dose of adsorbents, temperature, ionic strength, and dissolved organic matter content of the medium also impact adsorptive removal of PFAS from water. Clay adsorbents minimize the transport of PFAS to edible crops through decontamination of irrigation water and reduce PFAS bioavailability. Advanced clay modification approaches such as clay-biochar composites and microorganism-loaded clay-based photocatalysts hold potential to improve PFAS remediation via enhanced adsorption-transformation mechanisms and therefore reduce PFAS bioavailability. However, regeneration and safe disposal are issues to overcome for using clay-based PFAS adsorbents at scale.

6.05.P-Th-160 Thermal Treatment of PFAS in Environmental Media

Phillip Potter, U.S. Environmental Protection Agency

PFAS are ubiquitous, persistent, and bioaccumulate in the environment. Thermal treatment methods are currently applied to a variety of PFAS-containing materials, including sewage sludge and granular activated carbon (GAC), but may not be sufficient to fully mineralize organic fluorine. The efficiency of thermal treatment methods is typically assessed using targeted PFAS quantification, but this targeted approach can overlook products of incomplete combustion (PICs). Non-targeted analysis of PFAS is required to properly assess the efficacy of these thermal treatment methods and to determine the fate of these compounds in thermal treatment systems. Results from multiple thermal treatment systems involving PFAS-containing waste will be presented.

6.05.P-Th-161 Impact of Biological Treatment on Chemical Composition of Wastewater and Associations with Microbial Communities

Analyssa Esperanza Hernandez, Manuel Garcia-Jaramillo, Tala Navab-Daneshmand and Gerrad Jones, Oregon State University

Treated wastewater contains residual bioactivity, which is a growing concern for both environmental and human health. Bioactive chemicals are removed through advanced treatment technologies, which are cost prohibitive for many municipalities. One possible solution to increase treatment is to optimize the microbial community in biological wastewater treatment processes. Some studies have reported on the removal of targeted compounds during biological treatment, but there is limited knowledge on the microbial communities driving chemical change. Herein, we examined the change in the non-target chemical composition as a function of microbial community composition and biological treatment type (i.e., activated sludge (AS), facultative lagoons (LAG), and biological nutrient removal (BNR)). Samples were collected before and after conventional biological treatment processes from 12 wastewater treatment utilities (WWTPs) in Oregon. The non-target chemical composition was quantified using liquid chromatography high-resolution time-of-flight mass spectrometry. Microbial community composition was determined using 16S rRNA Illumina sequencing. The evaluation of the fate of non-target features revealed a complete removal of $86.08 \pm 2.98\%$ on average achieved across all WWTPs. However, over half ($54.28 \pm 4.12\%$) of the detected effluent features across all WWTPs were identified as potential transformation products. Treatment efficiency, as determined by removal of features, decrease in features, increase features, and formation of new features, was determined to be superior in BNR followed by AS and LAG. Dissimilarity and multivariate analysis revealed holistic chemical composition of samples collected before and after treatment were significantly different. BNR and AS were also both significantly different than LAG. Suspect screening was performed on samples to identify anthropogenic compounds. Network analysis revealed associations between suspect transformation and microbial communities. The associations identified between microbial community and chemicals identify potential microorganisms responsible for major chemical transformation. The results from this study will help engineers select microbial communities to seed into wastewater treatment facilities to help increase treatment efficiencies and to reduce their impact on receiving ecosystems.

6.05.P-Th-162 Organic Matter Quality Impacts on Poly- and Perfluoroalkyl Substance Sorption to Soil: Using Low Molecular Weight Proxies to Improve Understanding of Interactions

Erica R. McKenzie¹ and Yaseen Al-Qaraghuli², (1)Civil and Environmental Engineering, Temple University, (2)Tempe University

Per- and polyfluoroalkyl substances (PFAS) have gained global attention, particularly in communities that have PFAS contaminated drinking water or ecosystems. Groundwater near point sources has notably elevated PFAS concentrations and pump-and-treat is the most mature remediation strategy. Mechanistic knowledge of PFAS accumulation at solid-water interfaces has increased; while soil organic matter (OM) content is among the important factors impacting retention, however substantial variability is observed (e.g., compound specific

solid-water and organic carbon-water partitioning coefficients; K_d and K_{oc} , respectively). OM quality has received comparatively little attention, but preliminary results indicate that it can result in 0.5 log difference in K_{oc} values. In this study, 13 low molecular weight OM proxies were selected to probe specific OM characteristics and their impacts on PFAS partitioning. All experiments were started with the same OM proxy concentration (100 mg/L) and suite of 20 PFAS (2 ug/L; 9 PFCA, 5 PFSA, 3 FtS, N-MeFOSAA, N-EtFOSAA, and PFOSA) in the presence of sand, and were mixed for 3 days so that the organic matter and PFAS could reach equilibrium. Preliminary data analysis indicates that some organic matter proxies, generally characterized by containing polar alcohol functional groups, reduce sorption to the soils compared to the organic matter free reference condition. Counter to the prevailing thinking that soil OM provides sorption opportunities, this instead highlights that polar organic matter moieties reduce sorption. Some OM proxies notably increased PFAS sorption to the soils – specifically toluic acid proxies (methylbenzoic acid) – and aliphatic carboxylic acids also modestly increased PFAS sorption. Greater effects were generally observed for the longer chain length PFAS, and especially for the precursors. Future data analysis will include linking partitioning coefficients (K_d and K_{oc}) with PFAS and OM proxy molecular features.

6.06.P-Mo Late Breaking Science: Engineering, Remediation and Restoration

6.06.P-Mo-223 Application of Gaussian Mixture Models to Quantify the Background Threshold for Perfluorooctane Sulfonate in Surface Soil

Richard Hunter Anderson¹ and Mahsa Modiri Gharehveran², (1)Air Force Civil Engineer Center, (2)EA Engineering, Science, and Technology, Inc., PBC

Global studies on per- and polyfluoroalkyl substances (PFAS) reveal higher concentrations at sites impacted by aqueous film-forming foam (AFFF) and other point sources, but background concentrations are also present, attributed to emissions from manufacturing and industrial activities. These lead to local and long-range transport and accumulation in surface soil, necessitating an urgent delineation of background threshold concentrations for legacy PFAS. The detection frequencies predominantly highlight perfluorooctane sulfonate (PFOS) as a priority. This study, leveraging nation-wide metadata from the U.S. Air Force, employed Gaussian Mixture Models (GMM) to estimate the background threshold for PFOS concentration. A critical step involved the assessment of the multi-component distribution for the GMM using multiple goodness-of-fit criteria, such as the Bayesian Information Criterion (BIC), Akaike's Information Criterion (AIC), the corrected AIC (AICc), and Pearson's chi-squared test. Though three out of the four criteria indicated a preference for three components, the decision to opt for a two-component GMM was based on visual inspection and the observation of significant overlap between two distributions, indicating that a two-component solution was more appropriate. This mindful choice, guided by both empirical evaluation and intuitive reasoning, and aimed at avoiding model overfitting, was vital in reaching a solution that was both parsimonious and interpretable. The study also explored various techniques to handle non-detects and to assess how these methods could impact the background threshold of PFOS. These techniques included the substitution and imputation of censored data using approaches such as Maximum Likelihood Estimation (MLE), Uniform Random Imputation (URI), and Kaplan Meier techniques. The findings revealed a background threshold for PFOS that ranged between 3.5 ug/kg to 26 ug/kg across all techniques (except URI at 200 ug/kg) used for treating non-detects, within a broader concentration range extending up to ~50,000 ug/kg. By demonstrating an innovative statistical approach that intelligently incorporates different criteria for model selection, this research makes significant contributions to risk mitigation efforts at point source-impacted sites and lays the groundwork for future targeted regulatory actions.

6.06.P-Mo-224 The Efficacy and Ecological Impact of Mercury Sorbents on the Physiology and Reproductive Fitness of *Daphnia magna*

Shane Daniel Power, Louise M Stevenson, and Teresa Mathews, Oak Ridge National Laboratory

Mercury (Hg) is ubiquitous in waterways across the world, and the bioaccumulation of Hg within biota from contaminated environments has led to negative effects on human and wildlife health. To reduce local concentrations of Hg within the water column and leaching from stream banks, various sorbents have been developed and used that have high binding-affinity to this trace metal. While many studies have examined the remediating efficiency of Hg sorbents, there is a distinct lack of chronic exposures to sensitive aquatic biota analyzing the physiological effects of sorbent exposure. For this reason, we chose to evaluate the toxicity of two promising sorbents, pinewood-based Biochar and Granular Activated Carbon (FILTRASORB® 300). We exposed these sorbents with and without Hg to a sensitive freshwater model organism, *Daphnia magna*, throughout its lifespan to better understand the long-term effects of sorbents on aquatic biota and its effects on the chemistry of the water column. Using flow-through columns specifically designed to test the efficiency of sorbents, we followed the sorbent and Hg effects on daphnid survival, growth, and reproduction. We also measured the impact of sorbents on water quality, such as concentrations of dissolved organic carbon, trace metals, nutrients (nitrogen and phosphorus), and Hg. Preliminary results have shown differences in the fecundity of daphnids depending on their sorbent condition. Activated Carbon + Hg decreased reproduction by 23.3%, compared to our control. In contrast, both Biochar groups (with and without Hg) produced more neonates per individual compared to the control. *Daphnia* exposed to Biochar + Hg produced the most neonates per individual, with individuals producing 38% more neonates than our control group. Further analysis of water quality and filtration rate samples may explain the major differences in fecundity between the sorbent trials. Overall, this work demonstrates the importance of chronic exposure experiments, as the ecological effects of sorbents can vary over time due to their leaching rates and distinct chemical composition which could induce beneficial or negative effects on aquatic ecosystems.

6.06.V Late Breaking Science: Engineering, Remediation and Restoration

6.06.V-029 Selective Adsorption of Pb (II) In a Binary System With Cu (II) For Its Removal From Synthetic Wastewater

Relebohile Gladys Hlabana¹ and Gletwyn Rubidge², (1)National University of Lesotho, (2)Nelson Mandela University

Waste tyre is becoming a global environmental problem due to challenges in its disposal management and the fact that it poses fire hazards and metal leaching risk. Waste tyre can also breed mosquitos if it traps water thereby becoming an environmental health problem. There is also a portion of unused tyre in a waste tyre that is valuable and should be recycled. In South Africa waste tyre recycling is in three industries, one of which is crumb rubber where waste tyre is applied into various sized particles called crumb rubber. Crumb rubber can be applied in environmental remediation as an organic and inorganic pollutants' adsorbent. Considering the fact that South Africa's environment is loaded with heavy metal pollutants due to contributions by mining and agriculture as examples, it is postulated that crumb rubber produced in South Africa could be used to remediate the environment of these toxic metals. In this study adsorption of Pb (II) and Cu (II) from the synthetic waste water using waste tyre crumb rubber produced in South Africa was studied. Crumb rubber was activated using aqueous NaOH (0.02%) to make crumb rubber wettable followed by it being treated with 0.02 % acidified KMnO₄ for modification of its ability to sorpositively charged metal species. Trial sorption experiments on 50 mg / L Cu (II) were successful with sorption of over 70% in 250 minutes. Final optimization of the sorption of combined Pb (II) and Cu (II) was studied using a full factorial design and optimum conditions of sorption were found to be pH 5.4; [metal] 0.617 mmol / L for Pb (II); and pH 5.4; the optimization of Cu (II) was found to be pH 5.4, [metal] 0.777 mmol / L. In Pb (II) sorptions the mechanism of adsorption on the activated crumb rubber was found to be following the Freundlich model indicating that the adsorption seemed to occur on a multilayer

adsorbent, while the mechanism of copper(II) adsorption on to the activated crumb rubber was found to be following Langmuir model, indicating that the adsorption seemed to occur on a monolayer. With adsorption percentages ranging from 98.2 % to 100.0 %, for Pb (II), the activated crumb rubber produced under the conditions of this study, can be used for selective removal of Pb (II) from contaminated industrial effluents and stabilization ponds. It is applicable where Pb (II) contamination is extreme as compared to Cu (II) contamination.

Track 7: Policy, Management and Communication

7.01.P-We Approaches for Teaching Environmental Toxicology & Chemistry

7.01.P-We-167 C-BOYA: Citizen Science Using the *Allium* sp. Bioassay for the Assessment of Toxicity in Yucatan Groundwater

Gabriela Rodriguez-Fuentes¹, Elsa Norena-Barroso¹, Maria Concepción Gómez Maldonado¹, Eunice Danilu COUOH Puga¹, Loremy Yeuhdí Cauich-Sánchez¹, Jonathan Aldair Uribe-López¹, Nuno Simoes¹, Isaac Chacón⁶ and Juan José Sandoval-Giò³, (1)National Autonomous University of Mexico (UNAM), (2)Kalambio, A.C., (3), Tecnológico Nacional de México

Due to the karst nature of the aquifer and the increase in anthropogenic activities, the quality of groundwater available to the Yucatan Peninsula population has become a topic of great interest for authorities and civil society. In recent years, different studies have been carried out to monitor water quality in the area and the adverse effects that may occur in the biota due to several types of pollutants. In this project, it is proposed to expand the studies carried out, focusing specifically on evaluating the genotoxicity of the groundwater of Yucatan through the realization of bioassays with onion *Allium sp.*, documenting the genotoxic and cytotoxic effect of the contaminants present in the water through changes in the growth, the mitotic index, and the prevalence of nuclear aberrations in the roots. Likewise, it is proposed to take advantage of the simplicity and speed of the toxicological tests proposed to establish a pilot monitoring of citizen science that allows participating in monitoring activities, including water collection, assembly and development of bioassays, collection, and fixation of tissue samples, for subsequent microscopic analysis in the laboratory. In such a way that, in addition to generating data and protocols that can be used for subsequent studies, it contributes to achieving an approach of scientific work with the communities that use water so that they are actively involved in actions aimed at the conservation of this vital resource. Also contributing to enriching the teaching-learning process of students and teachers who participate as volunteers in the project, intending to organize a vast network of citizen monitoring in which academia and society participate together to generate valuable information that allows the conservation and sustainable use of natural resources.

7.01.P-We-168 Lessons Learned from Teaching Environmental Toxicology Across Multiple Modalities by Utilizing a Synchronous Hyflex Model

Joseph H Bisesi, University of Florida

Classroom instruction of scientific material has seen tremendous shifts over the past 20 years. These shifts have been driven by numerous factors including recognition of different learning styles and the emergence of online degree programs that require novel methods of interacting with students from a distance. Additionally, tightening budgets at universities have required faculty to do more with less, which often results in larger class sizes or teaching multiple levels of students (undergraduate and graduate) in the same classroom. With all these factors in mind, HSC 4507/PHC 6304: Environmental Toxicology Applications in Public Health was designed to combine undergraduate and graduate students as well as on campus and online student together in one classroom. The course utilizes a blended learning style (a.k.a. flipped classroom) where lectures are

prerecorded, and students are expected to watch them before coming to class each week. Classroom time is then used to review questions about the lecture and participate in integrated learning activities that help to reinforce concepts of environmental toxicology including small group discussions, interactive presentations on historical environmental disasters, and short current event presentations. By utilizing technology that enables two-way communication between online and on campus students, students from both on campus and online modalities participate in weekly synchronous sessions, often referred to as a hyflex model. This format has been used across 4 years which has provided invaluable opportunities for both self-reflection as well as integration of student feedback to help improve the course delivery format. The presentation will focus on the lessons learned by combining students from 4 different modalities (undergrad, graduate, on-campus, online) in a single synchronous classroom including both pros and cons of this approach. Additionally, because this course is taught to a large number of students who come from multiple majors across the University, there will also be a discussion of adapting an environmental toxicology course to students with different levels of prerequisite coursework and knowledge.

7.01.T Approaches for Teaching Environmental Toxicology & Chemistry

7.01.T-01 Failure Leads to Success: How to Use The Design-Make-Play Framework in a Variety of Class Settings to Enhance Learning

Corinna Singleman, Matt Greco and Nathalia Holtzman, Queens College

Through the integration of active learning activities and the cultivation of maker mindsets, the Make STEAM Q project offers a promising approach to enhance learning outcomes and student engagement in STEM disciplines. The project follows a Design-Make-Play framework, fostering a community of practice among faculty fellows through formal and informal learning. Students in STEM and non-STEM courses are exposed to active learning activities, specifically making and design thinking, to enhance student learning and increase engagement in STEM disciplines. One key aspect of the Make STEAM Q project is the emphasis on the cultivation of "maker mindsets" among students. Faculty fellows from Queens College incorporate long-term projects into their courses, encouraging students to embrace the process of making and design thinking. These projects span various disciplines, such as utilizing AutoCAD programming to design 3D printed molds for pottery, developing and producing pinhole cameras, creating mathematical art, and using programming in introductory design courses. These examples highlight the belief that students must actively explore, experiment, and inevitably encounter failures to learn and ultimately succeed in their projects and courses. The inclusion of failure as an integral part of the learning process is a fundamental principle of the Make STEAM Q project. Students are encouraged to view failure not as a setback, but as an opportunity for growth and learning. Troubleshooting and problem-solving become essential skills as students navigate through challenges and setbacks, enabling them to refine their designs and approaches. The faculty fellows provide guidance and support, fostering an environment where students can learn from their mistakes and apply newfound knowledge to overcome obstacles. By embracing failure as a stepping stone to success, students develop resilience, creativity, and critical thinking skills. The active learning activities in the Make STEAM Q project promote a growth mindset, where students are motivated to persist and overcome challenges, leading to deeper learning and a greater sense of accomplishment. By embracing the process of making and design thinking, students develop important skills and attitudes that extend beyond the classroom, empowering them to become lifelong learners and contributors to many fields within STEM.

7.01.T-02 Decreasing Barriers to STEM Education Through Do It Yourself (DIY) Laboratory Courses

Helen C Poynton and K. Garrett Evensen, University of Massachusetts, Boston

The rising cost of college, and the failure of financial aid to cover college costs means that the majority of college students are working students. Low-income students are more likely to be students of color and work excessive hours that impacts their grades and ability to schedule coursework. Many of our students at UMass

Boston have difficulties juggling life and working responsibilities with STEM courses and lab classes, while also acknowledging that having more research experiences is critical for their success in future careers or graduate school. Opportunities that allow students to learn laboratory or field research techniques while being flexible for working students are greatly needed. During the Fall 2020 semester, the COVID19 pandemic required that lab courses become remote. The advanced level Marine Pollution Laboratory course was not an exception. We decided to transition most of our laboratory activities to “Do it yourself” (DIY) field trips and kit-assisted lab exercises. At the conclusion of the semester, the students were surveyed (anonymously) to determine the effectiveness of the DIY lab exercises and the overall course structure. To our surprise, we found that the online course was as effective as an in-person course and could provide increased accessibility to lab courses for our low-income working student population. Because of the success of this lab course during the Fall 2020 semester, we decided to develop the course as an online laboratory in all upcoming semesters and a newly revamped online Marine Pollution Laboratory was run in Fall 2022. The course was successful in increasing participation of working students where 50% of the students stated that they would not have been able to take the class if it was in person. Despite it being completely online, 67% of the students said it was as “hands-on” or more than other laboratory courses and 67% said that they felt more empowered to do the experiments on their own. This presentation will review the components necessary to make to a successful DIY online laboratory course, tips that we have learned along the way, and results from our anonymous student evaluations highlighting the opportunities online labs provide and the power they can have in reducing barriers to STEM education.

7.01.T-03 From Failed Citizen Science to a Long-Term Undergraduate Research Program in Aiken, SC

Sarah Michele Harmon, University of South Carolina Aiken

This presentation describes how an undergraduate research capstone class took over a failing citizen science project and turned it into a multifaceted undergraduate research program with opportunities for students at all levels of interest and capability. The Horse Creek watershed in Aiken County, SC, was impaired due to fecal coliform pollution, and it was hypothesized by local officials that this was due to poorly maintained, failing sewer lines in an old textile mill village that had been abandoned by the textile company. Undergraduates from the University of South Carolina Aiken took the project over from a citizen science group and began monitoring for fecal coliforms, with the goal of supporting this hypothesis. The results were quite unexpected, thus leading to an expansion of the initial project to include a larger portion of the watershed, including our own campus and a popular equine recreational area. The ongoing project has grown significantly since its beginning in 2012 and has moved beyond simple fecal coliform enumeration to include microbial source tracking via qPCR, the presence of antibiotic residues via ELISA, and evaluation of bacterial antibiotic resistance using traditional culture methods and qPCR. These tools have allowed undergraduates to evaluate risks to downstream recreational users and have provided an excellent platform for class discussions on Best Management Practices, responsible stormwater management, and Environmental Justice. Through a collaboration with local city government, students have also learned that they can make a difference when they are able to identify “hot spots” that are quickly investigated and repaired by concerned city officials.

7.01.T-04 Application of Specification Grading in a Risk Assessment Course

Amanda D Harwood, Alma College

In recent years there has been an increased interest in novel grading strategies that better emphasize student learning outcomes. One such strategy is specifications (or specs) grading. While there are many variations in this grading scheme, there are three principles typically utilized in assessment: 1) Direct links to student outcomes 2) Use of a pass/fail grading system 3) Includes multiple assignment attempts. This year in Risk Assessment, a 300-level course for third- and fourth-year undergraduates, a version of specifications grading was used. In this system, all assignments were assessed as meeting mastery or not and the final grade was determined by number of assignments from each assessment group that met the mastery expectations. All

assignments had one free “re-do” for each assignment and had three additional “tokens” that could be used to re-do assignments or get 48 h extensions on assignments. Students were also given a standard numerical grade on their original assignments for comparison purposes. Comparisons were made between the grades in a traditional system, numerically calculated grades with re-dos, and the specifications grade. The specifications grade was higher than the traditional grade for 43% of students, the same for 33%, and lower for 24% of students. Students who did not see improvement from specifications grading were mostly students who would have received an A or AB regardless and those who would have received a lower grade were those who did not meet mastery on their mid-term exam and/or final projects. Therefore, grading distributions can affect the impact of specs grading. Anecdotally, students generally preferred specs grading and while creating the system was more challenging, grading itself was more straightforward.

7.01.T-05 Learning By Doing: Incorporating a Laboratory Practical into an Upper-Level Ecotoxicology Course

Sam Nutile¹ and Adam M. Simpson², (1)Pennsylvania State University, (2)Penn State Behrend

Teaching toxicology often involves incorporating laboratory exercises, such as toxicity tests and *in vitro* assays, into the course that give students hands-on experiences with the techniques used to gather data related to contaminant exposure. To save on time, faculty will often streamline the process of conducting such experiments by doing much of the required experimental design and prep work before students even enter the classroom. While this maximizes the time spent with students, fully comprehending the principles used in such experimentation necessitates building a test from the ground up. Finding a balance between conducting a diverse array of experiments while giving the students the opportunity to design their own experiment can be difficult. One means by which to accomplish both goals is through the incorporation of a laboratory practical into upper-level toxicology courses. To test the efficacy of this pedagogy, a lab practical asking students to design, execute, and interpret the data from a toxicity test will be incorporated into a 400-level Ecotoxicology course taught during Fall 2023. Over the course of the semester, students will be led through various experiments evaluating adverse effects (i.e., toxicity tests, *in vitro* enzyme assays, behavioral assays) in invertebrates (i.e., *Hyalella azteca*, *Chironomus dilutus*) exposed to different environmental contaminants (i.e., pesticides). During guided exercises, students will be given direction in how to determine test concentrations, prepare spiking solutions, prepare test organisms and exposure chambers, and analyze and interpret data. Using this information, students will be asked to prepare and execute a toxicity test of their own at the end of the semester. Students will be evaluated on the appropriateness of the experimental design in relation to their experimental objective, the preparation of spiking solutions, and analysis and interpretation of the data collected. Utilizing this approach, we aim to provide students with hands-on experience in experimental design and execution, as well as provide them with more robust examples of how toxicological concepts covered in lecture are applied in the lab.

7.01.T-06 Improving Student Outcomes with a “Maker Mindset”

Matt Greco, Corinna Singleman and Nathalia Holtzman, Queens College

The Make STEAM Q project aims to establish a community of faculty fellows across diverse disciplines who integrate "making and design thinking" activities into their undergraduate courses. Embedding making and design thinking in introductory undergraduate STEM courses increases student motivation and interest in STEM. Together with the support of New York Hall of Science (NYSCI) and Queens College's Makerspace, the Make STEAM Q team works towards fostering the development of maker mindsets among faculty fellows, ultimately (re)invigorating their students' interest and participation in STEM education. Faculty fellows engage in the creation of courses or course modules that incorporate making and design thinking, utilizing the tools and physical space available at the Queens College Makerspace. Guided by a Design-Make-Play framework, formal and informal learning experiences are integrated to cultivate a community of practice, emphasizing best practices in andragogy, which are instructional strategies tailored for adult learners. Throughout the project's

duration, professional development sessions enable faculty fellows to address their attitudes, teaching expectations, and preparedness to incorporate the design-make-and-play thinking approach into their courses. Students in courses supported by the project participate in pre- and post-course surveys which are analyzed in conjunction with data on implementation fidelity, project outputs, and faculty perceptions and beliefs. Initial regression analyses reveal statistically significant positive impacts of the design activities in treatment courses on course GPA for several courses. This abstract highlights the objectives and achievements of the Make STEAM Q project, which seeks to foster maker mindsets, integrate making and design thinking into undergraduate courses, address challenges in online learning, and facilitate knowledge exchange between institutions. By incorporating these components, the project contributes to advancing STEM education, empowering students with practical skills, and cultivating a passion for creativity and innovation in a rapidly evolving world.

7.02.P-Th Demystifying the Method Standardization and Accreditation Processes

7.02.P-Th-163 Behind the Curtain - Testing Standards and Method Development

Cameron A. Irvine¹, Tripp Fisher², Molly Lynyak³ and Tim Haley⁴, (1)RBI, (2)Brownfield Science & Technology, Inc., (3)ASTM International, (4)Latitude Legal

Have you ever wondered how standards are developed for various environmental actions, such as toxicity testing, analyses, or site assessments? Have you ever found a better way to implement a method and asked yourself how or why the prescribed method was determined and if it could be improved? Standards are useful references for implementing scientific investigations and they complement published scientific journal articles and regulatory methods. Experts in the appropriate technical field are important in developing these documents, but doing this work also relies on new scientists, technicians, technical editors, and those knowledgeable about science communication. This presentation will describe the who and the how of method development, committees and task groups, approval processes, and review requirements of ASTM International (formerly the American Society of Testing and Materials) as an example of standards development facilitated by a not-for-profit stakeholder-driven international organization. In addition to the nuts and bolts of standard method development and maintenance (keeping methods current is an advantage of frequent reviews) we'll discuss how to get involved with organizations that develop and manage standard methods and how anyone with material interest or with some technical subject knowledge, in any stage of their scientific career, is welcome to participate.

7.02.P-Th-164 A Former Quality Assurance Director's Perspective on Implementing the TNI Standard in a Commercial Toxicity Laboratory

Katie M Payne, Enthalpy Analytical

The consensus-based standard developed by The NELAC Institute (TNI) facilitates generation of environmental measurement data that is rigorous, robust, and uniform across entities. However, the TNI Standard can also be daunting to implement, particularly for those just becoming newly acquainted with it. Especially for small laboratories that serve only a single state or have limited analyte lists, the TNI requirements may be significantly more stringent than some state level certifications. The TNI Standard can also seem prescriptive and over-wrought in some areas while simultaneously vague and ambiguous in others. Each module contains pages and pages of quality systems requirements, and it can be challenging to balance the perhaps conflicting priorities of ensuring compliance and maintaining operational efficiency. The TNI Standard also presents both challenges and benefits to laboratories that focus on novel and unique testing where codified methods and guidance are either absent, under-developed, or outdated. Meeting project specific data quality objectives (DQO) ensures acceptability of the data by clients, regulatory agencies, and the public. Adaptations may need to be made to universal tenets (e.g., the quality systems module) and translated to a laboratory's specific needs. It is critical for laboratories to be able to communicate how their quality systems fulfill the TNI standards and

meet the requirements of this distinguished accreditation. This presentation will reflect upon the benefits, challenges, and lessons learned in implementing the TNI Standard in a commercial toxicity testing laboratory that performs both routine testing, as well as novel, technically challenging projects. It will also provide valuable insight and resources for approaching the TNI Standard and its component modules, encouraging creative thinking to leverage the systems and process that a lab already has in place and improve documentation to demonstrate fulfillment of the standard requirements.

7.02.T Demystifying the Method Standardization and Accreditation Processes

7.02.T-01 How to Remain Objective and Open; Overview of the Consensus Standard

Development Process

William Lipps, Shimadzu Scientific Instruments

In 1995 the US Congress enacted the National Technology Transfer and Advancement Act (NTTAA) that encourages formal adoption of national consensus standards for American regulatory agencies. OMB Circular A-119 states "if an applicable voluntary consensus standard exists in an area where the agency seeks to regulate, the agency should use this as the basis for a proposed rule rather than starting from scratch or adopting a different approach". Circular A-119 requires that all federal agencies must use consensus standards in their procurement and regulatory activities, except where inconsistent with law or otherwise impractical. Voluntary Consensus Standard bodies (VCSB) are domestic, or international organizations that plan, develop, establish, or coordinate standards using agreed upon standard operating procedures. A VCSB operates according to the following attributes: openness, balance of interest, due process, and an appeals process. This presentation covers these attributes of VCSBs, in particular ASTM International and Standard Methods, and provides a generalized overview of the process in developing consensus standards.

7.02.T-03 Validation and Standardization are Key Design Features for a New Approach Method: Lessons from the EcoToxChip Project

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Since 2016 our team has set forth to develop, test, and commercialize toxicogenomic solutions (i.e., EcoToxChips, and a data evaluation tool EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures of regulatory concern. EcoToxChips have been developed for early life stage species representing key vertebrate groups in ecological risk assessment (fish-fathead minnow and rainbow trout; bird-Japanese quail and double-crested cormorant; amphibian-Xenopus laevis and Northern leopard frog). To date, more than 1,800 EcoToxChips have been analyzed covering 28 distinct chemicals and 4 complex environmental samples. The objective of this presentation is to detail how key aspects of validation and standardization were built into the project and its deliverables. Foremost is that the project was driven by stakeholder needs (i.e., Environment and Climate Change Canada, along with others) and founded by social sciences research in the areas of institutional entrepreneurship and design thinking. Validation of the EcoToxChip Test System focused on case studies spanning: a) technical performance (e.g., inter- and intra-lab reproducibility and repeatability); b) biological interpretation (e.g., mechanisms of action); and c) user experience (e.g., ease and access of use of deliverables). Standardization of the EcoToxChip Test System was built around three prioritized users and their user journeys (i.e., internal R&D by industry, environmental monitoring, chemical screening) to best realize guidance documents that span problem formulation to exposure studies to EcoToxChip use and EcoToxXplorer.ca analysis. Taken together, insights from diverse case studies have enabled us to rapidly iterate, prototype, test, and improve the design of

the EcoToxChip Test System to yield a toxicogenomic New Approach Method that is accessible, standardized and user-validated. This study was conducted as a part of a large-scale Genome Canada-funded project (EcoToxChip project - www.ecotoxchip.ca).

7.02.T-04 Statewide Quality Assurance Studies to Support Whole Effluent Toxicity Testing in California, USA

Kenneth Schiff, Alvine Mehinto and Darrin J. Greenstein, Southern California Coastal Water Research Project (SCCWRP)

The State of California promulgated updated toxicity provisions in 2020 instituting stronger enforcement for whole effluent toxicity tests. Partly in response from stakeholders, the State instituted a three year study to assess the inter- and intra-lab variability of the 3-brood chronic toxicity test using *Ceriodaphnia dubia*. The goal of the study was to assess what lab techniques could be standardized to reduce variability and ensure comparability among laboratories. The study had multiple facets including: a) Inventory of accredited lab SOPs and QAPs, b) Electronic survey of accredited lab methods, c) Phone interviews of accredited laboratories key staff, d) Site visits to accredited lab, e) Inventory of historical testing data of accredited laboratories, f) Two split-sample intercalibrations of accredited laboratories, g) Decades of testing, training, and auditing by the project's Expert Science Panel, and h) Feedback from the project's Stakeholder Advisory Committee. After compiling historical data from more than 1,000 tests across California, then sending multiple rounds of split samples to each of the accredited laboratories, the study's Expert Science Panel provided consensus recommendations falling into four major categories: method best practices, accreditation requirements, training, and future studies to quantify variability. Method best practice recommendations aimed largely at laboratories consisting of 10 different techniques that ranged from strengthening adherence to existing method requirements to limiting method techniques that are considered flexible or for which the method is silent. No new method requirements or deviations were provided. Accreditation recommendations aimed largely at the State consist largely of upgrading performance evaluation (PE) sample analysis, which was a major task within the study. Currently, the state under-utilizes much of the PE information for the accreditation and quality assurance process. Training recommendations aimed at lab facilities, lab senior staff, lab technical staff, state auditors, and lab clients consisted of curriculum development, learning metrics, and especially documentation. Future studies are designed to follow up on the intercalibration study technical findings to better quantify potential sources of variability in the *Ceriodaphnia dubia* study such as age of females used to start the test brood boards, food and feeding, or the effect of water hardness.

7.02.T-05 Multi-Disciplinary Approaches to Collect Data that Supports New Anti-Sea Lice Therapeutant Product Development and Registration

Benjamin Patrick de Jourdan, Amber Garber, Ehab Misk and Claire Goodwin, Huntsman Marine Science Centre, Canada

Infestations by the parasitic sea lice (*Lepeophtheirus salmonis*) continue to be a challenge to most Atlantic salmon (*Salmo salar*) farming regions of the world, including Atlantic Canada. Annually, approximately 75% of the active marine finfish aquaculture sites in Canada used at least one drug or pesticide to mitigate effects on production performance. Under the Aquaculture Activities Regulations, the Canadian marine finfish aquaculture industry is permitted to use various drugs (authorized for use by Health Canada's Veterinary Drugs Directorate) and pesticides (registered for use by the Pest Management Regulatory Agency) to support sea lice control or management. Industry fish health managers understand the need to implement an Integrated Pest Management Program, which necessitates introduction of new therapeutants and technologies to minimize existence of resistant sub-populations of sea lice following frequently repeated treatments using a limited number of approved options. The development, and ultimately registration, of new therapeutants (e.g., in-feed drugs or bath applied pesticides) for sea lice control involves demonstrating efficacy, target animal safety, determining drug withdrawal periods to ensure human safety and examining the impacts to non-target

organisms and the environment. In this presentation, we discuss how we apply a standardized framework to collect data that addresses all of these facets in support of submission to different national regulatory authorities, including: 1) closing the life cycle of the ectoparasitic sea louse to perform standardized bioassays on different life stages and conduct a controlled and reliable infection model to determine *in vivo* efficacy testing with Atlantic salmon, 2) standardizing test methods with non-standard species (e.g., American lobster (*Homarus americanus*)) based on different exposure scenarios (e.g., water column, sediment or dietary) and 3) monitoring freshwater and marine benthic invertebrate communities following initial commercial scale treatment regimes at a hatchery or marine grow-out site. A rigorous QA/QC framework is applied while utilizing local species and environments to collect data through these studies. Our entire process meets the various regulatory requirements and improves public confidence in the use of these therapeutants in areas that overlap with other commercial activities.

7.02.T-06 Introduction to the NELAC Institute (TNI) Laboratory Accreditation Standards

Katie M Payne, Enthalpy Analytical

Industry practitioners may find it challenging that there are a wide range of accreditation types and accrediting bodies for environmental sample analyses. There are countless agencies which provide accreditation depending on matrix, geography, technical focus of the laboratory, and availability of codified methods. The NELAC Institute (TNI) is a non-profit organization whose mission is to foster the generation of environmental data of known and documented quality through an open, inclusive, and transparent process. TNI seeks to provide a consensus national standard for accreditation through a forum driven by community stakeholders. While TNI does not directly accredit laboratories or provide guidance on method-based interpretation, it does work to unify these functions across accrediting bodies. Membership is open to any individual interested in lab accreditation in the private, public, or academic sectors, and members span many environmental disciplines including sampling organizations and laboratories, consulting and engineering firms, and municipalities and government entities. Most of the work of TNI is done by volunteers working on committees tasked with specific functions (e.g., whole effluent toxicity, proficiency testing, etc.). This presentation will provide an overview of the purpose and evolution of TNI, how it is organized and the various programs it includes, how it functions in administering the National Environmental Laboratory Accreditation Program (NELAP), and the benefits it provides to its members.

7.03.P-Tu Healthy Environment - Indigenous Knowledge System (IKS) Informed Management of Environmental Contaminants

7.03.P-Tu-213 Weaving Indigenous Knowledges and Western Sciences in Ecotoxicology: A Regional Perspective from the Alberta Oil Sands

Alana Wilcox¹, Lydia Johnson², Ella Bowles¹, Jennifer F Provencher¹, Dominique A Henri¹, Steven Alexander³, Diane M. Orihel², Heather Castleden⁴, Jessica Taylor⁵, Steven J Cooke⁵, Philippe Thomas¹, Chris Herc⁶ and Lucas King⁶, (1)Environment and Climate Change Canada, (2)Queen's University, (3)Department of Fisheries and Oceans, (4)University of Victoria, (5)Carleton University, (6)Territorial Planning Unit, Grand Council Treaty #3

Knowledge describes the facts and values that govern how we understand, produce, and organize information. Western sciences have typically applied mechanistic and reductionist approaches, while Indigenous sciences emphasize the ethics and values that underlie the interconnectedness between all relations. Indigenous Knowledge Systems have strengthened western scientific understanding of social-ecological systems and over the last decade, efforts have been made to advance the value of both systems in all aspects of our scientific endeavors in the academy and government programs. As part of a collaborative investigation that identified ~320 studies weaving knowledges in the ecological sciences across Canada, a subset of regional systematic

reviews aimed to understand the scope and approaches to weaving in ecotoxicology. These reviews also explored studies conducted in the Alberta oil sands region of Canada, a prominent site of resource extraction and industrial activity on First Nations Treaty lands and Métis territories. Each review coded for 78 questions in six categories that include: (1) bibliographic information; (2) research themes; (3) study setting and design; (4) knowledge systems; (5) power relationships, colonization, and ethical considerations in research; and (6) benefits and challenges of weaving knowledge systems. Outcomes from the reviews revealed that studies spanned much of Canada's jurisdictional boundaries and researchers used a broad range of approaches to gather Indigenous Knowledges and Western scientific data, most notably through community-based participatory research. Metals and polycyclic aromatic hydrocarbons were commonly studied, particularly in aquatic wildlife. Additionally, researchers identified multiple challenges, including asymmetries of power, resource availability, and funding. Our findings show substantial barriers to weaving Indigenous and Western ways of knowing, particularly in research and monitoring related to environmental impacts from contaminants. While the weaving of knowledge systems can offer novel insights into our understanding of social-ecological systems, additional efforts are needed to foster and promote collaborative research that simultaneously forwards the plurality of scientific knowledge and addresses the ecological threats across regions with extractive industries.

7.03.P-Tu-214 Advancing Indigenous Sovereignty, Self-Determination, and Sustainability in Ohio through a Regenerative Approach

Briana Mazzolini-Blanchard¹, Dawn Knickerbocker² and James M. Lazorchak³, (1)Urban Native Collective, (2)Native Americans in Philanthropy & Urban Native Collective, (3)U.S. Environmental Protection Agency

Advocacy: Urban Native Collective (UNC)'s advocacy strategy is centered around education and partnerships that promote a clear path towards acknowledging and overcoming racial biases while establishing a deeper understanding of Indigenous culture. We advocate for solidarity, protection, and defense of human and non-human kind, land, water, and air. Our advocacy strategies are designed to create real and lasting change that uplifts the voices and experiences of Indigenous communities while challenging the harmful systems and structures that perpetuate inequality and injustice. **Community Support:** UNC's community support strategy is built around a commitment to advancing the wellbeing and empowerment of Native and Indigenous communities while building allies. We recognize that many Indigenous communities face significant challenges, including (1) limited access to resources like land, medicine, food, and water; (2) systemic inequalities; and (3) environmental degradation. We focus on addressing these issues and providing tangible support such as access to our community center, referrals to healthcare, land and hunting access, and social services. **Ohio Native Land Initiative:** The Urban Native Collective has developed an Ohio Native Land Initiative that seeks to establish a broader platform to promote Indigenous perspectives on sacred sites, encourage land repatriation efforts (Indigenous women-led work to restore sacred relationships between Indigenous people and our ancestral land, weaving traditional and cultural knowledge back in harmony with the land), and assert Native sovereignty as foundational to some of the region's most important natural resources and public spaces. The UNC's Initiative is an effort intended to (1) promote greater involvement with and protection for sacred Indigenous sites in Ohio; (2) broaden alliance-building efforts leading to deeper engagement with movements that support the protection of Indigenous sovereignty and cultural rights; (3) expand education and public outreach around Indigenous culture, lifeways, and native habitats; and (4) encourage serious efforts toward the re-indigenizing of public and private spaces in order to create opportunities that honor Indigenous lifeways and promote spiritual freedom.

7.03.T Healthy Environment - Indigenous Knowledge System (IKS) Informed Management of Environmental Contaminants

7.03.T-01 How Might We Begin Decolonizing Chemicals Management in Canada?

Elina Eronen¹, Gunilla Oberg¹, Susan Chiblow² and Deondre Smiles³, (1)University of British Columbia, (2)University of Guelph, (3)University of Victoria

A growing body of research is demonstrating that the ways in which environmental and health data is collected, analysed, utilized, stored, and shared has not only restricted the ability of Indigenous communities and experts to influence such processes, but has also lessened the relevance and usefulness of the data for Indigenous communities. These present data structures, which rest on colonial thought styles, are also complicit in harms done to Indigenous peoples, making the need for change vital. Our research aims to shed light on the ways in which Canada's current data practices form barriers to meaningful and respectful inclusion of Indigenous needs, expertise and knowledge. We do this by facilitating dialogue among experts in Canadian chemicals risk assessment (RA) about their present data practices. Using a modified brokered dialogue approach, two parallel information exchanges were initiated focusing on protocols, procedures and words: RA experts provided a 'Risk Assessment 101' session to the research team, tailored specifically for this project. The first author collected questions and comments from the audience, drawing on scholarship in data-justice, Indigenous data governance and data sovereignty. The questions were presented back to the RA experts, who prepared and delivered a 2nd presentation. In parallel, the team provided a "Data Justice 101" to the RA experts, also tailored specifically for this project. The first author collected questions and comments, which was presented back to the research team and a 2nd presentation in Data Justice was prepared. These two parallel iterative cycles (presentation, analysis, evaluation and feedback) continued until agreement was reached between the collaborators on actionable key findings that had the potential to lower the barriers to meaningful and respectful inclusion of Indigenous interests and expertise. We present our findings and discuss whether the approach presented here might be used to advance decolonization of chemicals management in Canada by increasing the awareness of harmful data practices, identifying barriers to meaningful and respectful inclusion of Indigenous interest and expertise and creating tangible suggestions for eliminating such barriers within Canada's chemicals management structures.

7.03.T-02 Loxiwe Lok'wali: From our Past to Inform our Future: Indigenous Science and Technologies

Gavin Woodburn, Environment and Climate Change Canada

Loxiwe (Clam gardens) are a human modified beach that showcase Indigenous technologies in mariculture and have been used for thousands of years. These unique intertidal features that decrease the natural beach slope and allow more habitat for clams and shellfish were constructed primarily by coastal Indigenous Nations stretching from Alaska down to Washington state, but other similar infrastructures can be seen around the globe. Loxiwe represent a significant depth of knowledge regarding the environment and its ecology by increasing clams and shellfish productivity by upwards of 400 percent than an unmodified beach. This technology maintains knowledge from thousands of years ago that carry onto the present by continuing their legacy as being a place for youth to learn from their Elders about important processes of the marine ecosystems, climate change, and much more. Currently, this is the beginning of a multi phase research project regarding clam gardens in Phillips and Fredericks Arm, BC which are located within the traditional territory of the Kwiakah First Nation and Laich-Kwil-Tach people. The first phase of this project will be to analyze how loxiwe can enhance food sovereignty for coastal Indigenous peoples and explore the idea of how or if they could be a driver in protecting endangered and culturally significant species of clams and shellfish. Phase two will look at loxiwe from the lens of climate change by examining how they can help us explain paleoenvironment through the holocene and into the future. By the end of this study, final results will be created in various ways such as a peer reviewed academic paper, peer reviewed academic video, and a creative video which will increase accessibility of knowledge and visual and audio representation of loxiwe and the cultural knowledge around them. In future

phases, research will explore the ways in which loxiwe inform us of going issues such as climate change and poor forestry processes. The purpose of these continued studies will remain focused on taking an Indigenous science approach by Bridging, Braiding, and Weaving Indigenous Science with Western science and have an emphasis on relationship building and a collaborative approach.

7.03.T-05 Indigenous Engagement to Inform Subsistence Human Health Risk Assessment and Focus Remedial Planning

Ariel Blanc¹, Amy Corp¹, Mark Larsen¹, Fiona Wong² and Eric Crawford³, (1)Anchor QEA, (2)Public Services and Procurement Canada, (3)Transport Canada

Ongoing indigenous engagement has played a critical role in conducting an accurate human health risk assessment and in focusing remedial planning for a working harbour on Vancouver Island. The harbour is located within the traditional lands of the Esquimalt and Songhees Nations and has been an important source of marine resources for the communities since time immemorial. Early engagement with the Esquimalt and Songhees Nations identified the traditional diet, serving sizes and frequencies, and resource considerations. Sea life were selected for inclusion in the risk assessment based on linkages to harbour conditions and temporal significance. Sea life were sampled from locations in the harbour selected for traditional importance and spatial coverage. Esquimalt and Songhees Nations community members participated in sea life sampling during which traditional processing information was shared along with observations of sea life conditions. Processing of sea life samples was conducted consistent with this shared knowledge. The risk assessment included two Esquimalt and Songhees subsistence scenarios. These scenarios characterized risk from current and historical rates of consumption of individual sea life types as well as the combined mixed diet. Risks above thresholds were identified for both scenarios from consumption of the mixed diet. Consumption of a portion of the mixed diet had risks below thresholds. For each risk driver chemical, the sea life contributing most to the mixed diet risk were identified. Numeric objectives were established as the level of risk reduction required to reduce the mixed diet risks to acceptable levels. Potential remedial actions were screened based on their ability to make measurable progress toward the numeric objectives and align with the understanding of the Esquimalt and Songhees expectations.

7.03.T-06 Derivation of Water Quality Guidelines for the Protection of Indigenous Use

Mandy L Olsgard¹, Thomas Dyck², Megan Thompson³ and Timothy Bebetidoh⁴, (1)Integrated Toxicology Solutions Ltd., (2)Integral Ecology Group, (3)Thompson Aquatic Consulting, (4)Athabasca Chipewyan First Nation - Denel Lands and Resource Management

Surface water and sediment quality criteria were defined to protect Indigenous water use by three First Nation communities in the Lower Athabasca Region (LAR) which has experienced intense oil sands surface mining development since the 1960s. The criteria were developed using two approaches; establishing the current condition and health risk protection. The presentation will focus on development of the latter criteria. Health risk criteria were defined by identifying valued components that reflect use of surface water by Indigenous community members; consumption of traditional foods, medicines, and surface water, trapping furbearing mammals that consume aquatic biota, the health of wildlife (birds and mammals) from ingesting surface water and diet items, and aquatic ecosystem health. Available surface water and sediment quality guidelines were reviewed to identify level of protection for the traditional valued components. It was found that humans were more sensitive than aquatic biota and wildlife species for 50% of substances with published surface water quality guidelines. When unavailable or determined to be non-protective of TLU, health risk criteria were derived using methods prescribed by regulatory agencies, using community specific ingestion rates of traditional foods (fish, and medicinal plants) estimated from a traditional food survey of community members. The study found risk- surface water quality guidelines for the protection of human health were available but not from governments in Alberta or Canada. Adopting human health water quality criteria from the United States

Environmental Protection Agency provided a good starting point for protection for of community members consuming fish and drinking water from surface water bodies. However, the traditional food consumption rates were higher than those used to derive US EPA criteria and therefore modified water quality guidelines using community specific consumption rates were derived. Methods, results, learnings, and potential application of the project will be presented.

7.04.P-We Metals: Application of Models and Bioavailability Measures – Recent Developments

7.04.P-We-170 Integrating Multivariate Analyses, Metals Bioavailability Models, and Community Ecology to Address Sublethal Toxicity in a Former Mine Pit

Steven S. Brown¹, Shaun A. Roark², Jeremy Rigsby³, Alexander Wilson-Fallon², Kevin Schanke³ and Martin Powers², (1)Anchor QEA, (2)Jacobs Engineering Group Inc., (3)FTN Associates, Ltd.

Wilson Mine is a former vanadium mine in the Ouachita Mountains near Hot Springs, Arkansas (USA), that was reclaimed to mitigate risks from metals in surface water in the surrounding watershed. Prior to reclamation, aquatic communities within the mine site were limited by poor water quality. Toxicity identification evaluations indicated various metals likely contributed to observed toxicity in East Wilson Pond, a mine pit lake. Historical pond treatment involved adding hydrated lime to reduce metal concentrations. Intensive watershed-wide reclamation efforts, including a sediment cap in the pond, were implemented beginning in 2015 and ending when lime treatment ceased in 2018. Post-reclamation, epilimnion water was characterized by circumneutral pH, reduced metal concentrations, and reduced toxicity. However, hypolimnetic water was still characterized by low pH and elevated metals, which degraded epilimnetic water during seasonal destratification and induced seasonal sublethal toxicity to *Ceriodaphnia dubia* at the gravity discharged outfall. The fish community remained stable but zooplankton and phytoplankton density and diversity declined post-2018. Bioavailability modeling was used with principal components analysis and concentration-response modeling to determine that Zn was the primary cause of seasonal sublethal toxicity and other correlated metals, particularly Mn, may contribute. These results informed additional management actions to further reduce metal concentrations. Beginning in Fall 2022, hypolimnetic water was air-lifted to the surface from approximately 30m to promote mixing with epilimnetic water and reduce the volume of poor quality hypolimnetic water during stratification. Subsequent sampling/analysis during destratification showed that bioavailable metal concentrations were reduced and seasonal toxicity was effectively eliminated. Plankton populations were evaluated during 2023 to assess the effects of mixing on pond biology. Reclamation actions at this site were strongly informed by data (science). An extensive dataset based on synoptic sampling/analysis supported the integrated use of multivariate analyses, metals bioavailability models, toxicity testing, and population/community ecology metrics. The improved understanding of relationships and mechanisms resulting from this work form the basis for recommending a relatively passive site-management plan which will minimize toxicity and improve biological conditions into the future.

7.04.P-We-171 Analysis of Biokinetic Parameters Reveals Patterns in Mercury Accumulation Across Aquatic Species

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Mercury (Hg) is a potent neurotoxicant and can impact humans through the ingestion of Hg-contaminated fish. Hg and especially its organic form methylmercury (MeHg) biomagnifies up food chains such that even small aqueous concentrations of Hg can result in significant concentrations of Hg in fish. Understanding Hg and MeHg bioaccumulation in aquatic species is crucial to minimize the contaminant's ecological and human health risk. Accumulation kinetics of Hg and MeHg have been studied for decades, resulting in an extensive literature of values. To better understand Hg and MeHg accumulation kinetics across aquatic species, we compiled

estimates of three biokinetic parameters: uptake rate (k_u), assimilation efficiency (AE), and efflux rate (k_e). These parameters describe contaminant uptake from aqueous (k_u) and dietary (AE) exposure and the rate of excretion (k_e). We found 59 papers for fish for 57 different species and 23 papers for aquatic invertebrates for 32 different species that include values for one or more of these parameters for Hg or MeHg. We collected 502 parameter values in total: 360 for fish and 142 for aquatic invertebrates. We used machine learning techniques to investigate the relationships between experimental and physiological variables on these parameter values, including but not limited to form of Hg (Hg or MeHg), salinity, pathway of exposure (dietary, aqueous, field exposure, etc.), temperature, exposure time, and organismal size. We also looked for phylogenetic patterns in the data and investigated if closely related species had similar or diverging parameter values. Following separate analysis of fish and aquatic invertebrate data, we found differences in which variables were driving variability in biokinetic parameter values. For k_u , aqueous exposure time significantly influenced values for invertebrates while the form of Hg influenced values for fish. For AE, the form of Hg was the only important variable explaining parameter values for both fish & invertebrates. Finally, k_e are the only parameter values significantly influenced by more than one variable, with water type (freshwater, brackish, or marine), organism weight, & form of Hg significantly impacting parameter values for fish and/or invertebrates. To our knowledge, this study represents the most extensive review of biokinetic parameters of Hg and MeHg accumulation in aquatic organisms and reveals novel patterns that may help us better understand and manage Hg accumulation.

7.04.P-We-172 Predicting Metal Toxicity to Aquatic Organisms in Rusting Arctic Rivers

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Climate change disturbances in the Arctic have the potential to increase the concentration and flux of organic carbon, nutrients, and trace metals in Arctic rivers. Recent observations from northwest Alaska indicate that waters draining permafrost landscapes may be susceptible to iron mobilization following thaw, resulting in abrupt color change of streams and river reaches. To assess the potential impacts of these disturbances on aquatic organisms, water quality data including water hardness, pH, dissolved organic carbon (DOC) concentration, and dissolved and particulate metal concentrations from pristine and impaired rivers were measured in the summer of 2022. Preliminary data from a subset of streams in the Agashashok, Kugururok, and Salmon river watersheds show that visually impaired streams have lower pH ($\text{pH} = 7.42 \pm 0.79$), higher sulfate concentration ($308 \pm 254 \text{ mg/L}$), and significantly higher average metal concentrations ($8590 \pm 6650 \text{ } \mu\text{g/L}$) in comparison to the visually pristine streams ($\text{pH} = 7.98 \pm 0.29$; sulfate = $53.9 \pm 38.5 \text{ mg/L}$; total metals = $153 \pm 82 \text{ } \mu\text{g/L}$). Impaired streams were elevated in metals including Fe ($3,010 \pm 2610 \text{ } \mu\text{g/L}$), Mn ($2690 \pm 2530 \text{ } \mu\text{g/L}$), Al ($2680 \pm 2450 \text{ } \mu\text{g/L}$), Zn ($326 \pm 212 \text{ } \mu\text{g/L}$), Ni ($163 \pm 118 \text{ } \mu\text{g/L}$), and Cu ($57.8 \pm 33.2 \text{ } \mu\text{g/L}$). The geochemical data suggest that the predominant mechanism driving these changes in water quality is the oxidative weathering of sulfide minerals, releasing acid that solubilizes metals. The recent onset of stream discoloration implies that climate-induced permafrost thaw may be exposing previously frozen sulfide minerals to active weathering. The potential toxicity of the metals to aquatic organisms was assessed using measured water quality data on these rivers and the U.S EPA's Biotic Ligand Model. Together, this aquatic toxicity model and geochemical data will allow for an initial assessment of the impacts of this climate change disturbance on aquatic organisms in Arctic Alaska.

7.04.P-We-173 Assessment of Intermittent Lead Exposures using the Integrated Exposure Uptake Biokinetic Model

Hyland Herring, GHD

The United States Environmental Protection Agency (USEPA) has long established approaches for evaluating lead exposure. These approaches have traditionally been used for constant and chronic exposures, however, in

certain situations the only exposures are intermittent or even transient. While transient exposures are typically not a concern/evaluated, routine yet intermittent exposures should be evaluated. USEPA updated its Integrated Exposure Uptake Biokinetic (IEUBK) Model in 2021 and released a guidance document titled *Assessing Intermittent or Variable Exposures at Lead Sites* in 2003, however, it has yet to specifically evaluate intermittent exposures. This work summarizes an evaluation of intermittent exposures that concluded clean-up levels on order of 1200 mg/kg to 2000 mg/kg for a recreator who visits a park two times per week. The 2021 updates to the IEUBK model resulted in the increase in the clean-up level from 1200 mg/kg to 2000 mg/kg. This clean-up level is in contrast to USEPA's recommended generic safe levels of 400 mg/kg in residential play areas and 1,200 mg/kg in bare soil in non-play areas. This poster specifically looks at assessment of intermittent lead exposures using the IEUBK model and evaluates the potential uncertainties to show how the calculated numbers are safe.

7.04.P-We-174 Use of the Zinc and Cadmium Biotic Ligand Model (BLM) to Evaluate Metals Toxicity in Whole Effluent Toxicity (WET) Testing

Ashley Romero, GEI Consultants, Inc.

The evaluation of potential parameters of concern in a discharge can be valuable in determining the cause of toxicity observed in whole effluent toxicity (WET) tests. GEI consultants, Inc (GEI) assisted MineWater LLC by providing an assessment of metals toxicity on the discharge from the London Mine Water Tunnel, located in Colorado, where zinc and cadmium concentrations were known to occur and cause toxicity in the quarterly compliance WET tests. In order to quantify the degree of toxicity of the contributing concentrations, and to evaluate if another unquantified parameter is contributing toxicity, two methods of analysis were employed, 1) comparison of site-specific analytical chemistry results to aquatic life criteria concentrations and, 2) the use of the Biotic Ligand Model (BLM) to predict metal toxicity based on zinc and cadmium bioavailability under varying water quality conditions. The first method is a screening process to determine if the concentration of a specific compound is above or below regulatory aquatic life criteria, thereby providing some indication of whether the metals could be contributing, or not contributing, to toxicity, respectively. The second method is a more accurate way of calculating the dissolved metal concentration that would be expected to cause toxicity at the site, based on the water quality chemistry in each sample and its influences on metal bioavailability. Challenges were encountered using both methodologies, however, the resulting analysis provided informative conclusions of the contributions of cadmium and zinc concentrations to toxicity observed in WET testing. Zinc was indicated to be the primary driver of toxicity, however, cadmium may also contribute toxicity in the timeframes where concentrations are higher. A summary of each approach, and associated data analyzed, discrepancies and any variations observed, and all outcomes will be provided.

7.04.P-We-175 Relationships in Selenium Concentrations Among Fish Tissues: Monitoring and Regulatory Implications

Claire Detering¹, Kevin Brix², Barry Fulton³, Marko Adzic⁴ and David K. DeForest¹, (1)Windward Environmental LLC, (2)EcoTox LLC, (3)Benchmark Environmental LLC, (4)Teck Resources Ltd.

Several regulatory jurisdictions in North America have adopted fish tissue-based selenium criteria or guidelines. The US Environmental Protection Agency (USEPA), for example, developed recommended selenium criteria of 15.1 mg/kg dry weight (dw) for fish eggs and ripe ovaries, 11.3 mg/kg dw for fish muscle, and 8.5 mg/kg dw for whole-body (WB) fish tissue. Similarly, Environment and Climate Change Canada has adopted guidelines of 14.7 mg/kg dw (eggs/ripe ovary) and 6.7 mg/kg dw (WB). Because selenium's primary mechanism of toxicity occurs via maternal transfer to eggs, which can impair larval development and survival, the Se criterion for fish eggs and ripe ovaries supersedes muscle and WB Se criteria. However, it can be logistically challenging to sample eggs or ripe ovaries in the field. In addition, it may be desirable to conduct non-lethal sampling of muscle tissue or to combine resources with other fish monitoring programs that may focus on collection of muscle or WB tissue in support of human health assessments. Relationships for estimating egg or ripe ovary Se

concentrations from muscle or WB Se concentrations is desirable. In this study, we compiled a database of selenium concentrations that could be paired in two or more tissue types (eggs/ripe ovaries, muscle, and/or WB). When available, we also gathered information on the reproductive status of the fish sampled. Relationships in selenium concentrations among fish tissues were derived for more than 15 freshwater fish species using linear regression. Several fish species exhibited an inverse relationship between ovary selenium concentrations and spawning status (i.e., ovary selenium concentrations were lower in reproductively mature fish near spawning). These results emphasize the importance in utilizing ripe samples when evaluating tissue concentrations relative to criteria, which were developed based on eggs and ripe ovaries. Selenium relationships among fish tissues developed in this study may be a helpful resource as states and provinces continue to adopt and implement federal recommended fish tissue selenium criteria.

7.04.T Metals: Application of Models and Bioavailability Measures – Recent Developments

7.04.T-01 Development of Multiple Linear Regression Models to Support Updating U.S. Environmental Protection Agency's Aquatic Life Ambient Water Quality Criteria (AWQC) for Metals

Christine Bergeron, Kathryn Gallagher, Joseph R. Beaman, Luis Cruz 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 to states and tribes 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. EPA intends to systematically update 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 has been working with the metals associations to develop MLR bioavailability models for individual metals starting with cobalt, lead, nickel, and zinc. EPA has reviewed the underlying toxicity studies, evaluated water chemistry ranges for pH, hardness, and DOC, investigated pooling taxa in the models, and plans to externally peer review the recommended MLR models for each metal. After peer-review of the models, EPA plans to develop updated, externally-peer reviewed Aquatic Life AWQC for metals to better support states, territories, and tribes. This presentation will discuss the current activities towards MLR model and criteria development.

7.04.T-02 Bioavailability and Toxicity Models of Copper to Freshwater Life: The State of Regulatory Science

Chris Mebane, U.S. Geological Survey

Efforts to incorporate bioavailability adjustments into regulatory water quality criteria in the USA have had four major procedures: hardness-based single-linear regression equations, water-effect ratios (WERs), biotic ligand models (BLMs), and multiple-linear regression models (MLRs) that use dissolved organic carbon, hardness, and pH. The performance of each with copper (Cu) is critically reviewed, emphasizing the relative performance of hardness-toxicity versus the MLR criteria equations. The WER approach was shown to be inherently high-biased. The performance of criteria versions was evaluated with numerous toxicity datasets that were independent from those used to develop the MLR models, including olfactory and behavioral toxicity, and field and ecosystem studies. Within the range of water conditions used to develop the Cu MLR criteria equations, the MLR performed well to both predict toxicity and protect sensitive species and ecosystems. In softwaters, the

MLR outperformed both the BLM and hardness models. In atypical waters with pH <5.5 or >9, neither the MLR nor BLM predictions were reliable, suggesting site-specific testing would be needed to determine reliable Cu criteria for such settings. The hardness-based criteria performed poorly with all toxicity datasets, showing no or weak capacity to predict observed toxicity. In natural waters, MLR and BLM criteria versions were strongly correlated. In contrast, the hardness-criteria version was often out of phase with the MLR and, depending on waterbody and season, could be either strongly overprotective or underprotective. The MLR-based U.S. EPA-style chronic criterion was more generally protective of ecosystems than other criterion versions. All evidence shows that Cu MLR-based criteria equations in their present form may be considered an “other scientifically defensible method” for Cu aquatic life criteria revisions.

7.04.T-03 Assessing the Bioavailability of Metals in Waters Impacted by Mining Waste Around the Tar Creek Superfund Site

Stacey Herriage, Justin Scott, Holden Husbands, Jason B. Belden and Matteo Minghetti, Oklahoma State University

The majority of metal bioavailability studies are lab-based, single metal experiments. The bioavailability of metals present in environmentally relevant mixtures is still poorly understood. The waters surrounding the now defunct lead and zinc mines in the Tar Creek Superfund site are ideal to address this knowledge gap. To begin our investigation, water and fish samples were collected from sites that represent the radius of flow from bodies of water closest to the sources of contamination to the southward direction, which we found reflect a gradient of change in the concentrations of metals as they reduce. The water and fish tissue samples were first analyzed for metal concentrations, and additional water chemistry parameters collected are being used to predict bioavailability using the Biotic Ligand Model (BLM). We found that the metal concentrations measured in fish tissues follow the same gradient trend as seen in the water samples, as well as high concentrations of zinc accumulation in all 4 tissues assayed. Freshly collected water from each sample site was used to directly expose RTgill-W1 cells, a fish gill cell line derived from rainbow trout (*Oncorhynchus mykiss*), and to 14-day-old fathead minnow larvae (*Pimephales promelas*) to measure bioaccumulation and evaluate acute toxicity. A significantly reduced viability by as much as 80% was observed in cells exposed to the water collected from the sources closest to mining sites whereas cytotoxicity in reference sites was identical to that of controls. Greater sensitivity was observed in the fathead minnow larvae with 100% mortality in those exposed to the most contaminated water. The gill cells and the larvae also accumulated metal reflective of the measured metal concentrations in the water. These methods allow for side-by-side comparisons of methods used to assess bioavailability and toxicity of metals in environmentally relevant mixtures under both acute and chronic conditions. Interestingly, although the cytotoxicity data confirm that the metal concentrations in those waters are elevated sufficiently enough to produce toxic effects, we have found a variety of fish species and other aquatic organisms in the most contaminated waters, which suggests that these organisms have evolved specific mechanisms to tolerate metal toxicity.

7.04.T-04 Withdrawn - Evaluating the Performance of the Nickel Bioavailability Models in Chinese Freshwaters Using Native Species

Jia He¹, Fengchang Wu¹, Elizabeth Traudt Middleton², Emily Rogevich Garman² and Christian E. Schlekat², (1)Chinese Research Academy of Environmental Sciences (2)NiPERA Inc.

Recently, the Chinese government has announced broad goals of improving environmental quality and there has been recent interest by the Chinese Ministry of Environmental Protection (MEP) to develop metal-specific thresholds applicable to Chinese surface waters. Since 2019, a substantial amount of data has been collected including water sampling to determine the chemistry ranges of local surface waters, nickel toxicity tests for algae, invertebrates, and fish species to establish baseline sensitivity values, generating toxicity predictions using bioavailability models established for other global jurisdictions, and performing a performance comparison between the models. The results of this extensive project were collated in a manuscript published in #SETACLouisville

early 2023 where it was observed that both the European-based nickel Biotic Ligand Model and the nickel Multiple Linear Regression models developed for Australian waters considerably decreased the intra-species variability for *D. magna* and *P. subcapitata*, the two species that 1) were the most sensitive to Ni exposure and 2) showed the greatest influence of water chemistry parameters. Although this data demonstrates that existing nickel bioavailability models can explain the observed variations caused by influences of water chemistry on nickel toxicity in standard test species, in order for a proposed bioavailability-based nickel water standard to be accepted by the MEP, the models must also be validated for native Chinese species. To address this data deficiency, the Chinese Research Academy of Environmental Sciences has performed additional toxicity tests to three native Chinese species (*S. vetulus*, *H. vulgaris*, and *N. pelliculosa*) in natural waters from the Yangtze River. Conducting toxicity tests in natural waters using native species not only satisfies criteria specified in Chinese regulation but also provides crucial data for the refinement of global bioavailability models. The toxicity results and implications for bioavailability models will be presented.

7.04.T-05 Use of Toxicity Identification Evaluation, Bioavailability Modeling, and Benthic Macroinvertebrate Assessment to Evaluate Aquatic Life use at a Former Mine Site

*Shaun A. Roark*¹, *Alexander Wilson-Fallon*¹, *Jeremy Rigsby*², *Kevin Schanke*², *Martin Powers*¹ and *Steven S. Brown*³, (1)Jacobs Engineering Group Inc., (2)FTN Associates, Ltd., (3)Anchor QEA

Indian Springs Creek (ISC) receives surface and subsurface water from Wilson Mine, a reclaimed former vanadium mine in the Ouachita Mountains near Hot Springs, Arkansas (USA). The stream was identified as impaired and a reclamation and enhancement project supported by monitoring has been implemented over the past decade. Instream macroinvertebrate sampling indicated the aquatic life use was not supported despite instream enhancements. Chemical analysis and biomonitoring indicated elevated minerals, metals, and seasonal sublethal toxicity to *Ceriodaphnia dubia* downstream from the inflow of subsurface water. A toxicity identification evaluation (TIE) study suggested that elevated minerals in ISC did not contribute to the toxicity; rather, manganese (Mn) was identified as likely the primary toxicant. Following the TIE study, a multiple-lines-of-evidence approach, including the Metals Bioavailability Assessment Tool (M-BAT), principal components analysis, and concentration-response modeling were used to investigate spatial-temporal relationships between metals concentrations and *C. dubia* toxicity. The approach was effective in confirming Mn as the primary cause of toxicity. ISC flow was diverted through a series of constructed wetlands which reduced Mn concentrations at the outflow of the wetlands by more than 100-fold and substantially and consistently reduced instream toxicity. Also, while enhancements in the lower portion of the watershed improved, instream habitat and benthic macroinvertebrate community structure, the ISC headwater still provides limited/poor habitat. The existing stream geomorphology and benthic habitat in this headwater segment of ISC will soon be enhanced by increasing the area of step pools and gravel-dominated substrate. This is expected to be the final reclamation activity in the ISC watershed. In the end, the ISC stream reclamation and enhancement project should reduce Mn concentrations below the preliminary site-specific EC20 (6,100 ug Mn/L), eliminate ambient toxicity, including sublethal effects, and improve instream habitat for benthic invertebrates and fish. This will allow the stream to be assessed for aquatic life use in the presence of elevated minerals and the absence of metals toxicity.

7.04.T-06 Development of Multiple Linear Regression Models for Predicting Chronic Iron Toxicity to Aquatic Organisms under Different Water Quality Conditions

*David K. DeForest*¹, *Lucinda Tear*¹, *Kevin Brix*² and *William J. Adams*³, (1)Windward Environmental LLC, (2)EcoTox LLC, (3)Red Cap Consulting

We developed multiple linear regression (MLR) models for predicting chronic Fe toxicity to aquatic organisms for deriving site-specific water quality guidelines (WQGs). The effects of DOC, hardness, and pH on Fe toxicity to three representative taxa (*Ceriodaphnia dubia*, *Pimephales promelas*, and *Raphidocelis subcapitata*) were evaluated. DOC and pH were identified as toxicity modifying factors (TMFs) for *P. promelas* and *R. subcapitata*, while only DOC was a TMF for *C. dubia*. Hardness was not a statistically significant term in MLR

models evaluated. MLR models based on EC10s and EC20s were developed and had adjusted R^2 of 0.68-0.89 among all species. Differences among species in the MLR models, especially in the significance of the pH term, precluded development of a pooled model. Instead, the species-specific models for *C. dubia*, *P. promelas*, and *R. subcapitata* were assumed representative of invertebrates, fish, and algae, respectively, and applied accordingly to normalize toxicity data for TMF conditions. The species sensitivity distribution (SSD) included a combination of laboratory toxicity data and toxicity data for insects from mesocosm experiments. The insects are the predominant taxa in the lowest quartile of the SSD. Using the European Union approach for deriving WQGs, application of MLR models to the SSD results in WQGs ranging from 114 to 765 mg l⁻¹ Fe across a range of TMF conditions (DOC: 0.5-10 mg l⁻¹, pH: 6.0-8.4), with slightly higher WQGs (199-910 mg l⁻¹) derived using the USEPA methodology. An important uncertainty in these derivations is the applicability of the *C. dubia* MLR model (no pH parameter) to aquatic insects and understanding the pH sensitivity of aquatic insects to Fe toxicity is a research need. An Excel-based tool for calculating Fe WQG using both European Union and USEPA approaches across the range of TMF conditions for which the models are applicable is available.

7.05.P-Tu New Developments in Pesticide Labeling and Risk Mitigation

7.05.P-Tu-215 An Introduction and Strategies in Washington State Regarding New and Forthcoming Pesticide Product Label Changes

Abigail Nickelson, Washington State Department of Agriculture

Recent label changes and mitigation approaches are a culmination of risk assessment processes by EPA, USFWS, NMFS, USDA and stakeholders. As new mitigation approaches have started to appear on pesticide product labels, state agencies are at the forefront of educating users and enforcement of the new labels. As an introduction to the challenges and opportunities these recent changes create, this presentation will give a description from the perspective of the Washington State Department of Agriculture (WSDA). WSDA has multiple programs all of which focus on aspects of education, natural resource protection and enforcement. February 2023 marked the first time in which bulletins were published for Washington State via Bulletins Live! Two. These bulletins have spurred work in all programs related to pesticide use within WSDA. Action plans for educating pesticide applicators have been started. Discussions on the enforcement of label language are ongoing. Engagement of stakeholders in pesticide stewardship partnerships are being assessed. Plans for effectiveness monitoring are being explored. This platform presentation aims to share strategies used in Washington State, their current state at the time of this meeting, successes and challenges encountered, as well as prompt discussion on the path forward.

7.05.T New Developments in Pesticide Labeling and Risk Mitigation

7.05.T-01 National Marine Fisheries Service (NMFS) Approach to Mitigating the Effects of Pesticides

Ryan DeWitt¹, Tony Hawkes², David H Baldwin¹, Thom Hooper³ and Karrin Goodman (Leazer)¹, (1)National Oceanic and Atmospheric Administration (NOAA), (2)National Marine Fisheries Service, (3)Office of Protected Resources - F/PR

Management of pesticide risks to Endangered Species Act (ESA)-listed species on a national scale is complicated by the fact that application-specific technology and site-specific factors highly influence risk. A one-size-fits-all approach to managing risk is not always necessary or desired. In recent biological opinions, NMFS has recommended a “pick-list” approach to provide applicators the flexibility to choose among a variety of options that can achieve the necessary level of risk reduction needed to comply with the ESA-related mitigation on Federal Insecticide, Fungicide, and Rodenticide Act labels. Risk reduction options include reduced application rates, lower risk application methods, vegetated filter strips etc. The “pick-list” approach can also be paired with EPA’s “Bulletins Live! Two” application to allow greater geographic specificity. In

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developing the approach, one challenge has been finding the appropriate level of prescriptiveness in the mitigation options. Too little definition may lead to requirements that don't make sense on-the-ground, whereas too much detail may cause disengagement of end-users due to complexity. In this presentation NMFS will provide an overview of the current mitigation approach, and discuss the various benefits and challenges associated.

7.05.T-02 State and SFIREG Roles in Shaping Effective Pesticide ESA Mitigation, Education, and Regulation

Gary Bahr¹, Hotze Wijnja², Beth Dittman³, Christina Zimmerman¹, James Cooper⁴, Kathryn Rifenburg⁵, Rajinder Mann⁶ and Dave Scott⁷, (1)Washington State Department of Agriculture, (2)Massachusetts Department of Agricultural Resources, (3)North Carolina Department of Agriculture and Consumer Services, (4)Florida Department of Agricultural & Consumer Services, (5)Oregon Department of Agriculture, (6)Minnesota Department of Agriculture, (7)Office of Indiana State Chemist

State Lead Agencies (SLAs) for pesticide regulation have equal primacy with the U.S. Environmental Protection Agency (EPA) in regulating pesticides in states and territories throughout the nation. The State FIFRA Issues Research & Evaluation Group (SFIREG) and the Environmental Quality Issues (EQI) working group represent SLAs in working with EPA on pesticide regulatory policy. SFIREG and EQI work to create and recommend solutions to EPA to resolve complex scientific and policy issues related to pesticide risk and mitigation measures for the EPA Endangered Species Act (ESA) and pesticide regulatory processes and actions. SFIREG and EQI track mitigation proposals as they're developed through EPA consultations with the U.S. Fish and Wildlife Service, National Marine Fisheries Service (the Services), and industry. To implement the goals of the EPA ESA work plan, various mitigation measures are proposed through a variety of assessments such as Biological Evaluations (BEs) and Biological Opinions (BiOps), and through registration and reevaluation of pesticides. SFIREG, EQI, and SLAs have opportunities to provide public comment through official federal register notices, and through regular SFIREG and working committee meetings and issue paper development. SFIREG and SLAs provide suggestions for EPA and the Services to include more flexible and diverse mitigation options to reduce pesticide ESA impacts. SFIREG and SLAs recommend that EPA and the Services consult with the diverse agencies and stakeholders at the state and regional levels to conduct education, gain trust at the local level, and to gain more knowledge of the diverse cropping and workable mitigation systems. There are diverse agricultural and conservation practices that are available to be more fully utilized. There are numerous examples around the nation where stakeholder groups have experience working with agriculture for natural resources and species protection. SFIREG proposes additional coordination and consultations to promote workable pesticide label-based mitigation and programmatic solutions, along with the utilization of research group findings, effective outreach, and the development of effectiveness monitoring and reporting systems.

7.05.T-03 Endangered Species Act: Actions by the Weed Science Society of America to Provide Science-Based Information to Regulators

Bill Chism, EPA Retired

The Weed Science Society of America (WSSA) created an Endangered Species Act (ESA) Committee to foster the protection of threatened and endangered species and their habitat while protecting critical weed management tools (e.g., herbicides) through developing partnerships which maximize the WSSA's ability to communicate science-based information to regulators. The committee has selected four areas to help provide information to our members and regulators. Communication: Many farmers and other pesticide applicators have little knowledge of the endangered species act which was signed into law in 1973, do not think their state has these species, and do not understand the potential implications for pesticide usage. The WSSA is creating

presentations and website to share educational materials for these groups. The website will provide links to lists and locations of threatened and endangered species on FWS and NMFS websites. The website will also advertise and help explain EPA's Bulletins Live Two webpage which describes pesticide usage restrictions to protect threatened and endangered species. Label Reform: Pesticide labels are too long, complex and difficult to understand often being over 100 pages. The WSSA will create a pilot program label workgroup to determine if standardizing the format, consistency, and simplicity would help users more accurately use pesticides and help EPA label reviews. Mitigation Information: The WSSA will communicate how pesticides can be used while minimizing the impact to endangered species, learn and share how to support the growth of threatened and endangered species and habitats, and provide the federal regulators with a better understanding of the times of year when herbicides are applied to help describe if there will be direct contact between the species and pesticide application. Phytotoxicity Testing: Very little information is available on the phytotoxic effects of herbicides on threatened and endangered plant species. The WSSA will work with researchers and registrants to collect that type of data which would allow federal agencies to make a more accurate estimate of risks to plants.

7.05.T-04 The PULA Path - From Likely Jeopardy to Product Registration

Colleen Priest¹, Twyla Michelle Blickley², Patrick Havens², Gerco Hoogeweg² and John Marton², (1)Corteva Agriscience and Intrinsik Ltd., (2)Corteva Agriscience

The Endangered Species Act requires federal agencies to ensure that any action will not jeopardize the continued existence of any listed (threatened or endangered) species, or adversely modify their designated critical habitat. In the context of pesticides, this means the EPA carries out an Effects Determination for all uses for new and existing actives as the defining Action by the Endangered Species Act. Refinements to conservative, screening-level risk assessments using species- and compound-specific data are the first step in the process of a predicted jeopardy/adverse modification (J/AM) analysis. Once the refinement options are exhausted and potential risk still exists for certain species, EPA may issue the registration with mitigations in place such that the action does not result in irretrievable or irreversible commitment of government resources that would prevent the implementation of any reasonable and prudent measures or alternatives as necessary to ensure the species are protected. Such mitigations may include general label changes (i.e., rainfall, wind speed and/or droplet size restrictions), label prohibitions, including off-labelling of counties or states, and pesticide use limitation areas (PULAs). Bulletins Live Two! was developed by EPA to provide a resource for growers/applicators for retrieving information on geographically-specific PULAs associated with the pesticide product. Additional precautions or restrictions are prescribed within the PULAs to avoid J/AM to listed species and/or their critical habitats. Habitat information, timing of flowering or species presence/absence, environmental parameters (e.g., elevation), and spatial information are typically used to refine PULAs with the goal of maintaining application flexibility for the growers. The PULA development and refinement process will be described from an industry perspective, using examples from species with predictions of J/AM in the recent biological evaluations released by EPA.

7.05.T-05 Prioritization of Resources for Assessing Potential Pesticide Risk and Mitigations for Federally Listed Threatened and Endangered Species

Matthew E Kern¹, Steve Kay², Christopher M Holmes³, Logan Insinga³, Dana Christian³, James L. Cowles⁴ and Kevin Henry⁵, (1)Balance EcoSolutions LLC, (2)Pyxis Regulatory Consulting, Inc. / Generic Endangered Species Task Force (GESTF), (3)Applied Analysis Solutions, LLC, (4)Tessenderlo Kerley, Inc. / NovaSource, (5)Tessenderlo Kerley, Inc.

Assessing the potential harm to species protected under the Endangered Species Act poses two main challenges that need to be addressed in a clear and transparent manner. The first challenge is the considerable number of species on the protected list that need to be evaluated. The second challenge is ensuring that assessments

provide enough information to determine the likelihood of risk to guide the implementation of specific actions to mitigate risk where specifically needed. There are several factors that can influence potential impact to species. In some cases, the risk to a particular species may be unlikely, requiring no additional actions for mitigation. However, in other cases, a species, or its habitat, may need extra measures to minimize risk and avoid jeopardy or adverse modification. Given the wide range of local conditions, use patterns, application methods and species attributes that occur for each species it is crucial to develop methods for prioritizing the allocation of resources for risk assessment and mitigation strategies for pesticides. The prioritization of resources is essential to adequately support risk conclusions and management decisions. To facilitate this process, specific attributes related to both the pesticide being evaluated and the species involved can be collected and organized. These attributes may include the sensitivity of species and their habitats to the pesticide, the overlap between habitats and areas where the pesticide is used, primary factors causing stress to the species, how likely the species is to be found in areas where the pesticide is applied, the size of available habitat areas, and information about pesticide usage. These attributes can be evaluated, assigned different weights according to their importance, and ranked to identify species that may require more, or less, effort in evaluating risk and potentially mitigations. This presentation provides an overview of how these attributes can be utilized to prioritize risk evaluation across different protected species and identify situations where mitigation measures are more likely to be necessary.

7.05.T-06 The Enlist Perspective: Driving New Developments in Pesticide Labeling and Risk Mitigation *Reuben Baris, Patrick Havens and Twyla Michelle Blickley, Corteva Agriscience*

On January 11, 2022 the US EPA approved amended registrations for Enlist One and Enlist Duo herbicides set to expire in January 2022, providing certainty for growers in the 2022 growing season. At the time of registration, EPA initiated formal consultation with the U.S. Fish and Wildlife (FWS) while simultaneously supporting the on-going uses of Enlist One and Enlist Duo. This action highlights the EPA's effects determination and first of its kind predictions of no jeopardy to any listed species nor adverse modification of critical habitat. There has been a steady shift over the last few decades by the EPA to evolve regulatory standards for evaluating pesticide registrations in the face of increasing litigation pressure. New policies implemented by EPA shift the paradigm for pesticide evaluations. The Enlist registration decisions, implementation, and consultation, including recent evaluations can be viewed as case studies for collective renewed focus on the process and to chart an efficient path for endangered species protection, agriculture, and pesticide regulation.

7.05.V New Developments in Pesticide Labeling and Risk Mitigation

7.05.V-032 Prioritization of Resources for Assessing Potential Pesticide Risk and Mitigations for Federally Listed Threatened and Endangered Species

Matthew E Kern¹, Steve Kay², Christopher M Holmes³, Logan Insinga³, Dana Christian³, James L. Cowles⁴ and Kevin Henry⁴, (1) Balance EcoSolutions LLC, (2) Pyxis Regulatory Consulting, Inc. (3) Applied Analysis Solutions, LLC, (4) Tessenderlo Kerley, Inc

Assessing the potential harm to species protected under the Endangered Species Act poses two main challenges that need to be addressed in a clear and transparent manner. The first challenge is the considerable number of species on the protected list that need to be evaluated. The second challenge is ensuring that assessments provide enough information to determine the likelihood of risk in order to guide the implementation of specific actions to mitigate risk where specifically needed. There are several factors that can influence potential impact to species. In some cases, the risk to a particular species may be unlikely, requiring no additional actions for mitigation. However, in other cases, a species, or its habitat, may need extra measures to minimize risk and avoid jeopardy or adverse modification. Given the wide range of local conditions, use patterns, application methods and species attributes that occur for each species it is crucial to develop methods for prioritizing

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7.06.P-We Risk Communication: Strategies and Platforms that Work for New and/or Complex Risk Management Needs

7.06.P-We-176 Why Crisis Communication is in a Crisis

Tamara House-Knight and Francis Ramacciotti, GHD

Environmental activism started as early as the 1960s when minority citizens organized to oppose environmental threats to their communities. Today this activism has an official name and is widely known as environmental justice. As the name implies, the objective of environmental justice is to provide fair treatment and meaningful involvement of all people regardless of race, color, national origin, or income with respect to the development, implementation, and enforcement of environmental laws, regulations, and policies. Within the last 10 years several states have enacted their own environmental justice laws, and just recently the federal government released their own environmental justice metrics. However, this has still led to inconsistent response to environmental releases in disadvantaged communities. In addition, impacted communities often are under-informed and or mis-informed and left feeling unheard, unseen, and that there is a lack of accountability for the harm caused. This presentation is intended to provide a comparison of responses to environmental impacts involving three real world examples (a petroleum spill, drinking water lead contamination, and vinyl chloride spill). It also serves as a practical guide for regulators, practitioners, and the general public to see how environmental justice, accountability, and risk communication are interconnected concepts related to the environment and society. Taken together, these concepts highlight the importance of ensuring that environmental policies and decisions are made in a fair, transparent, scientifically sound, and accountable manner, and that the public is equitably informed about potential risks associated with environmental hazards.

7.06.P-We-178 Connecting the Dots: PFAS Risk Framework and Communication Hub

Mark Ballentine¹, David W. Moore¹, Alan Kennedy², Manoj Shukla², Jodi Ryder² and Bill Gardiner², (1)U.S. Army Engineer Research and Development Center (ERDC), (2)U.S. Army Corps of Engineers

Per- and Polyfluoroalkyl substances (PFAS) in the environment continues to evolve and grow in complexity with no obvious risk management strategy to protect citizens worldwide. The growing number of PFAS compounds (more than 10,000) and the ever-changing and large amount of scientific data being generated increases risk-management uncertainty; this uncertainty slows timely, science-based risk-informed decision-making for military installations and site managers. The U.S. Army Engineer Research and Development Center is developing a science-based, rapid risk characterization framework for PFAS across a broad range of environmental media (water, soils, sediments) to enable better informed and cost-effective management solutions. The framework and communication hub will connect users to requisite resources and subject matter experts facilitating more timely decisions. Experimental case studies (from micro- to mesocosm) are planned to bridge the gap between high fidelity laboratory generated data and field observations improving understanding

of key mechanistic drivers required for derivation of risk estimates and reduce uncertainty. The data generated will be utilized in computational models to characterize fate and effect of PFAS in the environment. These computational models will ultimately be used to optimize an existing multi-media fate and transport modeling framework for field scale PFAS concentration screening. The multi-year effort's goal is to create a working PFAS risk framework prototype and communication hub to direct users to needed scientific data, tools, and standards.

7.06.P-We-179 What Contributes to Perceptions of Public Health Advisories Across the United States? A Case Study on Communicating Variability in Fish Consumption Advisories Triggered by Perfluorooctane Sulfonate

Joseph Makaure, Philip E Goodrum, Evelyn Reategui Zirena, Kenia Whitehead and Janet K Anderson, GSI Environmental, Inc.

Fish consumption provides both nutritional and recreational human health benefits. However, high levels of toxic environmental contaminants such as methyl mercury, PCBs, and more recently perfluorooctane sulfonate (PFOS) in freshwater fisheries have prompted health and environmental agencies across the U.S. to issue fish consumption advisories. Fish advisories can be an effective tool for risk communication to the public, with recommendations on limiting or avoiding fish consumption from specific water bodies to reduce human exposure to toxic contaminants. However, mixed messaging on the tradeoffs between health benefits of a fish diet and the potential exposure to toxic contaminants from fish consumption may be a source of confusion to consumers. The current study explores the hypothesis that consumers are more likely to trust an advisory if they understand both the science and policy components and uncertainties. We conducted a national survey of state agencies to compare PFOS fish advisories for non-cancer endpoints in recreationally caught fishes in order to identify examples of potential challenges in risk communication when advisories differ across state boundaries. Findings are incorporated in an online fish advisory calculator that we designed to help consumers understand the major differences in assumptions used by states to calculate human health risks from dietary PFOS exposure. The tool highlights interstate variations in minimum PFOS threshold concentrations that trigger fish consumption advisories. Among 17 states that have developed PFOS fish consumption advisories for both general and potentially more susceptible populations, the lowest PFOS concentrations that trigger the minimal advisory range 80-fold, from 0.5 to 40 parts per billion (ng PFOS/g wet weight). The tool presents information in graphics and tables to demonstrate how multiple factors can contribute to interstate variation in fish tissue thresholds including different policies on fish meal frequency categories and interpretations of the science on the toxicity of PFOS. This presentation will report on findings from a pilot study of the tool, based on feedback of participants from communities with high-end fish consumption rates. This pilot study demonstrates that user-friendly online tools may be one effective approach for addressing challenges in communicating health risks associated with dietary exposure to toxic emerging contaminants such as PFOS.

7.06.P-We-180 Ethylene Oxide Risk Communication: Reflections on a National Approach to Local Risk Comm at the USEPA

Madeline Beal, U.S. Environmental Protection Agency

In 2022 and 2023, US EPA undertook a significant risk communication effort to share more information sooner with many American communities about risk they face from Ethylene Oxide. This effort included intentionally incorporating risk communication best practice and using a strategic approach based on EPA's SALT Framework. The speaker will discuss the effort, successes, and lessons learned. This effort included a need for national consistency but also local responsiveness and used a phased approach to meet these different needs from different audiences.

7.06.T Risk Communication: Strategies and Platforms that Work for New and/or Complex Risk Management Needs

7.06.T-01 Scientific Dissemination Projects Through Fairs, Forums and Festivals

Martha Georgina Orozco-Medina, Javier Omar Martínez Abarca and Arturo Figueroa Montaña, Universidad de Guadalajara

In the context of training professionals with a comprehensive vision, the need to develop strategies for the application of knowledge in higher education students was identified, in such a way that they were motivated to participate in training projects outside the classroom so that they could develop creative abilities, critical thinking, documentary research, problematization, application of innovative methodologies, oral communication, exposition of topics of biological, scientific, toxicological and environmental interest, among others. Thus, the forums, fairs and festivals project consists of designing activities to expose a topic linked to the learning unit in question as part of the training process in their university career (specifically for the learning units of Environmental Toxicology, Teaching Methodology and the Learning and Knowledge Management Seminar for undergraduate students in Biology, Veterinarians, Zootechnicians and Masters in Environmental Health Sciences). In a complementary way and in the field of the COVID-19 pandemic, they were motivated to develop methods and techniques for the integration of digital materials and the organization of virtual events, so that the possibility of participating in forums, fairs and festivals broadened their horizons of application through digital media and with the help of social networks, thus reaching foreign audiences. With great enthusiasm throughout this experience, we have seen how the participation of students in these events increases the acquisition of new knowledge, broadens ties with the community, develops skills for the dissemination of scientific and environmental issues, influences the scientific literacy and motivates students for vocations in biological and environmental issues; In addition, there is a favorable increase in positive thoughts, improvement in their self-esteem, in their spirit of freedom and creative thinking and other aspects linked to the development of soft skills, which were multiplied in a way that was evident and satisfactory. These activities have evolved since they began in 2015, to date we continue to identify ways to innovate in linking scientific communication through creative strategies such as the organization of forums, fairs and festivals in different spaces, with different communities and with a diversity of themes and topics that arise from the inventiveness of university students.

7.06.T-02 Improving Communication of Risk and Uncertainty Across Federal Agencies

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Federal agencies tasked with safeguarding the well-being of the US public face the common challenge of effectively communicating the risks of various hazards, as well as the nature and extent of scientific uncertainty about these risks. Effective communication of risk and uncertainty enables the public to understand the likelihood of important hazards, assess the strength of available risk information, and take appropriate action to mitigate and respond to these hazards. Yet effective communication of risk and uncertainty to the public raises key questions about what information to communicate, why, when, how, and to whom. Effective risk and uncertainty communication is also challenging due to human factors including limitations in literacy and numeracy, cognitive biases and heuristics (mental short-cuts), the spread of misinformation and disinformation, and limited access to information among different communities and stakeholders. To address these many challenges, a federal inter-agency workgroup, convened by the US Office of Science and Technology Policy, is leading an initiative to improve the quality, consistency, timeliness, and appropriateness of efforts to communicate the risks and uncertainties of various hazards to the general public. The workgroup is identifying key needs, available resources, and potential strategies to promote a more intentional and coordinated approach

to communicating risk and uncertainty across federal agencies; increase translation of social and behavioral evidence on risk and uncertainty communication into best practices; and ensure meaningful engagement of community stakeholders in these efforts.

7.06.T-03 Trauma-Informed Risk Communication and Community Engagement

Amelia Hertzberg, Oak Ridge Institute for Science Education (ORISE)

Scientists and psychologists are becoming increasingly aware of the impact trauma has on the brain, cognition, and health. They estimate between 50 and 75% of American adults have experienced a traumatic event. Trauma affects brain chemistry, trust, perception of authority figures, and cognitive thinking, among other things. Communities dealing with a traumatic situation and/or with high levels of historic trauma, therefore, present a particular kind of challenge for risk communication and community engagement. Historic trauma is particularly prevalent in communities with high rates of poverty and marginalization, the same populations who are often among the most at-risk during situations requiring risk communication. Ignoring trauma as an audience factor leaves communicators underprepared and open to misunderstanding the effects of trauma as simple belligerence or stubbornness, leading to sub-optimal work at best, and re-traumatization and further injustices at worst. Applying some of the trauma-informed approaches used in the medical field to risk communication and community engagement practices will help communicators better understand their audiences and avoid these negative outcomes. This presentation will discuss how the S.E.L.F. technique from the Sanctuary Model, the 3 R's from the Neurosequential Model, and the H.E.A.R. approach from Minson's Receptiveness Model can be used in risk communications and discuss how trauma-informed approaches can also protect the communicator from experiencing vicarious trauma.

7.06.T-04 Misinformation and Disinformation in Risk Communication: Reflections from East Palestine OH

Michael Nye, U.S. Environmental Protection Agency

Misinformation and disinformation present a significant and growing challenge for effective risk communication, particularly in context of disasters and public health emergencies where they can lead to casualties or other irreversible effects. Both thrive in conditions of uncertainty, and both are easily spread through social media as people search for the information they need to make decisions about hazards, risk, and protective action. This session will first present a primer on the psychological and social dimensions of the human response to unforeseen circumstances, and the ways that information (both correct/official and mis/disinformation) are sought out to fill information vacuums, reduce anxiety about the unknown, and create feelings of control. We will then discuss several specific examples of misinformation and disinformation encountered by EPA and other Federal/State/Local staff during the response to the recent train derailment in East Palestine OH. The presentation will conclude with contextually relevant tips for combating misinformation and disinformation in risk communication and in the field and will offer opportunities for attendees to share their own experiences and examples.

7.06.T-05 From Uncertainty to Action: Enhancing Public Understanding of PFAS Risks in Colorado

Kristy Richardson, Shannon Barbare, Kelsey Barton and Mallory O'Brien, Colorado Department of Public Health and Environment

The rapidly evolving science around per and polyfluoroalkyl substances (PFAS) is a well-known risk communications challenge. The news media and activist groups may overemphasize risks while agency experts, health educators and communicators proceed cautiously to avoid alarming the public. Too often, this results in a reliance on technical language, a minimization of potential health effects, and a failure to provide actionable information to the public and other stakeholders. Since contamination was first discovered in 2016, the Colorado Department of Public Health and Environment (CDPHE) has worked to effectively understand concerns, communicate risk, and acknowledge uncertainty associated with PFAS exposure in Colorado. In June

2022, following the EPA's release of new health advisories for four PFAS, we worked with stakeholders to identify concerns and knowledge gaps. Focusing on plain language, we developed new materials closely targeted to three key audiences: the public, drinking water systems, and health care providers. This case study will outline our change in direction, how we made our materials more actionable and accessible, and subsequent outreach to key audiences.

7.06.T-06 Per and Polyfluoroalkyl Substances (PFAS) Research and Remediation Podcast Series

Theresa Guillette, Johnsie Lang and Craig Divine, Arcadis U.S., Inc.

Innovative approaches are needed for the transfer of information and novel results from the Department of Defense's Strategic Environmental Research and Development Program (SERDP) and Environmental Security Technology Certification Program (ESTCP) research and demonstration projects to end users. Knowledge sharing from these research projects is critical to implementation of lessons learned and prevention of costly rework in the remediation and risk assessment field. For example, a solution to a problem may be developed, however, lack of information transfer could prevent other researchers and the practitioner community from using the solution. Previously, the public distribution of information related to SERDP/ESTCP projects has focused on research manuscripts, webinars of specific projects, the Environmental Restoration Wiki or Enviro.wiki platform, and professional conferences. Podcasts are used weekly by over 20% of the US population (80 million, age: 12+ population) regularly. This communication channel has achieved steady growth from the early 2000s and is a particularly popular tool for science communication as podcasts have an adaptable format, broader audiences, are relatively easy to disseminate, and can be quickly produced. Science-themed podcasts have increased over the last five years, and academic journals are increasingly using social media platforms to broaden their audience base. Since 2011, SERDP/ESTCP funded programs have developed applied research and product development for improved environmental restoration from the emerging contaminants, Per and Polyfluoroalkyl Substances (PFAS). The goal of this project aimed to create a PFAS Research and Remediation Podcast series based on the SERDP/ESTCP funded projects. PFAS are a polarizing topic to discuss within popular media, and setting the right tone for the project was important in the Podcast creation and delivery. This presentation will document how the Podcast was developed, discuss key metrics of listenership over time, and suggest evolving best practices for #scicomm to diverse audiences.

7.07.P-Tu-217 End of Life Repercussions for Cosmetics and PCPs: Are the Appropriate Decision Frameworks Being Used?

Konrad Kulacki, Margaret Fleming and William L. Goodfellow, Exponent Inc.

Cosmetics and personal care products (PCPs) may be returned to retailers for a number of reasons. Upon return, these products are processed through one of several possible pathways, from resale, to recycling, to incineration. In California, (waste) products recycled into repurposed materials or chemically transformed by incineration before subsequent landfilling are subject not only to Resource Conservation and Recovery Act (RCRA) regulations to potentially define them as RCRA hazardous waste, but also to the Hazardous Waste Control Law (HWCL), Chapter 6.5 under the California Code of Regulations (CCR; 22 CCR Section 66261.3), to potentially define them as non-RCRA hazardous waste. While CCR largely mirrors RCRA when defining waste as ignitable, reactive, or corrosive, the two regulations differ in defining product toxicity. Specifically, while RCRA incorporates exposure into its classification of toxicity (and by association, risk), the HWCL only relies on hazard information (often using the fathead minnow acute toxicity test) and does not incorporate likelihood of exposure. This can lead to materials being classified as non-RCRA hazardous waste, and thus subject to additional regulatory scrutiny and requirements, even when there may be no chance of any relevant biological receptors being exposed to the materials. This presentation will expand on this topic, using returned cosmetic and PCPs as an example. We will discuss issues associated with the use of fathead minnow toxicity testing and the associated 2016 toxicity testing assessment that is (still) being relied upon to classify certain

products as toxic (non-RCRA hazardous waste). We will further highlight the importance of generator knowledge when classifying potentially hazardous waste materials in an effort to make informed decisions as to product end-of-life decisions and environmental stewardship.

7.07.P-Tu-218 Unwrapped: Implications of Regulatory Restrictions on Recycling and Additives in Plastic Packaging

Alexandra Steele, Konrad Kulacki and Christopher White, Exponent Inc.

The fate of plastics in the environment is an area of growing research and public concern. Plastics in the environment, the definition of recyclability, and producers' roles and responsibilities for the end-of-life management of their products, have also recently drawn increased attention from legislators and regulators. Plastics-related regulations are increasing in the U.S., primarily at the state level, signaling a changing reality for packaging and other single-use product manufacturers. Several states have passed or are considering Extended Producer Responsibility (EPR) legislation that will incentivize producers to either make their glass, paper, or plastic packaging and products more readily recyclable or else cover the actual costs of recycling these items. In addition, states are also developing legislation related to recyclability claims and labelling, as well as restricting the additives that may be used in plastic packaging. Multiple legal battles have surfaced regarding company claims around single-use plastic products and recycling; these new state regulations would restrict communications about recyclability of products and may spark product line changes to meet labelling requirements. Additionally, additive restrictions would add single-use plastic products to the growing lists of products (e.g., textiles, food packaging) where the use of certain chemicals, such as PFAS, may be regulated. In the absence of federal regulations, a complex patchwork of state regulations may require businesses to comply with an array of developing state regulations. Thus, the effects felt by producers will be dependent upon the details and structure of the policies in each state. These regulatory changes may have profound supply chain ripple effects among plastic and petrochemical manufacturers and present challenges for national brands. This presentation will explore this changing regulatory landscape across the U.S. and present case studies of law implementation in select states.

7.08.P-We How Does the SETAC Community Define Good Quality Data?

7.08.P-We-181 Risk Assessment of Nanomaterials - What Regulators Want!

Adelle Strobel, Sarah Gewurtz, Hava Friedman and Marc LaPointe, Environment and Climate Change Canada

Nanotechnology is the study and manipulation of matter at the nanoscale (1 to 100 nm). Government of Canada regulators conduct risk assessments on nanomaterials under The Canadian Environmental Protection Act, 1999 (CEPA) to account for the unique characteristics exhibited by these substances. In regulatory risk assessment, good quality hazard data are needed for confident decision making. A challenge for regulators is evaluating the quality of data consistently between substance assessments. As such, there are many tools and documents identified to standardize ecological test methods, identify validity criteria, and to encourage reporting. To assess the regulatory adequacy of ecotoxicity data, we consult Organisation for Economic Co-operation and Development (OECD) test guidelines, Environment and Climate Change Canada (ECCC) Biological Test Methods, and the nanomaterial Criteria for Reporting and Evaluating Ecotoxicity Data (nanoCRED) framework. In addition, we have developed internal tools, such as the Nanotechnology Checklist Key (NECK), which allows Canadian regulators to adjudicate hazard data to be included in each assessment, by applying international criteria and relevance verification to this data. This presentation aims to support researchers by defining good quality data that can be used in Canadian regulators' risk assessment of nanomaterials. The NECK supports best practices for determining good quality data including study design, mitigation of wet lab challenges, and reporting through publication. The application of robust validation tools and methods provides confidence to assessment conclusions as set by section 64 of CEPA.

7.08.P-We-182 Evaluation of Relevancy, Reliability, and Quality of Toxicity Data in the ECOTOX Knowledgebase

Dale J. Hoff¹, Jennifer Olker¹, Brian Kinzinger², Anita Pomplun², John Frisch² and Anne Pilli², (1)U.S. Environmental Protection Agency, (2)General Dynamics Information Technology

The USEPA's ECOTOXicology Knowledgebase (ECOTOX, www.epa.gov/ecotox) has provided support for ecotoxicological assessments of chemicals for decades through the identification and curation of toxicity results. ECOTOX's compilation of ecotoxicity data uses well-established protocols (Olker et al. 2022) to systematically identify, screen and curate relevant toxicity data from scientific literature. Curated ECOTOX data are reviewed using relevancy, reliability, and acceptability criteria. These criteria include key data quality attributes such as confirming species identities, chemical names and synonyms; and documenting the endpoint of a biological response with a description of the magnitude and duration of exposure and inclusion of control treatments. In addition to these attributes, other data fields (e.g., chemical purity and verification of exposure concentrations, water quality parameters) are extracted into ECOTOX to provide information to answer commonly applied study evaluation questions and complement formal study quality or risk of bias evaluations that would otherwise require a review of the original publications. Data analysis, synthesis, and interpretation of toxicity results are application- and assessment-specific, and thus are conducted outside of ECOTOX. To aid in study evaluation, several options were recently added to ECOTOX to make it easier to access and export needed information for study evaluations in an effort to support the application- and assessment-specific study evaluation and analyses that are conducted external to ECOTOX. Additional formats for data exports have been completed to further support EPA's regulatory offices needs. These formats include JSON result records that can be imported into commonly used systematic review and data evaluation tools. Further, internal evaluation templates are being completed to take advantage of objective curated information (e.g., methodology parameters) from toxicological studies to be used in tandem with flexible subjective data attributes of quality for specific use applications. Recent chemical searches of PFAS chemicals will be used to illustrate these functions and recent additions to the ECOTOX Knowledgebase. *This abstract does not necessarily reflect US EPA policy.*

7.08.P-We-183 The Semi-Automated Study Quality Assessment and Reporting Evaluation (SQuARE) Tool for Assessing the Quality of Individual Studies and Extracted Datasets

Sara M Vliet¹, Jordan Vasko², Patricia Ceger³ and Kristan Markey¹, (1)U.S. Environmental Protection Agency, (2)Battelle Memorial Institute, (3)RTI International

The evaluation of study quality is one of the most time-intensive components of the systematic literature review process, as it involves evaluation of study methods and reporting against broad, variable reliability and relevance criteria by human experts. Current approaches rely on idiosyncratic judgment calls that are highly specific and generate quality metrics that can only be utilized for the specific problem being addressed. Simultaneously, ensuring that crucial study characteristics and reporting requirements are met is essential in determining whether data are suitable for risk assessment. Because study quality evaluation often relies upon data that have been previously extracted, the opportunity exists to assess study quality criteria programmatically when the curated data are properly integrated. A key challenge to successful automation of study quality evaluation is developing workflows to enable a bench-level domain expert to identify and express the data integration and evaluation components necessary in a computer-readable format without extensive interventions from a data scientist. To this end, we present the Study Quality Assessment and Reporting Evaluation (SQuARE) tool, a simplified, semi-automated framework to integrate extracted data and flexibly evaluate it against custom 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 study quality with only a manual quality check by a domain expert after the initial criteria specification. To demonstrate the utility and applicability of the SQuARE tool, case studies using extracted in vitro and in vivo data spanning species and chemicals are presented. Although applied to toxicological data in the present study, the SQuARE tool is

customizable across diverse scientific domains. Overall, the SQuARE tool presents a framework and method for assessing the quality of both individual studies and extracted datasets, and has the potential to significantly reduce time and labor across fields engaging in literature review and to leverage existing database information for study quality information. This abstract neither constitutes nor necessarily reflects U.S. EPA policy.

7.09.P-We The Role of Deliberate and Unintentional Misinformation in Science and Communication: What Does it Look Like and How to Guard Against it

7.09.P-We-185 Misinformation in Science and Communication on Decision Making: As Scientists, What Are Our Roles as Gatekeepers?

William L. Goodfellow¹, Timothy J Canfield² and Patrick D. Guiney³, (1)Exponent Inc. (2)U.S. Environmental Protection Agency, (3)University of Wisconsin

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 tested 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 effectively implement to address misinformation in science communication.

7.09.P-We-186 Perceptions of Bias: Does the Standard of Certainty Change When Politics Comes into Play?

Michael Dourson, Toxicology Excellence for Risk Assessment

Bias comes naturally to all of us based on education, culture, and habit and so perceptions of bias in others is a reasonable expectation in any human endeavor. Perhaps not unlike other occupations, scientists are trained to reduce the underlying bias in their own work and to look initially beyond the bias in the scientific work of others so that the truth of a given observation can speak for itself. The discussion sections of scientific publications are an opportunity for authors to synthesize the results from testing a hypothesis in a larger world view, but woe to the authors that do not also discuss strengths and weaknesses of their experimental design and contrasting hypotheses. Science is not divorced from politics, nor is any other human activity really. But in contrast to science, politics often looks for bias first, and observations second, in any stated position. For example, scientists nominated for senate-confirmed positions might get criticized as being funded by one sector

of society when such funding might come from multiple sources. Regardless, the value of the actual scientific work to society is seldom discussed, but of course not only should it be, but such science is the key to moving forward with insight.

7.09.P-We-187 Maintaining High Quality in the International Journal – Environmental Toxicology & Chemistry – in the Face of Deception, Misleading Impact Factors, and the Limited Resources of a Non-Profit System

G. Allen Burton Jr., University of Michigan

Like so many aspects of life, impactful scientific publishing has become extremely challenging; considering the present system of rewarding publishing in the highest Impact Factor journals, competition across hundreds of similar journals, and the growing use of deceptive practices (e.g., falsifying authors, reviewers, editorial boards, data and text). During the early years of ET&C there were few competing journals and ET&C was considered a/the leader in the field. Then ES&T began overlapping content with ET&C and commercial publishers developed stronger marketing strategies than SETAC's publishers. These realities, plus a growing importance of the flawed Impact Factor system embraced by many institutions has created a formidable challenge for ET&C. The strategy we have chosen is to focus on maintaining high quality publications in areas historically of importance to SETAC – environmental toxicology and chemistry. Past efforts to expand into newer fields have been unsuccessful, as ET&C's title and associated perceptions ran counter. Additionally, our strategy includes increased publications of symposiums and special series, using all SETAC publication outlets, open access, and additional assistance to authors, reviewers, and editors. Uninvited series and Critical Reviews are closely screened. Manuscripts are monitored closely for plagiarism, fake data, and valid references. Maintaining high quality standards are largely due to our excellent group of Editors and Editorial Board members. Obviously, SETAC's motto "Environmental Quality Through Science" is only possible using high quality science – which must always be the focus of ET&C.

7.09.P-We-188 Science Communication Mishaps: How They Occur, and Can They Be Fixed or Avoided?

Serena Ciparis, U.S. Fish and Wildlife Service

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 gone 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.

7.09.P-We-189 A Journey Through the Two Decades of Testing Hypotheses Based on Non-Reproducible Science

Anne LeHuray, Chemical Management Associates, LLC

In the first years of the 21st century the City of Austin (CoA), TX, thought they had identified a problem – polycyclic aromatic hydrocarbons (PAHs) in city watersheds – and also believed they had identified the cause of the problem – parking lots treated with refined coal tar-based pavement sealant (RTS). While the PAHs were not present at levels warranting special attention, the CoA sought assistance from state and federal agencies. Working with the city, hydrologists at US Geological Survey’s (USGS) Austin office conducted a study and generated the hypothesis published in 2005 that RTS “may dominate loading of PAHs to urban water bodies in the United States.” Since that time there has been a steady stream of publications across many environmental science disciplines focused on this issue. For nearly 2 decades multiple approaches and forensic methods have been used to test the hypothesis that application of RTS is a significant source of PAHs in US urban sediments as well as hypotheses based on evaluations of air, water, ecosystems, and human health risk. A multi-year study of PAHs in CoA waterways was conducted in attempt to verify conclusions relied on by the CoA. Subject area experts have been commissioned to review studies and, if possible, reproduce visual and/or statistical results as well as model results. Commonly used computer input data have been subjected to validation. Public records requests have been filed and, when necessary, litigated to retrieve data relevant to understanding government-conducted studies and reports. Available data involving PAHs in environmental media in the US and elsewhere have been compiled and evaluated for relevant information. Results of evaluations have been published in peer-reviewed journals or made available online, as appropriate. The initial and some subsequent publications in what has become a substantial body of literature on the topic of RTS as a source of PAHs appears to have reached predetermined conclusions based on deliberate inclusion and exclusion of data. Published comments and peer reviewed publications reaching opposite conclusions are routinely overlooked. Impacts of less than rigorous peer reviews, selective citation, irreproducibility, confirmation bias, source-of-funding bias, and other systemic flaws will be demonstrated.

7.09.P-We-192 Environmental Justice and Increased Environmental Equity Scrutiny: A Case Study in Risk Communication and Misinformation

Dana McCue and Jordan Stark, EHS Support, LLC

An independent, non-profit newsroom that produces investigative journalism in the public interest published an on-line map in November 2021 titled “The Most Detailed Map of Cancer-Causing Industrial Air Pollution in the U.S.” Using publicly available air emissions data compiled by the United States Environmental Protection Agency (USEPA), the searchable online map identified more than 1,000 “hot spots of cancer-causing air”. The purpose of the map was to offer a tool for the public to understand the risks of breathing the air where they live. The mapping tool and companion articles with eye-catching headlines were picked up by other news organizations and local community advocacy groups on social media, which resulted in increased activity and focus on air pollution at the local, state and federal levels. One local news article was published with the title “*A knee on the neck may not kill people in South Memphis. But toxins in the air might*”. This case study provides a deep dive into this mapping tool. The purpose of this review was to identify key assumptions incorporated into the development of the mapping tool and any flaws, if any, in those assumptions. The scope of the analysis included reviewing the air modelling methodology and the modeled air concentration inputs, and confirming the cancer risk estimates and toxicity assumptions. The findings of the review indicated that the reporting/mapping is misleading to the community as it depicts an incomplete picture of air toxics, used USEPA’s model data inappropriately, and assumed overly conservative toxicity assumptions that overestimated potential risk.

7.10.P-Mo General: Policy, Management and Communication

7.10.P-Mo-202 Comparison of Aquatic Life Protective Values Developed for Pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Clean Water Act (CWA)

Christine Bergeron, Kathryn Gallagher, Douglas (Ethan) Harwood, Jean Holmes and Thomas M. Steeger, U.S. Environmental Protection Agency

The U.S. Environmental Protection Agency (EPA) has been working for several years to harmonize aquatic effects assessment methods for pesticides to provide a common basis for achieving water quality protection under the Clean Water Act (CWA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The objective of this collaborative effort within EPA is to ensure development of protective aquatic life values using current science while minimizing duplicative work within the agency, thereby promoting consistency in aquatic effects assessments for pesticides. EPA's evaluation and draft analyses support leveraging Office of Pesticide Programs' (OPP) Aquatic Life Benchmarks (ALB) developed under FIFRA for application as CWA section 304(a) National Recommended Ambient Water Quality Criteria for the protection of aquatic life ("Aquatic Life Criteria" or ALC) for pesticides. The comparative analyses for select insecticides and herbicides from different chemical classes and with different modes of action investigated several methods to compare ALB to values calculated based on ALC methods or other ALC-related approaches. The analysis demonstrated that the most sensitive ALB values fall within a factor of two for 304(a) ALC, and within a five to ten-fold range for other ALC-related approaches used when available toxicity data were limited and insufficient to develop an ALC. This range in values is approximately the same as the inherent variability observed in toxicity tests conducted within the same laboratory and across laboratories. These analyses demonstrate that EPA's process used to develop ALB values under FIFRA generates protective values for registered pesticides that are appropriate for use as national recommended ALC magnitudes for pesticides with CWA standard frequency and duration components for criteria. A decision to incorporate OPP ALB values as pesticide ALC for CWA purposes would provide states and tribes with criteria they can consider in their water quality standards to manage potential effects of most registered pesticides on aquatic life.

7.10.P-Mo-203 Evaluating Impacts to the U.S. Department of Defense (DoD) Mission from Changing Regulations and Toxicity Values for Vanadium and Cobalt

Emma Williams, EIT¹, Jessica Lambert¹, Emily Barrett¹, Catherine Vogel¹, Kelsey Hendrixson¹, Margaret Graham¹ and Natalia Vinas², (1)Noblis, Inc., (2)Office of the Assistant Secretary of Defense (EI&E)

The U.S. Department of Defense (DoD) Chemical and Material Risk Management Program (CMRMP) uses a three-tiered process for proactive identification, assessment, and management of emerging chemicals. As part of this process, the CMRMP identified vanadium and cobalt as emerging chemicals and conducted assessments to evaluate their impacts to critical DoD functions and to develop risk management options. These assessments qualitatively assessed risks to DoD and the defense industrial base (DIB) from potential increased regulation of cobalt and vanadium under the European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals regulation and changing toxicity values under the U.S. Environmental Protection Agency's Integrated Risk Information System Program. The Defense Logistics Agency lists both metals as strategic and critical materials due to their vital contribution to our national defense and economic well-being. These assessments include identifying the regulatory and toxicity drivers, gathering chemical usage data, assessing potential impacts to DoD, and recommending risk management options. The primary challenge in conducting these assessments involved collecting chemical and material (C/M) usage and application information for products and materials used by DoD and DIB. To address this challenge, search terms like chemical names and Chemical Abstracts Service Registry Numbers were used to query DoD data systems. These terms included eight vanadium compounds and 20 cobalt compounds. The

assessments identified C/M data gaps; however, this was expected due to DoD's data systems' reliance on safety data sheets. The CMRMP engaged with subject matter experts to better fill in and understand these gaps. Cobalt and vanadium are used in numerous commercial, industrial, and military applications. In the U.S., the primary use of vanadium and cobalt is alloy production, however, their use in batteries is growing rapidly due to clean energy initiatives. DoD's demand for cobalt and vanadium also includes secondary uses such as welding supplies, metal finishes, lubricants, paints, and coatings. This briefing will present results from the assessments including potential impacts and recommendations for risk mitigation. The relevance of this assessment extends beyond cobalt and vanadium - the tools used in these assessments have the potential to improve DoD's ability to collect C/M content and provide better visibility into the DIB supply chain.

7.10.P-Mo-204 Integrated Approach for Testing and Assessment (IATA) for Bioaccumulation

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Bioaccumulation is the net result of competing rates of chemical uptake into, and elimination from an organism. Bioaccumulation assessment is an essential endpoint in national and international chemical regulatory programmes and treaties, e.g., the identification of Persistent, Bioaccumulative and Toxic (PBT) substances and Persistent Organic Pollutants (POPs). Bioaccumulation assessment is both a scientific and regulatory challenge due to the intricacy of the subject, the availability of reliable and relevant data, and lack of guidance in how data should be evaluated and weighted to inform decision-making. This process becomes more challenging considering variations in accepted data and the varying threshold criteria of different regulatory jurisdictions. A weight of evidence (WoE) approach is recommended by most established regulatory jurisdictions, such as REACH program in Europe, The Canadian Environmental Protection Act and the United Nations Stockholm Convention; however, there is no clear guidance for implementing a WoE approach for B assessment. Multiple lines of evidence (LoE) can be used to quantify, measure, and qualify the common metrics of bioconcentration factor (BCF), bioaccumulation factors (BAF), biomagnification factors (BMF) and trophic magnification factors (TMF). These LoE can comprise *in silico* predictions, combinations of *in vitro* and *in silico* data, e.g., *in vitro-in vivo* extrapolation (IVIVE), and laboratory or field data. Quantitative Structure Activity Relationship (QSAR) models for BCF and BAF, and biotransformation half-lives, as well as mechanistic bioaccumulation toxicokinetic (TK) models are established. Additionally, there are internationally recognised OECD test guidelines for the laboratory determination of *in vitro* fish biotransformation rates, BCFs, and BMFs. An Integrated Approach for Testing and Assessment (IATA) for Bioaccumulation has been produced that encompasses the aforementioned LoE along with existing OECD testing guidance and WoE guiding principles to aid evaluators in the collection, generation, evaluation, and integration of multiple LoE for clear and transparent decision making in B assessment for aquatic and terrestrial environments. Case studies are presented to illustrate the applicability of the new IATA for B assessment.

7.10.P-Mo-205 Surface Water Quality Criteria for PFAS: Variation in International Approaches and Risk Management Challenges

Betsy Ruffle¹, **Kelly Vosnakis**¹, **Christine Archer**¹, **Belinda Goldsworthy**¹, **Josh Butler**², **Craig Warren Davis**² and **Trent A. Key**², (1)AECOM, (2)ExxonMobil Biomedical Sciences, Inc.,

Regulation of PFAS in surface water is a work-in-progress with relatively few promulgated criteria. Data gaps and uncertainty in PFAS toxicity, exposure, and environmental fate, as well as the continuous publication of new studies and scientific updates, pose significant challenges to agencies striving to establish policy and regulatory limits. Within the United States (US), the Environmental Protection Agency (USEPA) has not yet set national recommended ambient water quality criteria (NRWQC) for any PFAS with the exception of draft recommended aquatic life criteria released in April 2022 for PFOS and PFOA. In the absence of national

criteria, some US states have derived their own or are in the process of doing so. Outside of the US, the European Union (EU), and some countries (e.g., Australia, Canada) have developed standards and/or guidelines for management of PFAS in surface water. The published surface water quality criteria (SWQC) for protection of human and ecological receptors vary more than five orders of magnitude. The broad range reflects differences in the target population, protection goal, assumptions about toxicity and exposure, derivation methodology, as well as policy. In some cases, the numeric criteria are below ambient levels and analytical detection limits. The presentation will discuss factors driving the range of SWQC including key assumptions and approaches, as well as areas of uncertainty. Considerations for future PFAS SWQC development and risk management challenges will be discussed.

7.12.P-Tu Late Breaking Science: Policy, Management and Communication

7.12.P-Tu-223 Strategies for Consumer Product Biocide Companies to Uphold the Endangered Species Act

Ying-Fei Yang, Kendall Torres and Wendy E. Hillwalker, SC Johnson

Conservation of endangered and threatened species and their ecosystems is a critical issue across the globe. The United States Endangered Species Act (ESA) of 1973 provides a framework to protect fish, wildlife, insects and plants that are listed as threatened or endangered. As of 2022, a newly established ESA-Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) framework, including a multi-step risk assessment program focused on ESA protection goals, was implemented across US agencies – U.S. Environmental Protection Agency (USEPA), U.S. Fish and Wildlife Service (US FWS), National Marine Fisheries (NMF). This presentation provides a systematic approach to support registrants of consumer product biocide companies to uphold the ESA-FIFRA framework. The approach includes an evaluation of the analysis and decisions along the multi-step program within all available Biological Evaluations (BEs) and Biological Opinions (BiOps), with a focus on relevant consumer product usages: down-the-drain (DtD) &/or outdoor residential. Definitions, uncertainties, and recommendations for harmonization across biocide regulation programs, such as ESA and FIFRA, will be summarized. The proposed strategies enable consumer product biocide companies to proactively address registrant responsibilities while fostering environmental stewardship and the protection of species and their habitats.

7.12.P-Tu-224 Assessment of The Public's Role in Reducing Synthetic Microfiber Emissions from Clothing and Laundry to Inform Decision Makers, Industry Sustainability Strategies, and Government Policies

Soma Barsen, Yale University

Synthetic microfibers, shed during the wear and laundering of petrochemical fiber-based (synthetic) textiles and clothing, have garnered significant attention from media, scientists, policymakers, and industry stakeholders. Synthetic textiles represent an important source of land-based microplastics emissions. Worldwide, synthetic microfibers have been identified in nearly every environment and habitat investigated to date. There is mounting evidence suggesting that synthetic microfibers could have adverse effects on animal lifecycles and may pose a risk to human water and food sources. Previous research has assessed the occurrence and prevalence of synthetic microfibers in the environment, estimated the rate of microfiber emissions from household sources like washing machines, and delved into potential environmental consequences. Various experts and stakeholders, public and private, have put forward recommendations to address synthetic microfiber emissions from clothing and the general environmental impacts of the public's demand for new textile products. Interventions such as a microfiber capture filter technology to capture microfibers from washing machine greywater have been touted as an important stopgap to address ongoing emissions. Despite these efforts, there has been little action to implement such technological and policy interventions. The lack of action can be attributed to an important knowledge gap, specifically concerning the public as a key stakeholder group. Their

behaviors, attitudes, and preferences for interventions can significantly influence the success or failure of such initiatives. The current study uses a rigorous representative sample survey method to address that knowledge gap by investigating the public's behaviors, attitudes, intervention preferences, and the role of science communication in two large coastal urban centres, Metro Vancouver, British Columbia, Canada, and Los Angeles, California, USA. The solutions-focused study explores the drivers of the problem and the practicability, feasibility, and cost-effectiveness of interventions and policies to address synthetic microfiber emissions from households. The findings provide foundational insights into the views of the public on synthetic microfibers and microplastic pollution, social norms and behaviors that contribute to emissions, and potential responses to regulatory and policy initiatives.

7.12.P-Tu-225 Molybdenum Aquatic Life Water Quality Standards Across Global Regions and Regulatory Implications of Potentially Outdated Standards

John Gondek¹, Shaun A. Roark¹ and William J. Adams², (1)Jacobs Engineering Group, Inc., (2)Red Cap Consulting

Molybdenum (Mo) aquatic life standards can vary significantly based on the species-sensitivity distribution (SSD) used. Evaluations under the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program identified a set of reliable data for calculating chronic probable no-effect concentration (PNECs) for freshwater and marine environments. These chronic PNECs are greater than some acute Mo standards used by some states in the US and other jurisdictions, suggesting a re-evaluation of the data used to derive less recent Mo standards may be warranted. Here, we present an inventory of the current Mo aquatic life criteria regulatory landscape in the US, EU, Canada, and Australia. The focus of this review includes a comparison of the Mo SSDs and datasets used by these regions and identification of states/regions where existing standards may not reflect the latest science. These findings can be used to prioritize areas where updates to aquatic life standards are needed and to inform lake and stream impairment and permitting decision-making.

7.12.P-Tu-226 From Legislators to Scientists. A Soil Environmental Impact Made by Laws *Rosalina Gonzalez, Universidad de la Salle, Colombia*

In Colombia, on July 6th 2021, the environmental regulation number 0699 by The Ministry of Environment and Sustainable Development came in action. This regulation permits discharges of treated domestic wastewater to the ground, establishes the chemical parameters of wastewater and determines the maximum permissible limit values of some chemicals. The Ministry indicates that this law is a preventive measure to protect the soil and permits discharges of treated domestic wastewater to the soil. This is applied to rural housing associated to productive activities that generates domestic wastewater. This environmental law regulates the physicochemical parameters as: temperature, pH, Chemical Oxygen Demand, Biochemistry Oxygen Demand, Total Suspended Solids, Total Settling Solids, Fats and Oils, Detergents, Phosphorus, Nitrogen and Chlorine at different infiltration rates in soils. However, on June 4th 2021, the FAO/UN released the Global Assessment of Soil Pollution Report. This document indicates that one of the main drivers of soil pollution in Latin America and The Caribbean is the urban settings transport and wastewater disposal without an adequate system treatment. Where many chemicals are into the water that not are measured. In fact, the pandemic situation forced people to use aggressive chemicals as disinfectants such as sodium hypochlorite, ethanol, quaternary ammonium salts, and large amounts of non-biodegradable soaps. This is a worried situation, because the new regulation is not mentioning nothing about that. In addition, the regulation does not specify physicochemical characteristics of the soil, such as organic matter content, nutrients and minerals, which are key parameters that the soil provides to the ecosystem services. The project carried out presents study cases under different conditions caused by the impact to the soil assuming the compliance of the new environmental regulation. And the way to determine the effect of the soil when the discharges of this kind of wastes are on it.

7.12.P-Tu-227 Persistence and Organic-Carbon Partitioning Do Not Correlate to the Detection of Substances Found in Surface and Groundwater: Criticism of the Regulatory Concept of Persistent and Mobile Substances

Marie Collard¹, Louise Camenzuli², Delina Lyon³, David Saunders⁴, Nathalie Vallotton⁵, Pippa Kate Curtis-Jackson⁶ and Jing Hu⁵, (1)DSM-Firmenich, (2) ExxonMobil Biomedical Sciences, Inc., (3)Concawe, (4)Shell Global Solutions, (5)Dow Chemical Company, (6)Environment Agency (England)

The European Commission has taken action at the UN Global Harmonization System level to include new PMT/vPvM hazard classes (Persistent, Mobile and Toxic / very Persistent and very Mobile) to identify those chemicals that have an intrinsic propensity to readily reach potential sources of drinking water. EC builds its proposal on the so-called PM concept according to which Persistence (criteria of EU-REACH Annex XIII) and Mobility ($\log K_{oc} < 3$) would be proxies for a substance's degradation potential and transport velocity, two processes believed to drive the potential for contamination. Two studies (Arp et al., 2017; Schulze et al., 2018) have identified hundreds of substances with these PM properties via throughput QSARs while three subsequent studies have selected some sets of these substances for monitoring in surface and groundwater to support the concept (Montes et al., 2019; Schulze et al., 2019, 2020). In the present work, the Persistence of the aforementioned substances was reassessed based on all experimental data available. It was found that a substantial portion of the substances (15-35%) were erroneously concluded as P. The reinterpretation of the data indicates that a PM substance does not have a higher likelihood to be detected in surface or groundwater than a non-PM substance. In addition, the PM properties do not have any influence on the level of contamination. Besides, 20-70% of the substances selected because they were identified as PM were not found in waters despite being selected for their high emission pattern. As it stands, the regulatory proposal to include PM substances into UN-GHS is unlikely to appropriately identify substances of concern for drinking water sources. It is more likely that chemical presence in surface and groundwater is driven by emission patterns. The development of specific exposure models would better contribute to the protection of water resources and consumers. Arp et al., 2017. *Environmental Science: Processes and Impacts* 19, 939-955. Montes et al., 2019. *Analytical Chemistry* 91, 5176-5183. Schulze et al., 2018. *Science of The Total Environment* 625, 1122-1128. Schulze et al., 2019. *Water Research* 153, 80-90. Schulze et al., 2020. *Analytical and Bioanalytical Chemistry* 412, 4941-4952.

Track 8: Systems Approaches

8.01.T The Bourbon Effect: Chemistry, Sustainability and Life Cycle Analysis of Kentucky Bourbon and Other Spirits

8.01.T-01 Three Cheers: Chemical Case Studies at Buffalo Trace Distillery

John Medley, Buffalo Trace Distillery

This presentation will touch on three unique applications of chemical analysis in Sazerac's Center for Innovation and Technical Excellence located at Buffalo Trace Distillery. Routine proof testing relies on accurate measurements of density; the assumptions underlying this model do not necessarily apply to non-binary ethanol-water systems, so alternate techniques are required. Forensic investigation of unanticipated phenomena in bourbon, using analytical techniques such as FTIR, can be used to improve processes, both internally and at suppliers. Finally, in our continual effort to expand our understanding of how process variables impact the chemistry and sensory attributes of bourbon, preliminary learnings from our collaborative project to apply explainable artificial intelligence to the Single Oak Project will be discussed.

8.01.T-02 Spirit in Barrel Maturation - The "Maturome": An OMICS Approach Towards the Holistic Understanding of a Complex Engine

Gary Spedding, Brewing and Distilling Analytical Services

The maturation of white-dog, or raw white, spirit in wood is an incredibly complex system that can be likened to a biological organism. Thus, seeking parallels with "Bio-OMICS" - metabolome and metabolomics, a global approach towards an appreciation of the overall maturation process will require building the relational model from "big-data". A complete understanding of thermodynamics, chemical kinetics, chemical flux, involving thousands of reactions will take time (if ever) to fathom. However, it is time to start looking at diverse experimental approaches and linking an understanding of wood, ethanol-water-congener (solvent) and environmental aspects of the system. A brief outline of processes involved in maturation from the perspective of a current understanding of the maturation engine will be presented. A re-introduction to classic early experiments, both the knowledge gained and the limitations of such will be noted. Finally, hints as to how OMICS and big'-data manipulation can assist here in generating a more complete model of the process will be provided.

8.01.T-03 Studying the DNA of Whiskey Through Colloids and Interfacial Monolayers

Stuart Williams, University of Louisville

Bourbon whiskey itself is a chemically complex liquid containing thousands of constituents that define its flavor profile, yet these aromatic compounds make up less than 1% (by weight) of whiskey's composition. A significant portion of these compounds are water insoluble and micelles will form when whiskey is diluted. The presence of these colloids is well-known in the spirits industry and they are monitored for product quality control, including assessing "chill haze". Recently, we have used dynamic light scattering as an alternative to study chill haze. In addition, these colloids will form a chemical monolayer at the liquid-air interface during evaporation of a droplet. We observed this phenomenon when these monolayers collapsed and created a vibrant pattern of self-assembled microstructures we have termed "whiskey webs". Assembly is a function of the inherent properties of the whiskey as well as the extrinsic evaporation characteristics. Initially, we demonstrated that these structures form only for American whiskey (i.e., whiskey matured in new charred barrels), but a recent modification in our approach has enabled the study of webs created from other spirits.

8.01.T-04 Sustainability, Climate Change and Bourbon

Seth DeBolt, University of Kentucky

The James B Beam Institute for Kentucky Spirits at the University of Kentucky is the bourbon industry's research and development vehicle. On campus, the Beam Institute comprises teaching distillery and barrel warehouse, which represent a first of its kind enabling space for education and innovation. The Beam Institute is taking strides to develop Kentucky's workforce with numerous offerings to meet students where they are in their higher education journey. Competencies are transdisciplinary and focused by our industry partners. Current offerings include Apprenticeship, Certificate (engineering, chemistry, policy, business and economics, agriculture and food science), DISCUS leadership academy, short courses and webinars. Due to the Land Grant mission of the University, we align with grain farmers, foresters and processors to identify and meet challenges facing the industry. We have determined the genome of the white oak in partnership with Makers Mark and Independent Stave, supporting white oak sustainability while contributing valuable insight into the role and sourcing of white oak in the bourbon industry and beyond.

8.01.T-05 Enhancing Bourbon Wastewater Quality by Applying Stillage to Wetland Treatment Systems

Katherine J. Ristola and Tiffany Messer, University of Kentucky

Stillage is an unavoidable byproduct of bourbon and is becoming an increasingly difficult byproduct to dispose of due to the increase in bourbon manufacturing in recent years. A popular disposal method of stillage is by selling it as a nutrient rich supplement to cattle farmers. However, there is more supply than demand. Therefore,

the stillage is often treated as wastewater and is attempted to be broken down through multiple processes such as biological (aerobic and anaerobic), physic-chemical (absorption and oxidation), and/or thermal (combustion). Stillage poses an environmental problem because it is high in nutrients such as nitrogen and phosphorous and has high chemical and biochemical oxygen demand (COD and BOD). Due to the difficulty of processing stillage, it may still contaminate local streams and lead to potentially hazardous conditions (i.e., toxic algal blooms) to humans and the environment. Wetland treatment systems have the potential to be a solution as secondary or tertiary wastewater treatment processes. Wetlands are known for creating an environment that can handle fluctuations of effluent concentrations and volume, reduce nutrients, and require low maintenance once established. Therefore, the goal of this project is to determine if wetlands are potential treatment alternatives for stillage through constructed wetland wastewater treatment processes. We hypothesize wetland treatment systems will reduce nutrients found within stillage to allow for a more thorough breakdown of compounds before entering a stream. This study will be accomplished using experimental mesocosms that will contain 30 cm of soil, 3 different types of native wetland plants (i.e., soft stem bulrush, pickler weed, and native cattail), and stillage from 1 distillery located in Kentucky. The mesocosm experiments will be completed following stillage enrichments. Wetland surface water samples will be collected on days 0, 1, 2, 3, 5, 7, and 10 following stillage enrichments and be analyzed for N, P, and C species (i.e., nitrate, ammonium-nitrate, total nitrogen, total phosphorus, total carbon, total Kjeldahl nitrogen, phosphate, and dissolved organic carbon) throughout summer 2023. Further, water pH, dissolved oxygen, ORP, and conductivity will be monitored throughout the study to evaluate if conditions for denitrification are present. Potential findings from this project could provide an alternative and/or additive way to assist in distillery wastewater treatment processes.

8.01.T-06 A Sustainable and Profitable Approach for Handling Stillage from Distilleries

Jagannadh Satyavolu, University of Louisville

For every liter of alcohol produced in a distillery, about 15 liters of spent water (stillage) is released. Distillery wastewater is one of the most polluted waste products to dispose because of the low pH, high temperature, and high percentage of dissolved organic and inorganic matter. Its characteristics depend on the grains used during the ethanol production process. A limited number of large distilleries use drying and evaporation to dry the solids and concentrate liquor respectively - this is a high capital, expensive, and large carbon footprint operation. A majority of the distilleries get rid of the stillage by giving it away to farmers. Using technologies licensed from University of Louisville, BioProducts, LLC, Louisville, KY is providing a sustainable and profitable approach to treat the stillage. The integrated approach produces a low-calorie sugar (xylose), high surface area activated carbon, animal feed, and biogas - thus, turning the stillage in to a profitable stream for the distillers.

8.02.P-Tu Advancements in Life Cycle Assessment (LCA)

8.02.P-Tu-219 Safe and Rapid Development of Miniaturized Sensors Through Life Cycle Analysis, Hazard Assessment, and Environmental Resilience Approach: A Public-Private Effort

Jonna Boyda¹, Alan Kennedy¹, Wu-Sheng Shih², Rishi Patel³, Cynthia Price¹, Lauren Rabalais May¹ and Mark Ballentine¹, (1)U.S. Army Engineer Research and Development Center, (2)Brewer Science Inc., (3)Missouri State University

Nano-enabled technologies (NET) have wide ranging applications in electronics, medicine, and water and air treatments. While NET have been extensively researched, a streamlined process to understanding their performance and potential environmental risks remains elusive. A seven-year collaborative effort between the Engineer Research and Development Center (ERDC, Vicksburg, MS), Brewer Science Inc. (BSI, Rolla, MO), and Missouri State University (MSU, Springfield, MO) formed to investigate the impacts and determine low-environmental-impact production methods, specifically for low-cost, environmentally durable, and miniaturized nano-enabled sensors. This effort was split into four components: (1) nano-enabled sensor development; (2)

Life Cycle Analysis (LCA) across the life cycle of the sensor production to identify releases and expenditures and guide risk-based evaluations; (3) an analysis of NET properties, including potential releases, fate and transport, and toxicity; and (4) communication and dissemination of research findings as evidence to help inform future protocols, guidelines, and regulation decisions related to NET products and services. As this seven-year effort culminates, this presentation intends to highlight successful outcomes, including published standard guidance with the Organisation of Economic Cooperation and Development (OECD) and the International Organization for Standardization (ISO). Additionally, electronic executable tools (e.g. NanoGRID, AdMGRID, NANO INSURANCE) have been created for testing materials and understanding regulatory compliance and insurance acquisition. It is our goal to provide information that others can use going forward, including current technologies, environmental impact findings, and data gaps.

8.02.P-Tu-220 Identification of Phages in Wastewater Samples in Abu Dhabi, UAE

Thyago Hermilly Santana Cardoso¹, Vinay Kusuma¹, Kenneth Cuares¹, Walaa Mohammed², Shahin Sheik¹, Shalini Behl¹, Madhu Subramani¹, Nitin K Saksena³, Javier Quilez¹, Patrick Merel¹, Wael Elamin¹ and Albarah El-khani¹, (1)G42 Healthcare, (2)Oxford Nanopore Technology, (3)Aegros

Phages, also known as bacteriophages, have played a critical role in maintaining the balance of Earth's biosphere for centuries. They are viruses that infect bacteria and have been shown to be effective in controlling the growth of harmful bacteria, preventing bacterial overgrowth, and promoting a healthy microbial environment. Despite a wealth of evidence supporting the benefits of phage therapy, the discovery of antibiotics has led to a decline in the use of phages. However, with the current crisis of antibiotic resistance, the world is facing a shortage of effective antibiotics to fight infections. This has led to a resurgence of interest in phages as a potential solution to this problem. The aim of our study was to collect wastewater samples from different locations and perform metagenomic shotgun DNA sequencing to identify and classify phages present in the Emirate of Abu Dhabi using samples from households, industrial areas, and suburbs. We are also investigating possible correlations between phages and their hosts in the samples. The results of this study may provide valuable information for the development of phage-based therapies to treat bacterial infections and improve food safety in the region.

8.02.P-Tu-222 Challenges of Integrating Environmental Risk with Life Cycle Analysis

Lawrence Kapustka, LK Consultancy

Life Cycle Analysis considers activities along the path of discovery, extraction, transportation, use, and disposal -- the so-called cradle-to-grave accounting of effects. Often these activities occur across multiple continents, in diverse ecoregions, and over varied timeframes. Environmental stressors span the gamut of physical alterations of landscapes; release, or activation of toxic materials; and these may impinge upon biotic communities. The focus of the accounting tends to embrace hazard ranking. Environmental Risk Assessments generally require considering of place-based exposures that may occur in terrestrial, freshwater, or marine environments. The ERA considers valued ecological receptors or human health endpoints within these varied types, with the intent of detailing the probability of specific adverse effects to these receptors. Although some jurisdictions consider risk quotients to be risk assessments, they are merely screening tools. Most importantly, quotients are not scalable in terms of effects. Nor can they validly be added. The LCA can tally common metrics such as energy consumption, carbon footprint, water demand, or other material usages to provide an integrated score to be used to compare alternative materials. Cumulative effects on ecological receptors, however, is far from being straightforward as they may be primarily due to habitat alteration in one region, related to toxic materials in another (and with different receptors), and each within different social-ecological systems. This talk will focus on the different approaches and different goals of these two frameworks and point toward the need for expanded stakeholder engagement along product pathways.

8.03.P-We Assessing Environmental Impacts Along Mineral Supply Chains

8.03.P-We-194 The Incorporation of Ecological Risk Assessment into the Life Cycle Assessment and Adaptive Management of Metals and other Materials

Wayne G. Landis, Western Washington University

Ecological risk assessment is the fundamental method in which the calculate the probability of effects due to metals or other materials introduced to the environment. The pathways that can lead to the introduction of materials with those probabilities of loss are part of the life cycle assessment. Both tools are applied to manage integrated cause-effect pathways to arise at socially/culturally acceptable goals. Both tools need to be integrated to assess the chain of events that result in either a regulated or accidental release. Adaptive management is the tool by which both kinds of releases can be controlled to either prevent effects due to normal operations and especially in catastrophic releases. This presentation will illustrate how these three tools can be integrated with probabilistic equations that reflect causality or generate hypothesis on the probability of categories of outcomes. The applicability of Bayesian networks and other related tools will be tested and evaluated.

8.03.P-We-195 Meeting the Mineral Demands of Clean Energy Technologies: Maximizing Net Environmental Benefits While Ensuring Equitable Outcomes Across the Life Cycle of Mineral Extraction, Distribution and Use

John Toll, Windward Environmental LLC

One big difference between 21st and 20th century technology development is the level of attention being given to the liability side of the ledger. The clean energy transition is a case in point. Awareness of the environmental impacts of mineral extraction necessary to support large-scale adoption of cleaner energy technologies is having real effects on everything from commodity pricing, investment strategies and purchasing decisions; to environmental regulation policies and practices; to proactive material, infrastructure, and social welfare stewardship. Heightened awareness is a step in the right direction, but it won't be effective unless we couple it with 21st century assessment tools that are up to the challenge of guiding people to translate their awareness into meaningful actions and decisions that promote sustainable health, wealth, and happiness among all societies, wherever and however much they intersect with points along the commodity-to-clean energy supply chain. In this presentation, an environmental risk assessor will draw on his 35 years of professional experience to tackle difficult questions about how the professional community should deploy its assessment tools to ensure that the clean energy transition brings increased prosperity and environmental quality to communities around the world. This is a once-in-a-generation opportunity for environmental assessment professionals to rethink and transform our practices in ways that could profoundly affect the state of the world we leave to our grandchildren. This presentation will be a call to action to bring out the best our profession has to offer to society.

8.04.T User Showcase: How do the U.S. EPA's New Approach Methodologies (NAMs) Training Pilot Program and NAMs Tools Advance Research and Decision Making?

8.04.T-01 What is 'U.S. EPA's NAMs Training Pilot Program'?

Esra Mutlu, U.S. Environmental Protection Agency

The U.S. Environmental Protection Agency (EPA) has a long history of using New Approach Methodologies (NAMs) for hazard identification and modeling for exposure assessment. The agency continues to implement NAMs for new and existing chemicals to evaluate hazard, exposure, and environmental fate. A 'NAMs Work Plan' was created to prioritize agency efforts and resources to reduce the use of vertebrate animal testing. The plan includes efforts and resources toward the development and application of NAMs while continuing to protect human health and the environment. One of the primary 'work plan' deliverables is to develop NAMs

training courses and materials for a broad range of stakeholders. The EPA NAMs Training Pilot Program was developed to provide regular engagement opportunities for NAMs tool users and make NAMs resources and trainings more accessible and engaging. This presentation will provide an overview of NAMs Training Pilot Program; summary of post training survey result from NAMs trainings and information on a suite of widely used tools that contribute to NAMs which are developed in collaboration with various stakeholders. The presentation aims to highlight efforts to reach a broader audience by showcasing several NAMs tools and how they can be used while increasing the awareness of other various EPA resources that are freely available to public.

8.04.T-02 Using EPA Environmental Exposure and Ecotoxicity Estimation Tools to Inform Decisions Regarding Vertebrate Animal Testing and Potential Ecological Risk

Paul C. DeLeo¹ and Elke Jensen², (1)American Chemistry Council, (2)Dow Chemical Company

Reform of the Toxic Substance Control Act (TSCA) in the United States in 2016 expanded the scope and scientific standard for the assessment of the risk of injury to human health or the environment from chemicals in commerce by the U.S. Environmental Protection Agency (EPA). Consequently, EPA is tasked with evaluating ecological risks to organisms that may be exposed to chemicals in environmental media beyond the typical freshwater column, such as sediment-dwelling and terrestrial organisms, but for which ecotoxicity data are not typically available. Therefore, alternative approaches are needed to estimate ecotoxicity and potential environmental exposure of these organisms. We used a variety of NAM tools to estimate potential exposures and ecotoxicity of avian species to several high priority chemicals. For ecotoxicity data, we started with EPA's Analog Identification Methodology (AIM) model and the Generalized Read-Across (GenRA) approach within the EPA CompTox Chemicals Dashboard as tools for identifying chemical analogs for which existing toxicity data might be available. From there, we mined EPA's ECOTOX Knowledgebase as a source of avian ecotoxicity data for the subject chemicals and the analogues identified. In addition, we used EPA's Interspecies Correlation Estimation model (Web-ICE) for estimation of avian ecotoxicity using mammalian ecotoxicity data. To understand the likelihood that a chemical would occur in the environment and to estimate environmental concentrations and potential avian exposure, we used fugacity and multimedia fate models. The models used include the Level III fugacity model in EPA's Estimation Programs Interface (EPI) Suite and the PROTEX-HT model which evolved from that fugacity model. A tiered decision framework was developed using a variety of NAMs including EPA tools and externally developed models to address avian ecotoxicity data. It uses lessons learned from EPA Office of Pesticide Programs guidance documents to consider information regarding exposure potential and relative ecotoxicity. The results of these exercises were input into a tiered decision framework to inform whether additional *in vivo* testing would enhance the ecological risk assessment, or whether those estimates were appropriate for weight-of-evidence risk assessment.

8.04.T-03 Integrated Research to Advance New Approach Methods for Environmental Health Protection

Katerine Sali, Heidi Bethel, Kathie Dionisio and Annette Guiseppi-Elie, U.S. Environmental Protection Agency

The development of innovative technologies and approaches is required to assess the thousands of previously untested chemicals for environmental or human health hazards. A central goal of the U.S. Environmental Protection Agency's (EPA's) Chemical Safety for Sustainability (CSS) National Research Program is the advancement of new approach methods (NAMs) to rapidly collect chemical toxicity data while reducing, refining, and replacing vertebrate animal testing. The CSS research program comprises three topics, or areas of research focus: chemical evaluation, complex systems science, and knowledge delivery and translation. Within these topics are eight research areas assembled to address partner needs. These research areas are connected by interdependent research activities that accomplish one or more aims: advancing innovative science, data generation and informatics, and application for decision making that is protective of human and environmental health. Key components of the EPA NAMs workplan include developing NAMs to fill information gaps,

establishing scientific confidence in NAMs, and demonstrating application to regulatory decisions. This presentation provides a high-level overview of how NAMs research is integrated across the CSS research portfolio, how it connects to research conducted within other National Research Programs, and how this research is being made available for use by partners and stakeholders. To illustrate these relationships, the presentation demonstrates how tiered testing strategies incorporating a range of technologies spanning multiple CSS research areas are used to collect toxicity data to inform decision making. The presentation will highlight examples of how CSS researchers are developing and applying in vitro NAMs under efforts such as the New Chemicals Collaborative Research Program to investigate priority endpoints (e.g., endocrine disruption, inhalation toxicity, developmental neurotoxicity) and address one of EPA's cross-cutting priorities, contaminants of immediate and emerging concern. *The views expressed in this presentation are those of the authors and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.*

8.04.T-04 Chemistry Data Delivery from the US-EPA Center for Computational Toxicology and Exposure to Support Environmental Chemistry

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In recent years, the growth of scientific data and the increasing need for data sharing and collaboration in the field of environmental chemistry has led to the creation of various software and databases that facilitate research and development into the safety and toxicity of chemicals. The US-EPA Center for Computational Toxicology and Exposure has been developing software and databases that serve the chemistry community for many years. This presentation will focus on several web-based software applications which have been developed at the US-EPA and made available to the community. While the primary software application from the Center is the CompTox Chemicals Dashboard almost a dozen proof-of-concept applications have been built serving various capabilities. The publicly accessible Cheminformatics Modules (<https://www.epa.gov/chemical-research/cheminformatics>) provides access to six individual modules to allow for hazard comparison for sets of chemicals, structure-substructure-similarity searching, structure alerts and batch QSAR prediction of both physicochemical and toxicity endpoints. A number of other applications in development include a chemical transformations database and a database of analytical methods and open mass spectral data. Each of these depends on the underlying DSSTox chemicals database, a rich source of chemistry data for over 1.2 million chemical substances. We will provide an overview of all tools in development and the integrated nature of the applications based on the underlying chemistry data. *This abstract does not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

8.04.T-05 Discussion 1 of 2 - How do the U.S. EPA's New Approach Methodologies (NAMs) Training Pilot Program and NAMs Tools Advance Research and Decision Making?

Christina Baghdikian, Scarlett Vandyke, Esra Mutlu and Jessica Daniel, U.S. Environmental Protection Agency

This is a facilitated discussion between speakers and session participants. The session aims to provide information on EPA's NAMs Training Pilot Program, widely used EPA NAMs tools (e.g., CompTox Chemicals Dashboard, high throughput toxicokinetic (HTTK), and Generalized Read Across (GenRA)), data bases (e.g., ToxVal, SEEM3, ToxRefDB, and invitroDB), other NAM tools that integrate EPA resources, and; applications of these tools in research and/or to inform decisions to protect human, animal, and/or environmental health. The speakers from different sectors will demonstrate various case studies and provide highlights about how these tools are informing chemical assessments by groups internal and external to EPA. This follow up discussion aims to engage session speakers with session participants to identify gaps; recognize the needs of researchers and risk assessors to increase the awareness and use of these freely available NAMs tools; and answer questions of new and prospective users as considering the use of these applications and tools. This discussion will not

only help promote the development of new tools and improvement of already existing tools, but also will encourage others to begin thinking about how they might use these tools for risk assessment and regulatory decision-making.

8.04.T-06 Discussion 2 of 2 - How do the U.S. EPA's New Approach Methodologies (NAMs) Training Pilot Program and NAMs Tools Advance Research and Decision Making?

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